ORIGINAL ARICLE

Association between Caspase-3 and miR-155-5p expression in Kaposi's Sarcoma in Zambia

Pachalo Kevin Matandara[1], Shari Rajendra Babu[2], Musalwa Muyangwa-Semenova[2], Rehana Omar*[2]

[1] *Department of Biology, Domasi College of Education, Zomba, Malawi*
[2] *Department of Physiological Sciences, School of Medicine, Ridgeway campus, University of Zambia, Lusaka, Zambia*

ABSTRACT

Background: Kaposi's sarcoma (KS) is a vascular tumour driven by the human herpesvirus 8 (HHV8). KS is the most prevalent HIV/AIDS associated cancer in the world and the second most prevalent cancer in Zambia, with 16% incidence and 15% mortality. Data suggests that HHV8 promotes KS tumorigenesis through inhibition of apoptosis by HHV8-encoded microRNAs. Indeed, miR-155-5p has been shown to promote tumour progression and inhibit expression of pro-apoptotic caspase-3 in several tumours. This study, therefore, aims to investigate the association between miR-155-5p and caspase-3 in KS.

Method: An analytical cross-sectional approach was used to compare miR-155-5p and caspase-3 mRNA and protein expression in KS tissues versus adjacent controls using RT-PCR and immunohistochemistry, respectively. Comparison of miR-155-5p and caspase-3 expression levels was done using the Wilcoxon rank-sum test while Spearman's rank was used to test for correlation.

Results: No significant difference was observed between miR-155-5p (p= 0.39) and caspase-3 mRNA expression (p = 0.67) in KS vs normal controls, however, there was a significant negative correlation between miR-155-5p and caspase-3 mRNA in KS tissues (p = 0.01; rho = -0.85). Furthermore, there was no significant difference in caspase-3 protein in KS vs normal controls (p = 0.18). Importantly, a significant correlation between miR-155-5p and viral load in KS tissues was observed (p = 0.05; rho = 0.81). **Conclusion**: This suggests a potential inverse relationship between miR-155-5p and caspase-3 mRNA in KS. Additionally, miR-155-5p expression may be regulated by HIV viral load, however, the nature of this regulation remains to be investigated.

Corresponding author:
Rehana Omar.
Department of Physiological Sciences, School of Medicine, Ridgeway Campus, University of Zambia, Lusaka, Zambia.
E-mail: rehana@unza.zm

INTRODUCTION

Kaposi's sarcoma (KS) is a vascular tumour of endothelial origin that usually presents as purple, red or brown lesions on the skin or mucosal surfaces.[1] It can also develop in internal organs of the body, including lymph nodes, lungs, or the digestive tract.[2] Epidemiologically, KS can be stratified into (i) classic KS in older men, (ii) endemic KS in younger African men and children, (iii) iatrogenic KS in

Keywords: Kaposi's sarcoma, Human Immunodeficiency Virus (HIV), tumorigenesis, apoptosis, miR-155-5p, Caspase-3

This article is available online at: http://www.mjz.co.zm, http://ajol.info/index.php/mjz, doi: https://doi.org/10.55320/mjz.52.4.737

The Medical Journal of Zambia, ISSN 0047-651X, is published by the Zambia Medical Association

immunocompromised individuals for example organ transplant patients or those under chemotherapeutic interventions, and (iv) epidemic HIV/AIDS associated KS.[3,4] Endemic KS in adolescents and adults is aggressive and presents with large invasive skin lesions while endemic KS in children predominantly affects the lymphatic system.[5] There is quite an overlap in the clinicopathology of paediatric endemic and epidemic KS although epidemic KS tends to also show aggressive disease progression, immune reconstitution and inflammatory syndrome.[6]

Regardless of their epidemiology, all KS types are known to be driven by the Human Herpesvirus type 8 (HHV8).[7] Current data shows a 5%-20% global infection rate with HHV8 with the highest prevalence in sub-Saharan Africa.[8] Regionally, the seroprevalence of HHV8 is estimated to be 16% in China[9], 6.8% in Qatar[10] and from 10.4 % to 48.3% in parts of Sub-Saharan Africa.[11,12] It is two to three times more common in males and is more prevalent in homosexual communities in endemic regions.[13] Transmission of HHV8 occurs by continued, substantial exchange of saliva.[14, 15, 16] This double-stranded lymphotropic DNA virus establishes latency in the host for a lifetime and undergoes lytic replication in blood vessels, skin and soft tissue under favourable conditions such as malnutrition and immune deficiency.[13] Indeed, immunocompromised individuals are at a greater risk of KS, making it the most common HIV-associated cancer in the world, and the third most frequently diagnosed cancer amongst men in sub-Saharan Africa.[17,18] In HIV positive patients, T-cell suppression and HHV8 replication are required for KS tumorigenesis and HHV8 polymerase inhibitors can curtail tumour progression but have no impact on existing skin lesions.[19] Interestingly, T-cell suppression is most commonly observed in HIV-related and iatrogenic KS but less so in classic KS[20], which suggests that different molecular mechanisms are involved in different KS subtypes. Despite a tremendous reduction in incidence and mortality of HIV-associated KS due to the introduction of antiretroviral therapy (ART) for HIV[21,22], KS remains a highly prevalent malignancy and its prevalence in sero-positive HIV patients compared to iatrogenic HIV-negative patients may imply that HIV impacts the immune system in a more profound manner giving rise to higher susceptibility to KS. Together, this raises the possibility that the molecular mechanisms at play in these KS variants require more investigation to identify key drivers of KS and potentially design targeted therapies for improved prognostic outcomes.

In resource-limited settings, diagnosis of KS is usually clinical, based on the presence of cutaneous or mucosal lesions which may be mimicked by other non-KS lesions.[2.3] In Zambia, suspected KS patients are referred to and present themselves to a well-established Dermatology and Venereology Division for diagnosis, mainly through clinical features and histological confirmation or immunohistochemistry.[24] The current gold standard of cancer diagnosis is the histological examination of tissue, obtained either by radiologically guided biopsy or surgical excision, which are both invasive and expensive.[23] Therefore, there is still a need for other diagnostic and screening tools for early diagnosis as well as potential biomarkers that may be amenable to therapy. Indeed, delayed diagnosis, arising from a lack of reliable diagnostic assays for the detection of latent or early-stage diseases, has partly resulted in the relatively high disease morbidity and mortality in sub-Saharan African countries like Zambia.[23] To this end, this study focuses on microRNAs (miRNAs), which have been increasingly identified as reliable biomarkers for the early detection of multiple pathologies.[25]

