Research and Clinical Significance of the Differentially Expressed Genes TP63 and LMO4 in Human Immunodeficiency Virus-Related Penile Squamous Cell Carcinoma

Wenrui Xue, PhD[1] [iD]**, Xin Zheng, PhD**[1]**, Xiaopeng Hu, PhD**[2]**, and Yu Zhang, PhD**[1]

Abstract

To study the differential gene expression and clinical significance in human immunodeficiency virus-infected individuals (HIVIIs) with penile squamous cell carcinoma. At our hospital from 2019 to 2020, we selected six samples of HIV-related penile squamous cell carcinoma for the experimental group and six samples of non-HIV-related penile squamous cell carcinoma for the control group. Transcriptome sequencing of sample mRNAs was performed by high-throughput sequencing. Differential gene expression analysis, differential Gene Ontology (GO) enrichment analysis and differential Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were carried out, and the reads per kilobase per million reads (RPKM) value was used as a measure of gene expression. A total of 2418 differentially expressed genes were obtained, of which 663 were upregulated and 1755 were downregulated (absolute value of logFC >1 and p value <.05). On the basis of the significance of the GO enrichment analysis, we found that the tumor protein p63 (TP63) gene was significantly upregulated and that the LIM domain only 4 (LMO4) gene was significantly downregulated in the experimental group compared with the control group. KEGG pathway analysis of the differentially expressed genes revealed that DNA replication was the most significant pathway associated with the upregulated genes and cell adhesion molecule (CAM) metabolism was the most significant pathway associated with the downregulated genes. The gene expression profiles of HIV-related penile squamous cell carcinoma and non-HIV-related penile squamous cell carcinoma are significantly different and involve significant GO enrichment and KEGG metabolic pathways, and this is very meaningful for the study of non-AIDS-defining cancers (NADCs). Differential expression of genes may be an important target for the prevention of penile squamous cell carcinoma in HIVIIs.

Keywords

Human immunodeficiency virus-infected individuals, penile squamous cell carcinoma, TP63, LMO4, gene therapy

Penile cancer is rare but contributes significantly to morbidity and mortality. In Western countries, such as the United States and Europe, the total incidence in men is less than 1.0/100000 (Hakenberg et al., 2018). Approximately 95% of penile cancers have squamous cell histology. From 1998 to 2018, the 5-year overall survival (OS) rate for penile cancer is greater than 90% in the absence of inguinal lymph node metastasis but decreases to 29%–51% when inguinal lymph nodes are involved, while the 5-year survival rate for pelvic lymph node metastasis is as low as 0%–17% (Marchioni et al., 2018). HIV-related penile cancer patients are special,

except for the low 5-year survival rate of patients with penile cancer with lymph node metastasis, and the decreased immunity caused by HIV infection further

[1]Beijing Youan Hospital of Capital Medical University, Fengtai District, Beijing China
[2]Beijing Chaoyang Hospital of Capital Medical University, Chaoyang District, Beijing China

Corresponding Author:
Yu Zhang, Department of Urology, Beijing Youan Hospital, No 8 of Xitou Tiao, Beijing, 100069, China.
Email: wenruixue@hotmail.com

affects their surgical opportunities and postoperative radiochemotherapies. Due to the low patient numbers, no prospective randomized studies are available.

AIDS, also known as acquired immunodeficiency syndrome, is associated with a series of clinical manifestations, such as opportunistic infection and malignant tumors. When the human body is infected with HIV, the number of CD4T lymphocytes continually decreases, seriously damaging the cellular immune function (Lucas & Nelson, 2015). According to the tumor combinations, these cancers can be divided into AIDS-defining cancers (ADCs) and non-AIDS-defining cancers (NADCs) (Ji & Lu, 2017). With the popularization of antiretroviral therapy, the incidence of immunosuppression-related ADCs has decreased, and tumors in human immunodeficiency virus-infected individuals (HIVIIs) tend to become NADCs (Noy, 2019). HIVIIs often delay or give up treatment for their own malignancies due to concerns about privacy leaks and inadequate access to medical care. A Diagnosis and Treatment Center for AIDS was established at our hospital in 2014 to provide treatment help for HIVIIs and to provide an important source for the collection of NADC-relevant pathological specimens.

Individual immune function is closely related to cancer risk and treatment. The reduced immune monitoring and increased susceptibility to carcinogenic viruses in HIVIIs explain why people living with HIV are more likely to develop cancer than the general population (Puronen et al., 2019). Organ transplant patients on immunosuppressive regimens have a significantly higher risk of developing cancer than ordinary patients, and a similar phenomenon exists regarding the development of tumors due to immune deficiency in HIVIIs. This indicates that immune deficiency plays a very important role in HIVII-related carcinogenesis (Gleber-Netto et al., 2018).

When the human body is infected with the HIV virus, the HIV gene is integrated into the cell. Like all retroviruses, HIV reverse transcribes its genome and permanently inserts itself into the selected chromosomal location in the infected cell. This leads to viral persistence and the long-term presence of virus-infected cells (Haworth et al., 2018). Targeted pattern cloning amplification occurs under certain conditions in gene pathways important for virus replication and persistence. The unique integration with cancer genes does not increase over time, suggesting that HIV preferentially integrates into cancer-related genes and other genes that promote cell proliferation. It is believed that the identifications and influences of these genes play an important role in HIV. The possible molecular mechanism by which HIV infection leads to penile squamous cell carcinoma is unclear, and we believe that high-throughput sequencing technology will substantially benefit to these patients.

In this study, we evaluated the genomic profile to understand oncogenes that may be associated with HIV.

Materials and Methods

Ethics and Consent

The research was reviewed and approved by the Ethics Committee of Beijing Youan Hospital Capital Medical University. The Ethics Committee archive number is LL-2019-176-K, and the approval number is [2020]035.

All the participants provided written informed consent.