MiRNAs are involved in highly regulated processes, such as proliferation, differentiation, apoptosis, and metabolic processes, and they are found in serum, plasma, and other body fluids that have a stable form to protect them from endogenous RNase activity.[26] MiRNAs can also stably exist in skeletal muscles, heart muscles, adipose tissues, B-cells and other tissues of the body.[27] As such, miRNAs have been intensively investigated in the past decade as possible biomarkers and therapeutic targets of

different types of cancers through liquid biopsies and serve as disease detection, progression, and other monitoring tools with rapid and non-invasive ease.[28] The sensitivity and accessibility of circulating microRNA have put them at an advantage as potential diagnostic biomarkers of cancers[29] such as melanoma[30] and non-small cell lung cancer.[31] The success of circulating microRNAs in disease monitoring is underscored by the substantial increase in biomarker identification and use for early detection, progression and treatment of HIV-associated malignancies by the WHO in collaboration with research institutions such as the National Cancer Institute.[32] HHV8-encoded miRNAs are known to produce multiple oncogenic proteins that downregulate tumour suppressors while activating signalling pathways that promote cancer development and progression.[13] In KS, HHV8-encoded miRNAs have been shown to downregulate caspase-3 mRNA expression, which is an important mediator of apoptosis.[33]

Of interest to this study is miR-155-5p, whose overexpression has been observed in several cancers, such as breast cancer[34], lung cancer[35,36], glioma[37], B-cell lymphoma and chronic lymphocytic leukemia[38], and oral squamous cell carcinoma[39] suggesting a potential role in the tumorigenesis of these cancers. Interestingly, studies have reported that inhibition of miR-155-5p promotes apoptosis of cancer cells by inducing caspase-3 protein activation.[40,41] Whether there is an association between miR-155-5p and caspase-3 mRNA expression in KS tumours is currently unknown. Investigating the expression patterns of both miR-155-5p and caspase-3 would provide some insight into the molecular drivers of KS and hence therapeutic targets as well as identify microRNAs as potential diagnostic markers of KS. The current study, therefore, investigated the expression and correlation of miR-155-5p and caspase-3 mRNA in KS tumours.

METHODS

Study Design and Participants

This was a cross-sectional analytical study conducted at the University Teaching Hospital in Lusaka, Zambia from May to October 2023. Due to low numbers of willing participants during the data collecting period, convenience sampling was done to recruit patients who were newly diagnosed with KS. Using theoretical change in miR-155-5p expression of 7.12 (SD ± 2.32) copies per cell in cancerous tissues and that of 3.20 (SD ± 2.90) copies per cell in normal adjacent tissues sample size was calculated using a two independent mean formula of $n_i = 2\ (Z_{1-\alpha/2}+Z_{1-\beta}/\ ES)^2$ where ES (Effect size), n_i (Sample size for one group), α (Significance level at 95 %), and β (Power at 80%). The effect size was calculated using the formula $ES = |\mu 1-\mu 2|/\sigma$ where μ_1 stands for mean for the first group (normal adjacent tissues), μ_2 stands for mean for the second group (cancerous tissues) and σ (Standard deviation). The calculated total sample size was 8, thus 16 tissues (8 cancerous tissues and 8 adjacent normal tissues). However, the study recruited 9 participants (9 cancerous tissues and 9 adjacent normal tissues). All study participants were 18 years of age or older as they were recruited from an adult outpatient clinic and were also chemotherapy/radiotherapy-naïve at time of sample collection. During recruitment, a study questionnaire was used to obtain consenting participants' demographic information, including age and gender. Clinical information including KS morphotype, HIV status, CD4 counts, HIV viral loads, and comorbid conditions were also obtained. Before enrollment into the study, the participants gave written informed consent which also permitted the investigators to obtain an additional KS tissue biopsy for the study. The study procedures were approved by both the National Health Research Authority (NHRA) of Zambia (Ref. No. NHREB0009/27/03/2023) and The University of Zambia Research Ethics Committee (UNZABREC) (Ref. No. 3752-2023).

Immunohistochemistry

A 5mm formalin-fixed and paraffin-embedded (FFPE) punch biopsy specimen used for the

standard histological confirmation of KS was subjected to immunohistochemistry for caspase-3 expression. The FFPE KS tissues were cut into 4µm sections and mounted on adhesive slides, then baked at 60? overnight. Deparaffination, hydration, and antigen retrieval was done on a semi-automated machine (PT Link, Agilent) according to the manufacturer's instructions. The slides were then rinsed with wash buffer (dilution 1:20), first for 1 minute and then 5 minutes. Peroxidase activity was blocked with hydrogen peroxide (dilution 1:100) in methanol for 5 Minutes. The slides were incubated overnight at 60°C in a humid chamber with the primary antibody, Rabbit anti-caspase-3 antibody (dilution 1:2000) **(**Atlas antibodies, HPA002643, Lot: B117459). This was followed by incubation with the secondary antibody, anti-Rabbit labelled Polymer (Dako, EnVision+ System-HRP, Ref: K4003) in Tris-buffered saline solution with 5% bovine serum albumin. Colour was then developed with a solution of diaminobenzidine (DAB). The sections were counterstained with haematoxylin. After examination of the stained slides, scoring was done using the intensity of the stains and percentage of stained cells (Figure 1). Staining intensity was numerically scored as 0 (no staining), 1 (weak), 2 (moderate), and 3 (strong). The percentage of stained cells was numerically classified as 0 (<5%), 1 (5-25%), 2 (26-50%), 3 (51-75%), and 4 (>75%). The product of nuclear staining percentage and staining yielded the total staining. To ensure that the results obtained were not affected by external factors, the samples were processed using batch-made reagents for all the samples and an automated machine for deparaffination, hydration, and antigen retrieval. As much as possible, each replicate was analysed in a single session

Quantification of miR-155-5p and caspase-3 Transcripts

Total RNA was extracted from frozen KS tissue samples that were stabilized in RNA later, using All Prep® DNA/RNA Mini kit (Qiagen) in accordance with the manufacturer's instructions. RNA was reverse-transcribed using the LunaScript RT Super mix kit (Biolabs) in accordance with the manufacturer's instructions to generate first-strand cDNA. Quantitative real-time PCR was performed using the Luna Universal qPCR Master Mix (Biolabs) according to the manufacturer's instructions. U6 was used as a normalization control for miR-155-5p, while GAPDH was used as a normalization control for caspase-3 mRNA. The sequences of the PCR primers used are presented in **table 1**.

Table 1. PCR primers used in the study

	PRIMER	SEQUENCE
1	miR-155-5p forward	5'-ACACTCCAGCTTAATGCTAATCGTGATAG -3'
2	miR-155-5p reverse	5'-CTCAACTGGTGTCGTGGA-3'
3	U6 forward	5'-CTCGCTTCGGCAGCACA-3'
4	U6 reverse	5'-AACGCTTCACGAATTTGCGT-3'
5	CASP-3 mRNA forward	5'-AGAGGGGATCGTTGTAGAAG-3',
6	CASP-3 mRNA reverse	5'-GTTGCCACCTTTCGGTTAAC-3'
7	GAPDH forward	5'-CACCCTCAAGATTGTCAGC-3'
6	GAPDH reverse	5'-TAAGTCCCTCCACGATGC-3'

Primers were purchased from Inqaba Biotechnical Industries (Pty) Ltd and the sequences used are previously validated primers. All PCR experiments were performed in duplicate. The relative mRNA expression was determined using the comparative Livak method ($2^{-\Delta\Delta Ct}$). While the standard procedure for PCR was strictly adhered to and replicates were performed in one session, the involvement of different personnel in conducting the PCR runs and sample preparation could have an impact on the validity of the results.

Data analysis

STATA version 17 was used for all statistical analyses, while GraphPad prism 9 was used for generating the figures. Baseline characteristics were analysed using descriptive statistics. Comparison of expression levels of the markers of interest in KS

tissue to normal tissue was done using the Wilcoxon rank-sum test. We used Spearman's rank correlation to determine whether expression of miR-155-5p and caspase-3 were correlated. P values <0.05 were considered statistically significant.

RESULTS

Participant Demographics

Our study population consisted of more males than females, with a median age of 39 years, and the majority were HIV positive. Among the HIV positive individuals, slightly more than half (55.0 %) were on ART at time of recruitment. The rest of the baseline demographic and clinical characteristics are shown in **table 2**.

Table 2: Demographics of study participants

Characteristics	Values
Gender, Male n (%)	6 (66.70%)
Gender, Females n (%)	3 (33.30%)
Median Age (years)	39 [31-57]
KS HIV positive	6 (66.70%)
KS HIV negative	3(33.30%)
HIV infection duration, median (months)	3[1-36]
On ART	5 (55.50%)
ART treatment duration, median (months)	3[1-36]
CD4 Count (Cell/µl), median (IQR)	222 [132-507]
HIV Viral Load (Copies/ml), median (IQR)	0 [0-469]

Analysis of mRNA expression of miR-155-5p and caspase 3 in KS tumours versus normal tissues

There was no significant difference (p = 0.39) in the expression of miR-155-5p in KS tumours compared to adjacent normal skin tissue (**figure 1A**). Additionally, no significant difference (p = 0.67) was observed in mRNA copies of Caspase-3 in KS tumours compared to the normal adjacent tissue (**figure 1B**).

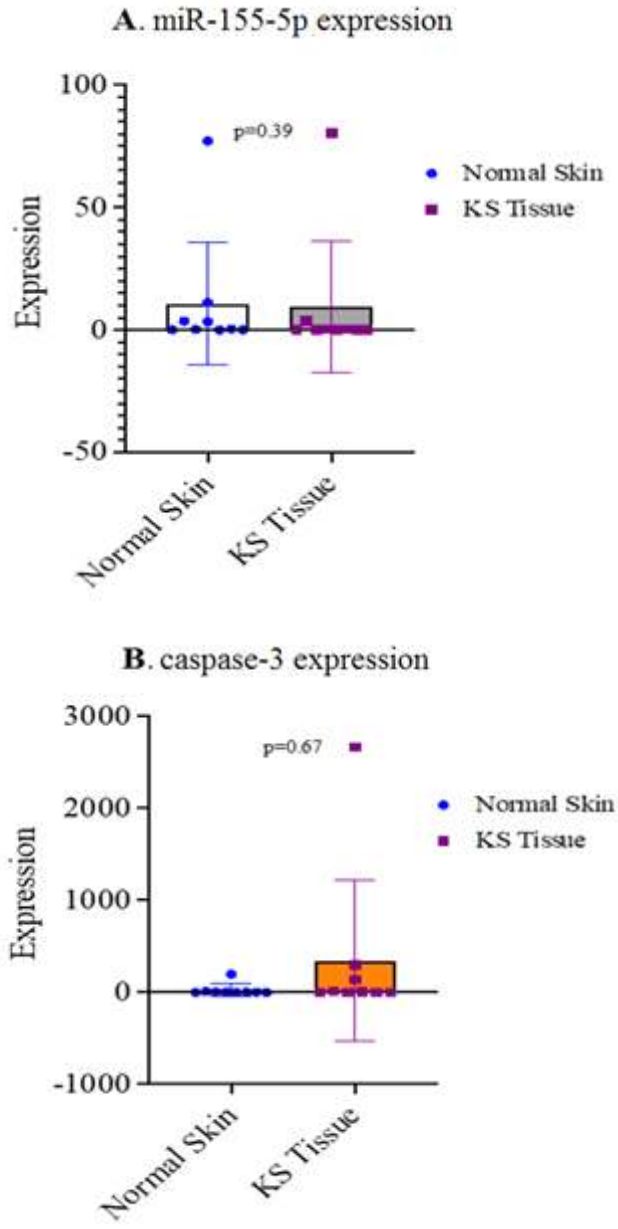

Figure 1. Expression of: A) miR-155-5p; and B) Caspase-3 mRNA in KS tumours and adjacent normal tissue. miR-155-5p expression was normalized against U6, while GAPDH was used as a normalization control for Caspase 3 mRNA using $2^{-\Delta\Delta Ct}$ (*p<0.05), n=2.

Caspase 3 and miR-155-5p mRNA expression are negatively correlated in KS tissue

To further investigate the association between expression of miR-155-5p and caspase-3 mRNA in KS and normal adjacent tissue, spearman correlation was performed and a statistically

significant negative correlation (p = 0.01; rho = -0.85) between mRNA levels of Caspase-3 and miR-155-5p in KS tumours observed (**figure 2A**). Interestingly, no significant correlation (p = 0.64; rho = -0.18) was seen in the normal adjacent tissues (**figure 2B**).