Materials

From 2019 to 2020, 12 penile cancer specimens were collected from the urology department of our hospital. The pathological type was squamous cell carcinoma, and the patients were 48–75 years old. All patients were subjected to the following procedures before the operation: medical history and physical examination, chest radiography, electrocardiogram, abdominal color ultrasound, routine blood tests, blood biochemistry assessment, coagulation, and T cell count detection. All cases were of the T3N1M0 pathological stage, and all patients underwent penile-sparing surgery and cisplatin + 5-fluorouracil chemotherapy. Six samples of HIV-related penile squamous cell carcinoma were selected for the experimental group, and six samples of non-HIV-related penile squamous cell carcinoma were selected for the control group. All pathological specimens were kept in a liquid nitrogen tank and then stored in a –80° refrigerator.

Methods

RNA-Seq library construction mainly includes three steps: total Ribonucleic Acid (RNA) extraction, RNA library construction, and computer sequencing.

Total RNA Extraction. The TRIzol extraction method was used to obtain high-quality total RNA. By default, the TRIzol volume is defined as "1 volume."

RNA Library Construction. The matching sequencing library must be constructed by using an Illumina sequencer for high-throughput sequencing. The main construction processes of the mRNA-seq library are shown in Figure 1.

RNA-Seq Analysis Process. Large amounts of sequencing data are generated by high-throughput sequencing, and important information is obtained from the data by means of biological information analysis. The main bioinformatics analysis protocols for transcriptome sequencing are shown in Figure 2.
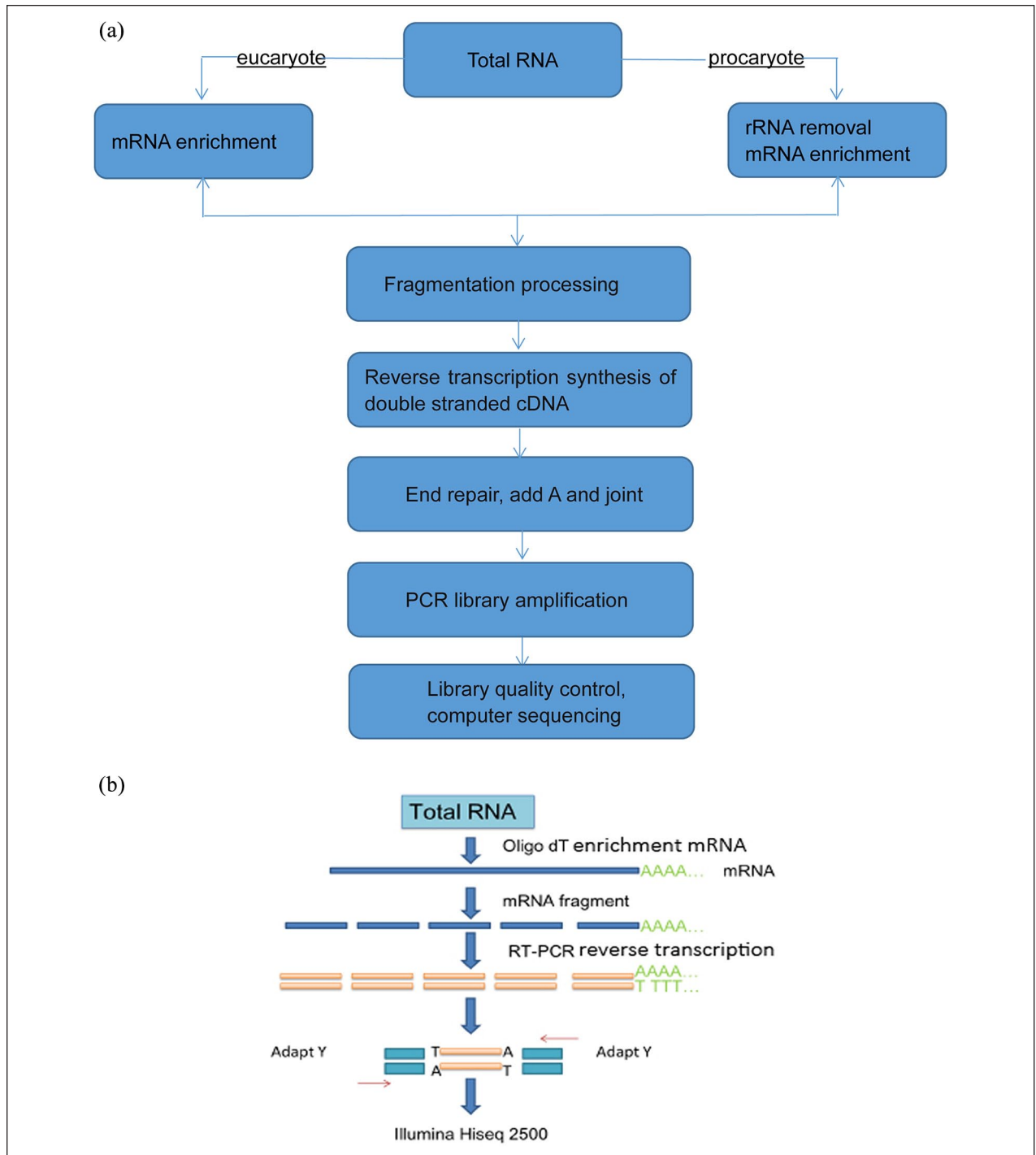
Figure 1. mRNA-seq library construction process.

Identification of Differentially Expressed Genes. We compared the experimental group with control group by using the DESeq 2 R package (http://www.bioconductor.org/packages/devel/bioc/html/DESeq2.html, Version 1.27.19) to identify the differentially expressed genes, its strict threshold as absolute value of logged fold changes (logFC) >1 and p value $<.05$. Then we draw the heat map and volcano map using the heatmap R package and ggpubr R package in R software.