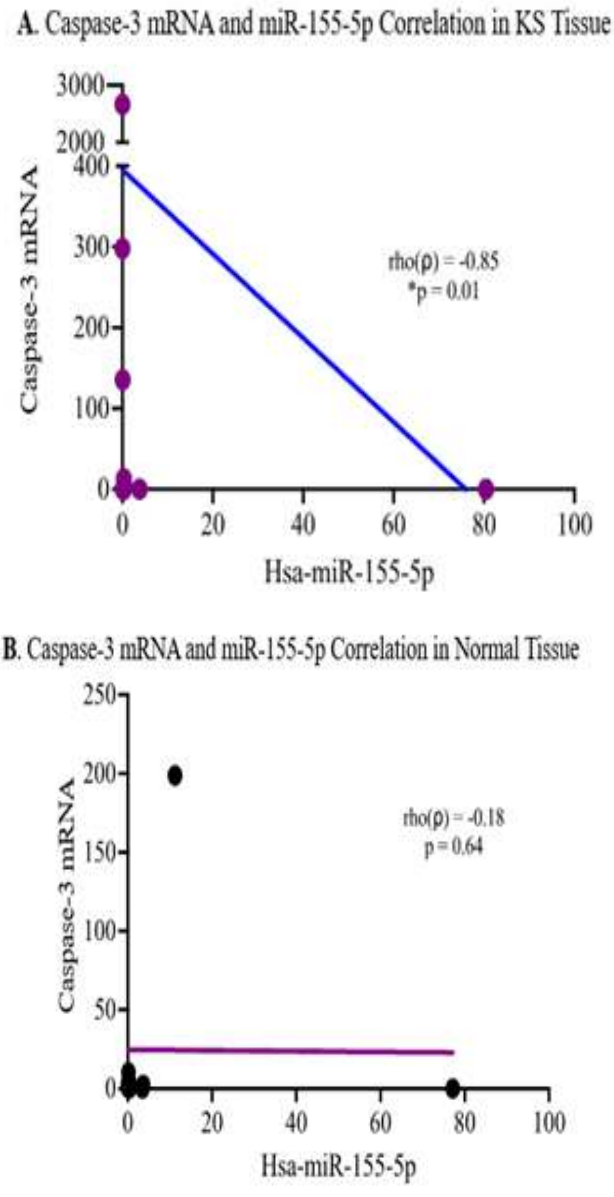

Figure 2: Correlation of miR-155-5p expression and Caspase-3 mRNA expression in KS tissues (A) and Normal adjacent tissues (B). A Spearman correlation was done to compare the expression of miR-155-5p and Caspase 3 mRNA in KS tissues and Normal tissues. The graphs indicate mean expression of miR-155-5p and Caspase 3 mRNA normalized to U6 and GAPDH respectively using $2^{-\Delta\Delta Ct}$ (*p < 0.05), n=2.

Protein expression of Caspase-3 in KS tumours versus normal adjacent controls

To investigate the expression of caspase-3 protein in KS vs normal adjacent tissue, immunohistochemistry was performed. A higher expression of caspase-3 was observed in KS tissues compared to normal tissue; however, this was not statistically significant (**figure 3**).

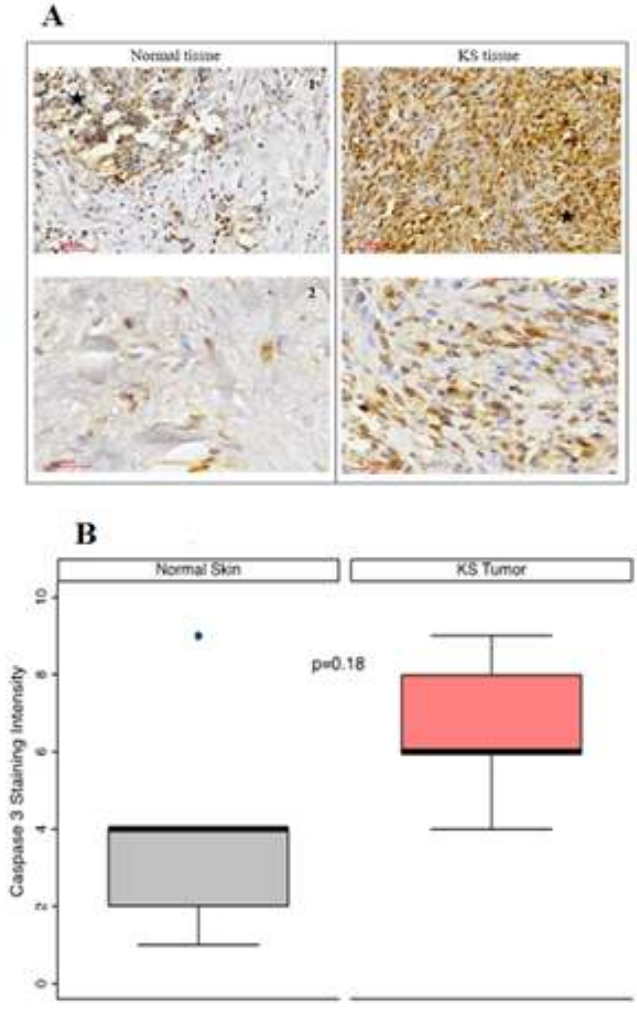

Figure 3: Expression of Caspase-3 protein. **A**, Normal tissue (1); Caspase -3 expression demonstrating less than 50% staining (*), X20 mag. Normal tissue (2); Caspase-3 expression with few cells staining, X40 mag. KS tissue (1); High expression of caspase-3, greater than 50%, in tumour cells (*), X20 mag. KS tissue (2); demonstrating expression of caspase-3 (greater than 50%) in tumour cells, X40 mag. Immunohistochemistry with caspase-3 antibody. **B**, Boxplot quantifying mean expression of caspase-3 protein. Caspase 3 expression was analysed in normal tissues and KS tissues using IHC. Scoring was done using the formula: Total score= Staining intensity x %

nuclear staining. Statistical analysis by student's t-test comparing mean expression of caspase-3 protein between normal tissue and KS tissues (*p < 0.05).

Expression of miR-155-5p is positively associated with HIV viral load

Since a high proportion of our HIV-positive study participants were ART-naïve with high viral loads, we further assessed whether HIV viral loads were correlated with miR-155-5p and caspase-3 mRNA levels. We observed a significant positive correlation (p = 0.049; rho = 0.81) between miR-155-5p and HIV viral loads in HIV-positive KS patients (**figure 4A**). On the contrary, an inverse, but statistically insignificant negative correlation (p = 0.12; rho = -0.69) was observed between caspase-3 mRNA levels and HIV viral loads (**figure 4B**).

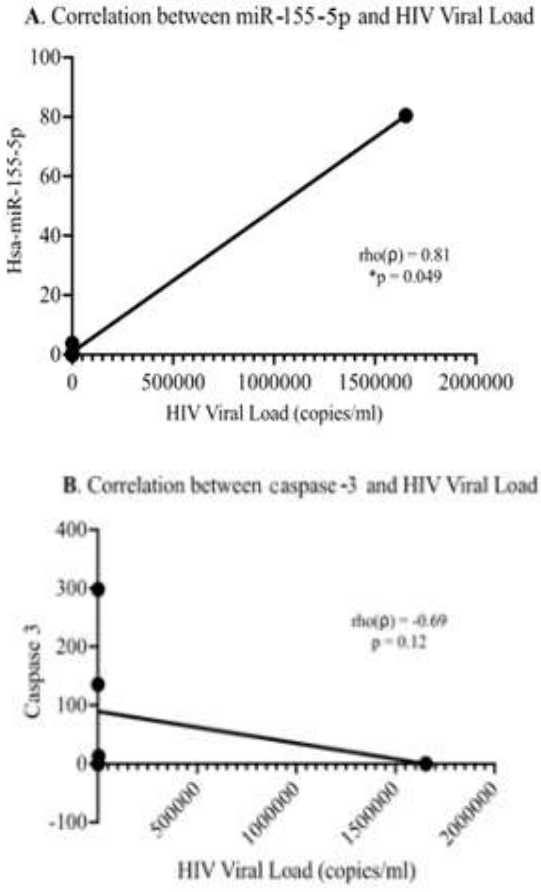

Figure 4: Correlation between miR-155-5p expression and HIV viral load (A) and correlation between Caspase-3 mRNA and HIV viral load (B) (*p < 0.05). U6 was used as a normalization control for miR-155-5p, while GAPDH was used as a normalization control for Caspase-3 mRNA (*p < 0.05), n=2.