Analysis of GO Enrichment and KEGG Pathway for Differentially Expressed Genes. After raw data were obtained,
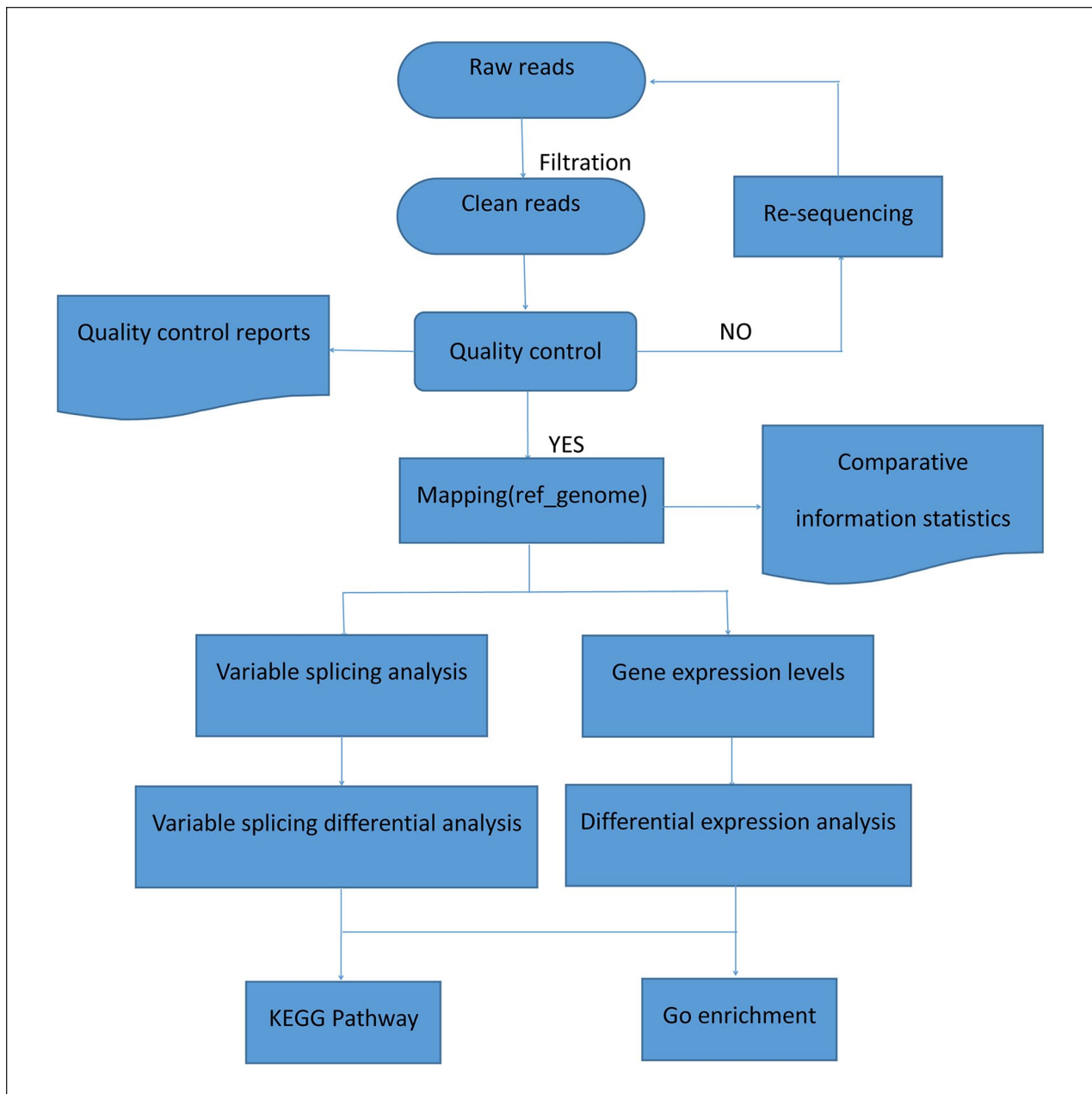
Figure 2. Analysis process of RNA-Seq.

clean data were first filtered to obtain high-quality sequencing data. Clean data were compared to the reference genome of the project species to obtain comprehensive transcriptome information, and gene expression quantification, Gene Ontology (GO) enrichment analysis, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were performed.

The Database for Annotation, Visualization and Integrated Discovery (DAVID) (http://david.ncifcrf.gov, Version 6.8) provides an analytical platform with a comprehensive source of gene annotation information (Huang

et al., 2009). Analysis of differential gene expression is mainly performed through GO enrichment and KEGG pathway analyses. GO is the international standard classification system of gene function (Gene Ontology, 2015). After screening the differential genes according to the purpose of the experiment, the distribution of GO terms associated with the differentially expressed genes helped to clarify the differences in gene function among the experimental samples. In organisms, different genes perform their specific biological functions through orderly coordination, and the most important biochemical

metabolic and signal transduction pathways associated with differentially expressed genes can be determined by pathway significant enrichment. Hence, the abundant information in the KEGG pathway database helps to elucidate system-level biological functions of genes, such as metabolic pathways, transmission of genetic information, and cellular processes, which greatly improves the practically and applicable value of the database (Kanehisa et al., 2019). ImageGP (http://www.ehbio.com/ImageGP/) is used to draw Enrichment Plot for GO and KEGG.

Quantitative Reverse Transcriptase Polymerase Chain Reaction. The expression levels of genes were quantitated using the AceQ qPCR SYBR Green Master Mix (R323-01, Vazyme, China) by the ABI 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA). Target gene levels were normalized against β-actin standards and calculated using the 2-ΔΔCt method.

Results

We used the absolute value of logFC >1 (logFC is log2FC; FC is the change in multiple reads per kilobase per million reads [RPKM] values of the same gene in different samples) and $p < .05$ as standards to indicate that the genes were differentially expressed. We identified 663 upregulated genes and 1755 downregulated genes (Figure 3).

The GO functional enrichment analysis showed that compared with the genomic background, the GO functional items were significantly enriched among the differentially expressed genes, and the biological functions were significantly related to the differentially expressed genes. The GO enrichment analysis of the differentially expressed genes is presented in Figure 4. The KEGG database helps us to understand the biological functions of genes at the system level, and the KEGG pathway analysis of differentially expressed genes is presented in Figure 5.