DISCUSSION

Expression of caspase-3 protein, an executioner caspase of apoptosis, has been observed to be low in KS tumors. MiR-155-5p is known to regulate caspase-3 expression in some cancers, but its effect in KS tumours remains unknown. In this study, the expressions of miR-155-5p, caspase-3 mRNA, and caspase-3 protein in KS tissues and ipsilateral normal skin were investigated. No significant difference was observed in the expressions of miR-155-5p and caspase-3 mRNA in KS tumours compared to adjacent normal tissue. However, a significant negative correlation between miR-155-5p and caspase-3 mRNA in KS tissues suggests a possible regulatory relationship in KS.

This study also showed no significant correlation between miR-155-5p and caspase-3 protein expression. This could be due to the small sample size, lack of molecular stratification of the KS subtypes, and lack of grading of the samples. It is interesting to note that although not significantly different, KS tissues had higher levels of caspase-3 protein (**figure 3**). Increased caspase-3 protein expression has been associated with advanced pathological stage, larger tumour sizes, and poor outcomes in several cancers. In support of the known tumour suppressor role of caspase-3, a study observed that down-regulated caspase-3 in KS tumours correlated with the inhibition of apoptosis. Contrary to these observations and indeed our expectations, the results in the current study show a higher expression of caspase-3 protein in tumour tissues than normal adjacent tissues albeit non-significant. Other studies have shown that in oral tongue squamous cell carcinoma (OTSCC) and buccal mucosa squamous cell carcinoma (BMSCC), levels of caspase-3 and cleaved caspase-3 were significantly higher in tumour tissues than in normal adjacent tissues. A possible explanation could be provided by a recent study that suggests that caspase-3 plays an important role in the initiation of

the HHV8 replication. The study showed that proteolytic cleavage of the transcriptional factor Sp3 under apoptotic conditions resulted in Sp3 C-terminal fragments with domains that can interact with HHV8 to initiate the viral lytic cycle, suggesting a transcriptional role of caspase-3. The study showed that activation of caspase-3 was critical in the proteolytic cleavage of Sp3. The observed elevated levels of caspase-3 in KS tissue in the current study may be a compensatory mechanism as a result of the increased viral lytic cyles. Furthermore, caspases have been shown to downregulate type 1 interferons which promotes HHV8 lytic replication. This was shown in a 2022 study, which suggests that caspases interfere with the cytosolic DNA sensor, cGAS, which recognizes viral replication DNA and activates systemic immune response via induction of type 1 interferon. It is important to note that Type 1 interferon is responsible for the expression of several interferon-stimulated genes that play an important role in inhibiting HIV infection.[-].

Other investigations into the potential roles of caspase-3 show that Sub lethal activation of caspase-3 seems to confer cell protective effects in response to chemical or environmental cellular stressors. In irradiated normal murine MCF10A breast cells, and mouse embryonic fibroblasts, sublethal levels caspase-3 have been shown to play a facilitative role in persistent DNA damage marked by gamma-H2AX foci, resulting in genetic instability. Interestingly, mice with double knockout *caspase-3* has significantly reduced skin carcinogenesis when induced with Dimethylbenz (a) anthracene + 12-O-tetradecanoyphobol-13-acetate. This study showed that caspase-3 triggers the translocation of endonuclease G from the mitochondria to the nucleus, where the Src-STAT3 pathway gets phosphorylated, ultimately resulting in oncogenic transformation. These data are corroborated by a more recent study, which suggests that consistent and sublethal activation of caspase-3 plays an essential and facilitative role in genetic instability and malignant oncogenic transformation in mammalian cells and mouse mammary tumour virus-polyomavirus middle T antigen (MMTV-PyMT) breast cancer mouse model. This suggests that aside from their well-known pro-apoptotic role, caspases have an inherent duality as tumour promoter or tumour suppressors. It would be interesting to investigate the molecular mechanisms that regulate this switch.

Additionally, since tumour development in KS is influenced by the efficiency of the immune system, our results may be attributed to ART treatment, especially for ART-treated cases. Caspases have also been shown to be overexpressed as a response to drugs to enhance cell death so it may be worthwhile to investigate the impact of ARTs on caspase-3 expression in KS. Although only caspase-3, and not both caspase-3 and active cleaved caspase-3, was stained in the current study, the results may be suggestive of caspase-3's activities beyond apoptosis in KS. Indeed, the lack of molecular subtyping of our samples hinders conclusive remarks and future studies would do well to stratify the KS subtypes and identify individual pathways that drive tumour formation as well as diagnostic and/or therapeutic biomarkers.

This study also investigated whether infection with HIV was associated with the expression of miR-155-5p and caspase-3, as KS is more common in people living with HIV. A significant positive correlation between miR-155-5p and plasma HIV viral loads was observed, however, there was no significant correlation between caspase-3 mRNA and plasma HIV viral loads. In agreement with these findings, a higher expression of miR-155-5p has also been observed in HIV-infected individuals. These observations are further supported by previous reports that show that the miR-155 family which comprises miR-155-5p is significantly highly expressed in ART-naive HIV patients with progressive disease compared ART-naive long term non-progressor HIV positive patients. Pawar *et al*., demonstrated that knockdown of *miR-155* in peripheral blood mononuclear cells from these two

HIV-1 cohorts resulted in significantly increased expression of Toll-like receptors (TLRs) and innate immune response markers such as interferon regulating factors, NF-κB and TNFα. Furthermore, the authors showed that Type 1 interferon was significantly positively correlated with CD4 count and significantly negatively correlated with viral load. Together these data suggest that HIV viral load regulates miR-155-5p and that this in part results in immune dysregulation in KS.

In sum, these data warrant more investigation into the role of miR-155-5p with regards to immune dysregulation and the role of caspase-3 in this signalling axis in different stages and KS subtypes to determine its oncogenic potential and expression pattern for diagnostic purposes.

CONCLUSION AND LIMITATIONS

In conclusion, our data suggests that caspase-3 mRNA could be a target of miR-155-5p in KS. The strong negative correlation between the two suggests that miR-155-5p potentially down regulates caspase-3 mRNA. Given the strong positive correlation found between HIV and miR-155-5p, the latter may serve as a possible biomarker for Epidemic KS and indeed a strong potential therapeutic target for partial immune reconstitution in KS patients. Expression of miR-155-5p may also be useful as a prognostic indicator of HIV-associated KS as well as a diagnostic biomarker. The high expression of caspase-3 protein in KS tissues may indicate that, although playing a direct role in apoptosis, caspase-3 may have dual roles and act as a tumour promoter or confer protective effects on KS tissue under sub lethal expression levels. It may also suggest caspase-3's activities beyond apoptosis such as promoting persistent DNA damage and oncogenic transformation and downregulate key immune response pathways in KS. While this study used a statistically derived sample size, it did not stratify the KS samples and future experiments on epidemiological subtypes would produce more robust data into the molecular mechanism/s that drive each specific subtype. This will have the potential to be translated into clinical practice for subtype specific diagnostic, monitoring and prognostic biomarkers.

It is important to note that the samples used were not graded by disease stage, nor were they stratified by subtype. Future studies would be better performed in graded samples to identify how early the expression of miR-155-5p is detected as this would help identify its potential as an early diagnostic marker. Due to limited laboratory infrastructure, it was not possible to perform cell culture assays to verify the role of caspase-3 in KS. Furthermore, due to small size of cleaved caspase-3, western blotting is required to verify that caspase-3 is activated. Immunohistochemistry alone used antibodies to caspase-3 and does not differentiate between the native and cleaved forms. Lastly, convenient sampling was used because of the study participants' insufficiency and the study acknowledges that external validity is negatively impacted and larger studies that properly represent the population would provide more accurate data. Even though the study's generalizability would be limited by its small sample size and convenient sampling, it nevertheless offers insight into the cytogenetic and molecular processes of KS. Therefore, it may be better to work with bigger sample populations to have conclusive data based on the subtypes, ART intervention, viral load and disease stage.

ACKNOWLEDGEMENTS

This paper forms part of the requirements for the Master of Science in Biochemistry. Our heartfelt gratitude goes to members in the School of Medicine and the Department of Physiological Sciences at the University of Zambia. A special acknowledgement is made to Dr. Owen Ngalamika and Dr. Chibamba Mumba from the Dermatology and Venereology Unit and the Department of Pathology respectively for their contribution in data analysis. We extend our gratitude to the members of the Kaposi's Sarcoma Laboratory at the University Teaching Hospital. Finally, we are grateful to all the study participants.

FUNDING

This study was funded by Domasi College of Education, an institution and Ministry of Higher Education, Malawi and Kaposi's sarcoma project at the University Teaching Hospital.

AVAILABILITY OF DATA AND MATERIALS

The data and materials used in this study are available from the corresponding author on reasonable request.

COMPETING INTEREST

The authors declare no competing interests.

AUTHOR'S CONTRIBUTION

Pachalo Matandara was involved in study design, data acquisition, analysis, manuscript writing and approval of the manuscript for publication

Shari Rajendra Babu conceptualized, designed, critically interpreted data, and was involved in manuscript writing and approval of the manuscript for publication

Musalwa Muyangwa-Semenova was involved in the study design, critical data analysis and interpretation, manuscript writing and approval of the manuscript for publication

Rehana Omar conceptualized, designed, critically interpreted data, revised and approved the manuscript for publication

REFERENCES

1. Orem J. Cancer prevention and control: Kaposi's sarcoma. Ecancermedicalscience. 2019;13:951.
2. Vally F, Selvaraj WMP, Ngalamika O. Admitted AIDS-associated Kaposi sarcoma patients: Indications for admission and predictors of mortality. Medicine (Baltimore). 2020 Sep 25;99(39):e22415.
3. Jakob L, Metzler G, Chen KM, Garbe C. Non-AIDS associated Kaposi's sarcoma: clinical features and treatment outcome. *PloS One*. 2011 Apr 12;6(4):e18397.
4. Curtiss P, Strazzulla LC, Friedman-Kien AE. An Update on Kaposi's Sarcoma: Epidemiology, Pathogenesis and Treatment. *Dermatol Ther*. 2016 Dec;6(4):465–70.
5. Jackson CC, Dickson MA, Sadjadi M, Gessain A, Abel L, Jouanguy E, et al. Kaposi Sarcoma of Childhood: Inborn or Acquired Immunodeficiency to Oncogenic HHV-8. *Pediatr Blood Cancer*. 2016 Mar;63(3):392–7.
6. El-Mallawany NK, Villiera J, Kamiyango W, Peckham-Gregory EC, Scheurer ME, Allen CE, et al. Endemic Kaposi sarcoma in HIV-negative children and adolescents: an evaluation of overlapping and distinct clinical features in comparison with HIV-related disease. *Infect Agent Cancer*. 2018;13:33.
7. Catrina Ene AM, Borze I, Guled M, Costache M, Leen G, Sajin M, et al. MicroRNA expression profiles in Kaposi's sarcoma. *Pathol Oncol Res POR*. 2014 Jan;20(1):153–9.
8. Khodadad N, Hashempour A, Akbarinia S. In silico analysis of human herpes virus-8 genome: a comparison of the K1, VR1, and VR2 regions for genotyping and global geographical distribution. *Sci Rep* [Internet]. 2025 Jan 2 [cited 2025 May 16];15(1):326. Available from: https://www.nature.com/articles/s41598-024-84376-2
9. Fang Y, Li W, Zhang Y, Zhou C, Wu H, Zhang Y, et al. Seroprevalence of Kaposi's sarcoma-associated herpesvirus and risk factors in Jiuquan area, China. *J Med Virol*. 2022 Dec;94(12):6016–22.

10. Zedan HT, Elkhider A, Hicazi A, Amanullah F, Al-Sadeq DW, Nizamuddin PB, et al. Seroprevalence and detection of Human herpesvirus-8 (HHV-8) among healthy blood donors residing in Qatar. *J Infect Public Health* [Internet]. 2024 Dec [cited 2025 May 9];17(12):102590. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1876034124003241
11. Malonga GA, Dienta S, Traore FT, Maiga Z, Ba A, Faye O, et al. Human Herpesvirus 8 seroprevalence among blood donors in Mali. *J Med Virol*. 2022 Sep;94(9):4554–8.
12. Mamimandjiami AI, Mouinga-Ondémé A, Ramassamy JL, Djuicy DD, Afonso PV, Mahé A, et al. Epidemiology and Genetic Variability of HHV-8/KSHV among Rural Populations and Kaposi's Sarcoma Patients in Gabon, Central Africa. Review of the Geographical Distribution of HHV-8 K1 Genotypes in Africa. *Viruses.* 2021 Jan 25;13(2):175.
13. Rewane A, Tadi P. Herpes Virus Type 8. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 [cited 2025 May 16]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK556023/
14. Kedes DH, Operskalski E, Busch M, Kohn R, Flood J, Ganem D. The seroepidemiology of human herpesvirus 8 (Kaposi's sarcoma-associated herpesvirus): distribution of infection in KS risk groups and evidence for sexual transmission. *Nat Med*. 1996 Aug;2(8):918–24.
15. Martin JN, Ganem DE, Osmond DH, Page-Shafer KA, Macrae D, Kedes DH. Sexual transmission and the natural history of human herpesvirus 8 infection. *N Engl J Med*. 1998 Apr 2;338(14):948–54.
16. Butler LM, Osmond DH, Jones AG, Martin JN. Use of saliva as a lubricant in anal sexual practices among homosexual men. *J Acquir Immune Defic Syndr* 1999. 2009 Feb 1;50(2):162–7.
17. Cesarman E, Damania B, Krown SE, Martin J, Bower M, Whitby D. Kaposi sarcoma. *Nat Rev Dis Primer.* 2019 Jan 31;5(1):9.
18. Kalubula M. Epidemiology of Kaposi's sarcoma in Zambia, 2007 - 2014. *Malawi Med J* [Internet]. 2020 Jun 30 [cited 2025 Apr 1];32(2):74–9. Available from: https://www.ajol.info/index.php/mmj/article/view/197192
19. Ensoli B, Stürzl M, Monini P. Reactivation and role of HHV-8 in Kaposi's sarcoma initiation. *Adv Cancer Res*. 2001;81:161–200.
20. Schneider JW, Dittmer DP. Diagnosis and Treatment of Kaposi Sarcoma. *Am J Clin Dermatol*. 2017 Aug;18(4):529–39.
21. Bohlius J, Valeri F, Maskew M, Prozesky H, Garone D, Sengayi M, et al. Kaposi's Sarcoma in HIV-infected patients in South Africa: Multicohort study in the antiretroviral therapy era. *Int J Cancer*. 2014 Dec 1;135(11):2644–52.
22. The University of Zambia, Mukololo L, Phiri LT, The University of Zambia, Chisha M, The University of Zambia, et al. Kaposi's Sarcoma Trends in the Era of Highly Active Antiretroviral Therapy in Zambia. *Univ Zamb J Agric Biomed Sci* [Internet]. 2022 Jul 1 [cited 2025 Apr 14];6(3):33–42. Available from: https://journals.unza.zm/index.php/JABS/article/view/978