On the basis of the significance of GO enrichment, the most significantly upregulated term in the experimental group compared with the control group was epidermal development, and the GO code was 0008544, corresponding to the tumor protein p63 (TP63) gene. The most significant downregulated term was regulation of cell activation, and the GO code was 0050865, corresponding to the LIM domain only 4 (LMO4) gene.

KEGG pathway analysis of the differentially expressed genes between the experimental and control groups revealed that DNA replication was the most significant pathway associated with the upregulated genes and that cell adhesion molecule (CAM) metabolism was the most significant pathway associated with the downregulated genes.

## Validation of Genes in the Clinical Cohort

According to the analysis, the differentially expressed genes TP63 and LMO4 may be targets to prevent the development of penile squamous cell carcinoma in HIVIIs. To verify the validity and reliability of the results, the expression of the two genes were analyzed between six samples of HIV-related penile squamous cell carcinoma and six samples of non-HIV-related penile squamous cell carcinoma through RT-PCR. As shown in Figure 6, the results demonstrated that the expression of TP63 gene was significantly upregulated in the experimental group, while the expression of LMO4 gene was significantly downregulated compared with the control group.

Discussion

HIV accessory genes are known to extensively alter the internal composition of infected cells by hijacking normal phosphorylation and ubiquitin processes, mediating viral gene transcription, and suppressing immune surveillance and detection (Haworth et al., 2018). These data suggest that the presence of genes associated with these pathways may be associated with HIV-infected cells.

The TP63 gene belongs to a P53 family of transcription factors that also includes P73 and the tumor suppressor gene P53 (Dötsch et al., 2010), which share a high degree of homology and are important for cell homeostasis (Koneva et al., 2018). TP63 is an important marker for the development of basic tumor biology by regulating genetic processes (Hoadley et al., 2014). TP63 can be synthesized from two different start sites, leading to two different N-terminal protein domains, the transactivation (TA) domain, and lack this amino-terminal (DN) domain. Both the TAp63 and DNp63 isoforms have α, β, and γ splice variants in the carboxyl terminal region, which leads to multiple TP63 isoforms (Su et al., 2013). These isoforms play a corresponding role in regulating tumor development and other physiological and pathological processes (Gatti et al., 2019). DNp63 can act as a sequence-specific transcription activator or suppressor. DNp63α isomers, in detail, can activate specific target genes via a second transcription activation domain (Lena et al., 2015). In addition, DNp63 interacts with a variety of epigenetic factors to effectively repress transcription (Saladi et al., 2017). TP63 is considered a transcriptional regulator of the basal gene program and is upregulated in basal subtypes of bladder, breast, and ovarian cancers. The enrichment of immune pathways in TAp63-expressing tumors suggests a link between TAp63 and tumor immune infiltrates (Bankhead et al., 2020).

LMO4 belongs to the LIM-only family of transcriptional coregulatory proteins and consists of two LIM protein–protein interaction domains that act as connexins
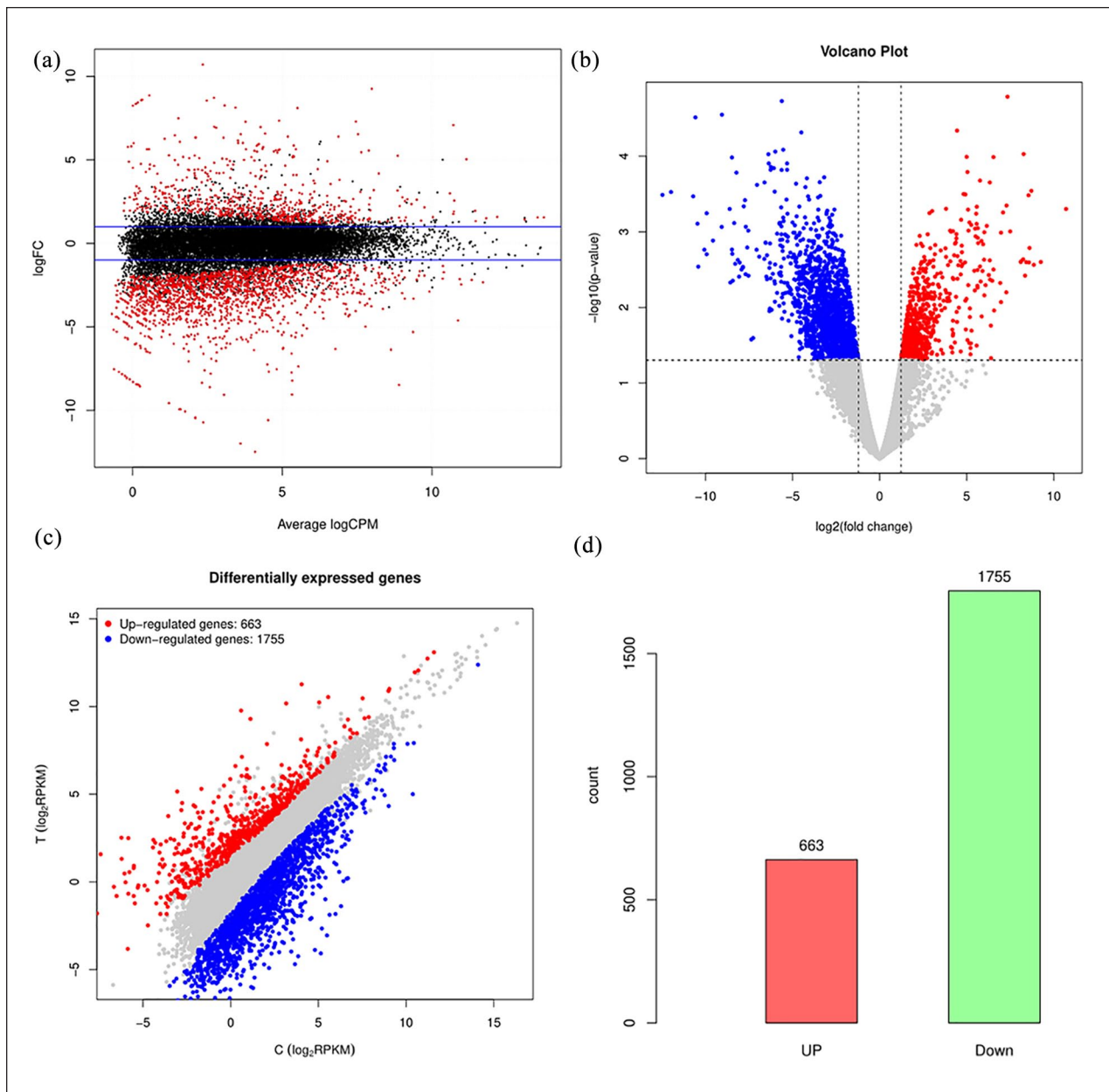
Figure 3. (a) log ratio (M) and mean average (A) (MA) Plot of differentially expressed genes. *Note.* Average log CPM = Average log2 counts-per-million; logFC = log-fold-changes between each pair of RNA samples. Two blue lines representing the up-down threshold for differentially expressed genes (logFC > 1 indicates upregulation, logFC < -1 indicates downregulation). (b) Volcanic map of differentially expressed genes. *Note.* Log2(fold change) = The logarithm of the difference multiples based on 2; -log10(*p*-value) = *p* value negative logarithm based on 10. The gray dots represent genes that are not differentially expressed, the blue dots represent genes that are differentially downregulated, and the red dots represent genes that are differentially upregulated. (c) Scatterplot of expression between comparison groups. The horizontal and vertical coordinates represent the treatment group and the control group, respectively; a logarithm of the expression RPKM based on 2. The gray dots represent genes that are not differentially expressed, the blue dots represent genes that are differentially downregulated, and the red dots represent genes that are differentially upregulated. The upper left corner indicates the number of up-down genes. (d) Number of up-down differentially expressed genes.