23. Muwonge H, Kasujja H, Niyonzima N, Atugonza C, Kasolo J, Lugaajju A, et al. Unique circulating microRNA profiles in epidemic Kaposi's sarcoma. Non-Coding RNA Res. 2022 Jun;7(2):114–22.
24. Ngalamika O, Minhas V, Wood C. Kaposi's sarcoma at the University Teaching Hospital, Lusaka, Zambia in the antiretroviral therapy era. *Int J Cancer.* 2015 Mar 1;136(5):1241–2.
25. Rupaimoole R, Slack FJ. MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. *Nat Rev Drug Discov*. 2017 Mar;16(3):203–22.
26. Meza MN, Carrillo JAB. Biomarkers, Obesity, and Cardiovascular Diseases. In: Wang M, Witzmann FA, editors. Role of Biomarkers in Medicine [Internet]. Intech; 2016 [cited 2025 Apr 8]. Available from: http://www.intechopen.com/books/role-of-biomarkers-in-medicine/biomarkers-obesity-and-cardiovascular-diseases
27. Huang Y, Yan Y, Xv W, Qian G, Li C, Zou H, et al. A New Insight into the Roles of MiRNAs in Metabolic Syndrome. *BioMed Res Int* [Internet]. 2018 Dec 17 [cited 2025 Apr 8];2018:1–15. Available from: https://www.hindawi.com/journals/bmri/2018/7372636/
28. Larrea E, Sole C, Manterola L, Goicoechea I, Armesto M, Arestin M, et al. New Concepts in Cancer Biomarkers: Circulating miRNAs in Liquid Biopsies. *Int J Mol Sci*. 2016 Apr 27;17(5):627.
29. Pozniak T, Shcharbin D, Bryszewska M. Circulating microRNAs in Medicine. *Int J Mol Sci.* 2022 Apr 3;23(7):3996.
30. Lim SY, Boyd SC, Diefenbach RJ, Rizos H. Circulating MicroRNAs: functional biomarkers for melanoma prognosis and treatment. *Mol Cancer* [Internet]. 2025 Mar 28 [cited 2025 May 16];24(1):99. Available from: https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-025-02298-7
31. Abdipourbozorgbaghi M, Vancura A, Radpour R, Haefliger S. Circulating miRNA panels as a novel non-invasive diagnostic, prognostic, and potential predictive biomarkers in non-small cell lung cancer (NSCLC). *Br J Cancer* [Internet]. 2024 Nov 2 [cited 2025 May 16];131(8):1350–62. Available from: https://www.nature.com/articles/s41416-024-02831-3
32. Dlamini Z, Mbele M, Makhafola TJ, Hull R, Marima R. HIV-Associated Cancer Biomarkers: A Requirement for Early Diagnosis. *Int J Mol Sci.* 2021 Jul 29;22(15):8127.
33. Suffert G, Malterer G, Hausser J, Viiliäinen J, Fender A, Contrant M, et al. Kaposi's sarcoma herpesvirus microRNAs target caspase 3 and regulate apoptosis. *PLoS Pathog*. 2011 Dec;7(12):e1002405.
34. Yan LX, Huang XF, Shao Q, Huang MY, Deng L, Wu QL, et al. MicroRNA miR-21 overexpression in human breast cancer is associated with advanced clinical stage, lymph node metastasis and patient poor prognosis. RNA N Y N. 2008 Nov;14(11):2348–60.
35. Zang YS, Zhong YF, Fang Z, Li B, An J. MiR-155 inhibits the sensitivity of lung cancer cells to cisplatin via negative regulation of Apaf-1 expression. *Cancer Gene Ther*. 2012 Nov;19(11):773–8.
36. Shao C, Yang F, Qin Z, Jing X, Shu Y, Shen H. The value of miR-155 as a biomarker for the diagnosis and prognosis of lung cancer: a systematic review with meta-

analysis. *BMC Cancer*. 2019 Nov 14;19(1):1103.