in multiprotein complexes (Wang et al., 2019). Sequence analysis of the mouse LMO4 gene revealed that it spans approximately 18 kb and consists of at least six exons, including two alternatively spliced 5′ exons. Ste20-like kinase (SLK) is a kinase that plays a key role in cell migration and focal adhesion turnover (Quizi et al.,
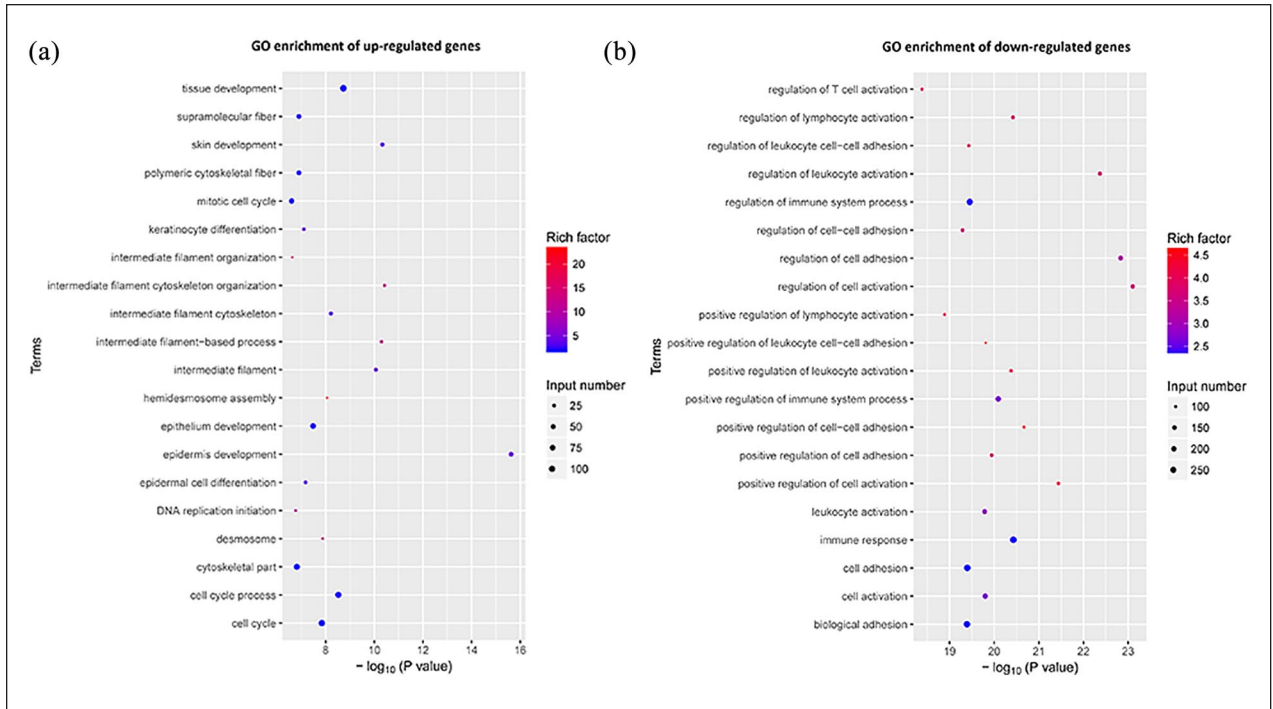
Figure 4. The horizontal axis indicates the significance of enrichment, expressed as -log10; the larger the value, the more significant the enrichment. The longitudinal axis represents the GO terms of enrichment. The dot size indicates the number of different genes contained in the GO terms, and the dot depth indicates the degree of rich factor enrichment.
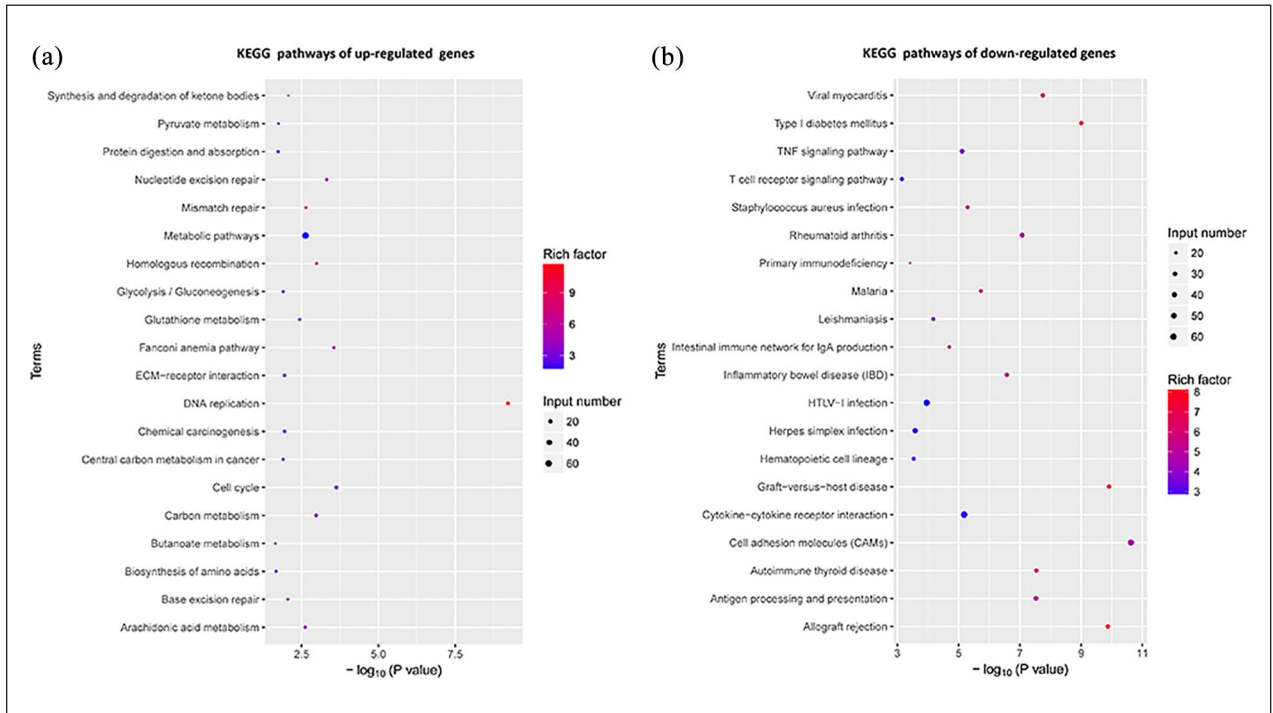
Figure 5. The horizontal axis indicates the significance of enrichment, expressed as -log10; the larger the value, the more significant the enrichment. The longitudinal axis represents the KEGG pathways. The dot size indicates the number of different genes contained in the KEGG pathways, and the dot depth indicates the degree of rich factor enrichment.
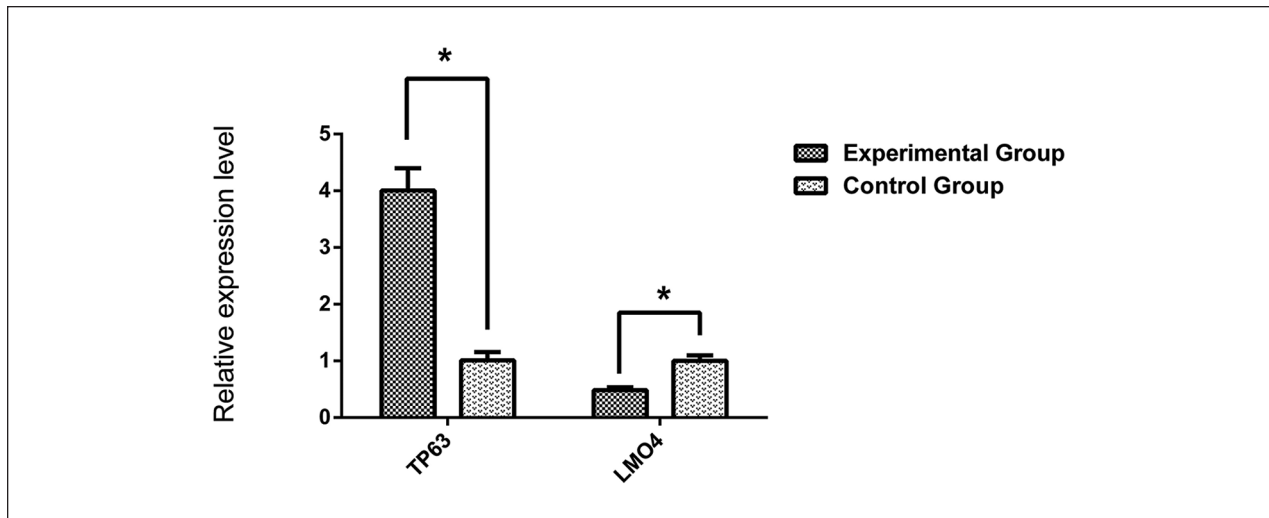
Figure 6. The bar plot exhibits the expressions of TP63 gene and LMO4 gene evaluated by RT-PCR in six samples of HIV-related penile squamous cell carcinoma and six samples of non-HIV-related penile squamous cell carcinoma. *Note.* LMO4 = LIM domain only 4; TP63 = tumor protein p63. *$p < .05$ versus control.