37. Ling N, Gu J, Lei Z, Li M, Zhao J, Zhang HT, et al. microRNA-155 regulates cell proliferation and invasion by targeting FOXO3a in glioma. *Oncol Rep*. 2013 Nov;30(5):2111–8.
38. Due H, Svendsen P, Bødker JS, Schmitz A, Bøgsted M, Johnsen HE, et al. miR-155 as a Biomarker in B-Cell Malignancies. *BioMed Res Int*. 2016;2016:9513037.
39. Wu M, Duan Q, Liu X, Zhang P, Fu Y, Zhang Z, et al. MiR-155-5p promotes oral cancer progression by targeting chromatin remodelling gene ARID2. *Biomed Pharmacother Biomedecine Pharmacother*. 2020 Feb;122:109696.
40. Milani R, Brognara E, Fabbri E, Manicardi A, Corradini R, Finotti A, et al. Targeting miR-155-5p and miR-221-3p by peptide nucleic acids induces caspase-3 activation and apoptosis in temozolomide-resistant T98G glioma cells. *Int J Oncol*. 2019 Jul;55(1):59–68.
41. Xu W, Song C, Wang X, Li Y, Bai X, Liang X, et al. Downregulation of miR-155-5p enhances the anti-tumour effect of cetuximab on triple-negative breast cancer cells via inducing cell apoptosis and pyroptosis. *Aging*. 2021 Jan 5;13(1):228–40.
42. Yang L Hong, Wang S Lu, Tang L li, Liu B, Ye W le, Wang L ling, et al. Universal Stem-Loop Primer Method for Screening and Quantification of MicroRNA. Hoheisel JD, editor. PLoS ONE [Internet]. 2014 Dec 30 [cited 2025 May 22];9(12):e115293. Available from: https://dx.plos.org/10.1371/journal.pone.0115293
43. De Santis R, Liepelt A, Mossanen JC, Dueck A, Simons N, Mohs A, et al. miR-155 targets Caspase-3 mRNA in activated macrophages. *RNA Biol*. 2016;13(1):43–58.
44. Ramos da Silva S, Bacchi MM, Bacchi CE, Elgui de Oliveira D. Human bcl-2 expression, cleaved caspase-3, and KSHV LANA-1 in Kaposi sarcoma lesions. *Am J Clin Pathol*. 2007 Nov;128(5):794–802.
45. Huang JS, Yang CM, Wang JS, Liou HH, Hsieh IC, Li GC, et al. Caspase-3 expression in tumorigenesis and prognosis of buccal mucosa squamous cell carcinoma. *Oncotarget*. 2017 Oct 13;8(48):84237–47.
46. Pu X, Storr SJ, Zhang Y, Rakha EA, Green AR, Ellis IO, et al. Caspase-3 and caspase-8 expression in breast cancer: caspase-3 is associated with survival. *Apoptosis Int J Program Cell Death*. 2017 Mar;22(3):357–68.
47. Liu X, He Y, Li F, Huang Q, Kato TA, Hall RP, et al. Caspase-3 promotes genetic instability and carcinogenesis. Mol Cell. 2015 Apr 16;58(2):284–96.
48. Chen LY, Chen LW, Hung CH, Lin CL, Wang SS, Chang PJ. Caspase-Mediated Cleavage of the Transcription Factor Sp3: Possible Relevance to Cancer and the Lytic Cycle of Kaposi's Sarcoma-Associated Herpesvirus. Miranda J, editor. Microbiol Spectr [Internet]. 2022 Feb 23 [cited 2025 May 17];10(1):e01464-21. Available from: https://journals.asm.org/doi/10.1128/spectrum.01464-21
49. Tabtieng T, Degterev A, Gaglia MM. Caspase-Dependent Suppression of Type I Interferon Signalling Promotes Kaposi's Sarcoma-Associated Herpesvirus Lytic Replication. *J Virol*. 2018 May 15;92(10):e00078-18.
50. Tabtieng T, Lent RC, Kaku M, Monago Sanchez A, Gaglia MM. Caspase-Mediated Regulation and Cellular Heterogeneity of the cGAS/STING Pathway in Kaposi's Sarcoma-Associated

Herpesvirus Infection. mBio. 2022 Dec 20;13(6):e0244622.

51. Wu J, Chen ZJ. Innate Immune Sensing and Signalling of Cytosolic Nucleic Acids. Annu Rev Immunol [Internet]. 2014 Mar 21 [cited 2025 May 17];32(1):461–88. Available from: https://www.annualreviews.org/doi/10.1146/annurev-immunol-032713-120156
52. Kane M, Zang TM, Rihn SJ, Zhang F, Kueck T, Alim M, et al. Identification of Interferon-Stimulated Genes with Antiretroviral Activity. Cell Host Microbe [Internet]. 2016 Sep [cited 2025 May 17];20(3):392–405. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1931312816303493
53. Aso H, Ito J, Koyanagi Y, Sato K. Comparative Description of the Expression Profile of Interferon-Stimulated Genes in Multiple Cell Lineages Targeted by HIV-1 Infection. Front Microbiol [Internet]. 2019 Mar 12 [cited 2025 May 17];10:429. Available from: https://www.frontiersin.org/article/10.3389/fmicb.2019.00429/full
54. Krapp C, Hotter D, Gawanbacht A, McLaren PJ, Kluge SF, Stürzel CM, et al. Guanylate Binding Protein (GBP) 5 Is an Interferon-Inducible Inhibitor of HIV-1 Infectivity. Cell Host Microbe [Internet]. 2016 Apr [cited 2025 May 17];19(4):504–14. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1931312816300609
55. Khalil H, Peltzer N, Walicki J, Yang JY, Dubuis G, Gardiol N, et al. Caspase-3 Protects Stressed Organs against Cell Death. Mol Cell Biol [Internet]. 2012 Nov 1 [cited 2025 May 17];32(22):4523–33. Available from: https://www.tandfonline.com/doi/full/10.1128/MCB.00774-12
56. Liu X, He Y, Li F, Huang Q, Kato TA, Hall RP, et al. Caspase-3 Promotes Genetic Instability and Carcinogenesis. Mol Cell [Internet]. 2015 Apr [cited 2025 May 17];58(2):284–96. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1097276515001689
57. Liu X, He Y, Li F, Huang Q, Kato TA, Hall RP, et al. Caspase-3 Promotes Genetic Instability and Carcinogenesis. Mol Cell [Internet]. 2015 Apr [cited 2025 May 17];58(2):284–96. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1097276515001689
58. Cartwright IM, Liu X, Zhou M, Li F, Li CY. Essential roles of Caspase-3 in facilitating Myc-induced genetic instability and carcinogenesis. *eLife*. 2017 Jul 10;6:e26371.
59. Zhu C, Fan F, Li CY, Xiong Y, Liu X. Caspase-3 promotes oncogene-induced malignant transformation via EndoG-dependent Src-STAT3 phosphorylation. *Cell Death Dis*. 2024 Jul 9;15(7):486.
60. Wilson EMP, Sereti I. Immune restoration after antiretroviral therapy: the pitfalls of hasty or incomplete repairs. *Immunol Rev*. 2013 Jul;254(1):343–54.
61. Li L, Wang S, Zhou W. Balance Cell Apoptosis and Pyroptosis of Caspase-3-Activating Chemotherapy for Better Antitumor Therapy. *Cancers*. 2022 Dec 21;15(1):26.
62. Yan L, Liu Y, Ma XF, Hou D, Zhang YH, Sun Y, et al. Triclabendazole Induces Pyroptosis by Activating Caspase-3 to Cleave GSDME in Breast Cancer Cells. *Front Pharmacol*. 2021;12:670081.
63. Jin C, Cheng L, Höxtermann S, Xie T, Lu X, Wu H, et al. MicroRNA-155 is a biomarker of T-cell activation and immune dysfunction in HIV-1-infected patients. *HIV Med*. 2017 May;18(5):354–62.

64. Boucher J, Rousseau A, Boucher C, Subra C, Bazié WW, Hubert A, et al. Immune Cells Release MicroRNA-155 Enriched Extracellular Vesicles That Promote HIV-1 Infection. Cells. 2023 Jan 31;12(3):466.

65. Pawar P, Gokavi J, Wakhare S, Bagul R, Ghule U, Khan I, et al. MiR-155 Negatively Regulates Anti-Viral Innate Responses among HIV-Infected Progressors. *Viruses*. 2023 Nov 1;15(11):2206