2013). Mechanistically, SLK functions through complex phosphorylation regulation (Luhovy et al., 2012). LMO4 can directly bind to SLK in vivo and in vitro and activate its kinase activity (Baron et al., 2015). LMO4 can also interact with LIM domain-binding protein (Ldb1) and play a role in cell proliferation and motility (Deane et al., 2004); for example, LMO4 is overexpressed in samples of patient tissue and oral squamous cell carcinomas (Meier et al., 2006). Deletion of LMO4 impairs Ldb1 and SLK recruitment in migratory cells. LMO4 is closely related to the occurrence of many malignant tumors, such as pancreatic cancer, non-small cell lung cancer, and head and neck cancer (Simonik, 2016; Wang, 2016), and can affect the differentiation of T cells, leading to acute leukemia (Ding et al., 2018).

On the basis of the significance of the GO enrichment analysis, we found that the TP63 gene was significantly upregulated and that the LMO4 gene was significantly downregulated in the experimental group compared with the control group. The transcriptional regulation property of TP63 gene and phosphorylation property of LMO4 gene. These results demonstrate that the TP63 and LMO4 genes may change the internal composition of HIV-infected cells by mediating viral gene transcription and phosphorylation pathways. HIV-encoded proteins may interact with the TP63 and LMO4 proteins and modulate their function in different ways. TP63 and LMO4 have been intensively studied as transcriptional targets in squamous cell carcinoma. They can regulate the expression of diverse tumor-related proteins and are involved in extracellular matrix and tumor microenvironment remodeling as well as in growth factor-mediated signal transduction (Gatti, 2019; Zhang, 2019).

Among the KEGG pathways, two terms linked to the regulation of cell proliferation and survival, including DNA replication and cell adhesion molecule metabolism, were identified. This suggests that HIV integration may promote the proliferation and persistence of infected cells. Although HIV has not been reported to directly cause carcinogenic transformation, the incidence of cancer in HIVIIs is higher than that in patients without HIV. HIV can persist and permanently insert itself into selected chromosomal locations within infected cells; it can also reverse transcribe its genome, like all retroviruses. Once HIV successfully integrates into a chromosomal site, all subsequent cells generated by cell division contain the same viral integration site (Mullins & Frenkel, 2017). Approximately 80% of the integration sites have viral integration within gene transcripts, and approximately 12.5% of these genes are related to cancer development (Wagner et al., 2014). It is unclear whether a link exists between the HIV site selection and potential oncogenic development, but Wagner et al. (2014) reported that HIV preferentially integrates into cancer-related genes and other genes that promote cell proliferation.

After HIV infection, the virus can have a synergistic effect on tumorigenesis, and human immunodeficiency virus type 1 (HIV-1) is among the most common synergistic oncogenes (Sowd et al., 2016). HIV-1 is a terrible pathogen that can cause persistent infections and evolve rapidly by integrating the original viral genome into chronically infected cells (Engelman & Singh, 2018). During viral replication, HIV-1 can mutate and recombine at a high frequency, promoting viral persistence and regulating HIV replication while usurping the cellular mechanisms of HIV replication during gene expression

(Shmakova et al., 2020). Further research on HIV-1 replication may help to identify new targets for antiretroviral therapy that will enable continued viral inhibition in patients with treatment failure (Bale & Kearney, 2019).

Since 2017, the use of transgenic T cells to treat previously incurable diseases such as cancer has multiplied. This success is now driving the use of the same technology to treat HIV infection (Ahlenstiel & Turville, 2019; Kiem, 2012). While antiretroviral combination therapy can significantly reduce the circulating viral loads in HIV-infected patients, highly replicable viruses remain. Wagner et al. (2014) noted that mechanisms allowing the persistence of HIV include long-term latent infection of cells, low levels of HIV replication, and proliferation of HIV-infected cells. Integrating HIV into specific genes may promote the proliferation of HIV-infected cells and slow the decay of the virus in antiretroviral therapeutic processes. A variety of approaches such as antiretroviral therapy, latency-reversing agents, structured treatment interruptions, therapeutic vaccines, chimeric antigen receptor, broadly neutralizing antibodies and immune checkpoint blockers are being tested to cure HIV, and the effectiveness of gene therapy is supported by most data. Gene therapy has the advantages of specificity and persistence and has the potential to protect patients from subsequent infections. A patient in Berlin that was positive for both HIV and acute myeloid leukemia (AML) received two stem cell transplants from a donor homozygous for a CCR5delta32 mutation. Eight years after his second transplant, he still had no HIV or AML infection. This case provides a strong proof-of-principle that a cure for HIV is possible and can be achieved through gene therapy (Johnston, 2016). Some articles have pointed out that in genome-wide studies, HIV integration is beneficial for the transcription sites of active genes and for the establishment of HIV replication and latency (Haworth, 2018; Maldarelli, 2014), thus promoting pathways related to tumorigenesis (Wagner et al., 2014). Understanding the relationship between HIV and tumors may provide a method for reducing the risk of HIV tumors or even a genetic pathway for treating HIV. As the principle suggests, gene therapy can cure HIV, and the improvement of current methods may lead to the optimization of transduction efficiency and persistence in vivo. Gene therapy strategies are linked to human pathology at a fundamental level to correct and improve the underlying genetic factors of any disease by delivering DNA and RNA molecules. The history of HIV gene therapy is particularly interesting, as targeted viruses are quickly chosen together as part of targeted strategies. It is generally accepted that the combination approach is the most promising for the functional treatment of HIV infection.

Our small sample size made it difficult to compare the genomic locations of TP63 and LMO4 mutations, and whether these differences in presentation and prognosis are related to the systemic effects of HIV-mediated immunosuppression or to specific biological characteristics of the primary tumor is still unclear. Additional studies with larger sample sizes are needed to validate our experimental results. Genetic studies on NADCs provide another possibility and opportunity to explore the relationship between HIV and tumors in regard to biological processes and molecular pathways. Whether the inhibition of related pathways or genes can induce immune reconstruction to combat early penile cancer and prevent the occurrence of NADCs is worthy of further study.

Conclusion

Gene therapy for HIV has extremely great potential. Herein, we evaluated genomic profiles to better understanding oncogenic genes that may be associated with HIV. These results may provide a genetic pathway to reduce the risk of NADCs and even provide more ideas for HIV treatment. The differentially expressed genes TP63 and LMO4 may be targets to prevent the development of penile squamous cell carcinoma in HIVIIs.

Authors' contributions

Wenrui Xue was a main contributor in the design, implementation and writing of the manuscript. Xiaopeng Hu and Xin Zheng did the experiments and data collection. Yu Zhang contributed much to the revised version of our manuscript for updating the literature and revising the paper. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ORCID iD

Wenrui Xue https://orcid.org/0000-0001-7289-9500

Availability of data and materials

The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

References

Ahlenstiel, C. L., & Turville, S. G. (2019). Delivery of gene therapy to resting immune cells for an HIV cure. *Current Opinion HIV AIDS*, *14*(2), 129–136. doi: 10.1097/COH.0000000000000531

Bale, M. J., & Kearney, M. F. (2019). Review: HIV-1 phylogeny during suppressive antiretroviral therapy. *Current Opinion HIV AIDS*, *14*(3), 188–193. doi: 10.1097/COH.0000000000000535

Baron, K. D., Al-Zahrani, K., Conway, J., Labrèche, C., Storbeck, C. J., Visvader, J. E., & Sabourin, L. A. (2015). Recruitment and activation of SLK at the leading edge of migrating cells requires Src family kinase activity and the LIM-only protein 4. *Biochimica et Biophysica Acta*, *1853*(7), 1683–1692. doi: 10.1016/j.bbamcr.2015.04.003

Bankhead, A., III, McMaster, T., Wang, Y., Boonstra, P. S., & Palmbos, P. L. (2020). TP63 isoform expression is linked with distinct clinical outcomes in cancer. *EBioMedicine*, *51*, 102561. doi: 10.1016/j.ebiom.2019.11.022

Deane, J. E., Ryan, D. P., Sunde, M., Maher, M. J., Guss, J. M., Visvader, J. E., & Matthews, J. M. (2004). Tandem LIM domains provide synergistic binding in the LMO4:Ldb1 complex, *EMBO J*, *23* (18), 3589–3598. doi: 10.1038/sj.emboj.7600376

Ding, K., Wu, Z., Li, X., Sheng, Y., Wang, X., & Tan, S. (2018). LMO4 mediates trastuzumab resistance in HER2 positive breast cancer cells. *American Journal of Cancer Research*, *8*(4), 594–609.

Dötsch, V., Bernassola, F., Coutandin, D., Candi, E., & Melino, G. (2010). p63 and p73, the ancestors of p53. *Cold Spring Harbor Perspectives*, *2*(9), a004887. doi: 10.1101/cshperspect.a004887

Engelman, A. N., & Singh, P. K. (2018). Cellular and molecular mechanisms of HIV-1 integration targeting. *Cellular and Molecular Life Sciences*, *75*(14), 2491–2507. doi: 10.1007/s00018-018-2772-5

Gleber-Netto, F. O., Zhao, M., Trivedi, S., Wang, J., Jasser, S., McDowell, C., Kadara, H., Zhang, J., Wang, J., William, W. N., Jr., Lee, J. J., Nguyen, M. L., Pai, S. I., Walline, H. M., Shin, D. M., Ferris, R. L., Carey, T. E., Myers, J. N., & Pickering, C. R. (2018). Distinct pattern of TP53 mutations in human immunodeficiency virus-related head and neck squamous cell carcinoma. *Cancer*, *124*(1), 84–94. doi: 10.1002/cncr.31063. Epub 2017 October 20.

Gatti, V., Fierro, C., Annicchiarico-Petruzzelli, M., Melino, G., & Peschiaroli, A. (2019). DeltaNp63 in squamous cell carcinoma: Defining the oncogenic routes a ecting epigenetic landscape and tumour microenvironment. *Molecular Oncology*, *13*(5), 981–1001. doi: 10.1002/1878-0261.12473

Gatti, V., Bongiorno-Borbone, L., Fierro, C., Annicchiarico-Petruzzelli, M., Melino, G., & Peschiaroli, A. (2019). p63 at the crossroads between Stemness and Metastasis in breast cancer. *International Journal of Molecular Sciences*, *20*(11), 2683. doi: 10.3390/ijms20112683

Gene Ontology, C. (2015). Gene ontology consortium: Going forward. *Nucleic Acids Research*, *43*(Database issue), D1049-56. doi: 10.1093/nar/gku1179

Haworth, K. G., Schefter, L. E., Norgaard, Z. K., Ironside, C., Adair, J. E., & Kiem, H. P. (2018). HIV infection results in clonal expansions containing integrations within pathogenesis-related biological pathways. *JCI Insight*, *3*(13), e99127. doi: 10.1172/jci.insight.99127

Hoadley, K. A., Yau, C., Wolf, D. M., Cherniack, A. D., Tamborero, D., Ng, S., Leiserson, M. D. M., Niu, B., McLellan, M. D., Uzunangelov, V., Zhang, J., Kandoth, C., Akbani, R., Shen, H., Omberg, L., Chu, A., Margolin, A. A., Van't Veer, L. J., Lopez-Bigas, N., & Stuart, J. M. (2014). Multiplatform analysis of 12 cancer types reveals molecular classification within and across tissues of origin. *Cell*, *158*(4), 929–944. doi: 10.1016/j.cell.2014.06.049. Epub 2014 Aug 7

Hakenberg, O. W., Dräger, D. L., Erbersdobler, A., Naumann, C. M., Jünemann, K. P., & Protzel, C. (2018). The diagnosis and treatment of penile cancer. *Deutsches Ärzteblatt International*, *115*(39), 646–652. doi: 10.3238/arztebl.2018.0646

Huang, D. W., Sherman, B. T., & Lempicki, R. A. (2009). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nature Protocols*, *4*(1), 44–57. doi: 10.1038/nprot.2008.211

Ji, Y. J., & Lu, H. Z. (2017). Malignancies in HIV-infected and AIDS patients. *Advances in Experimental Medicine and Biology*, *1018*, 167–179. doi: 10.1007/978-981-10-5765-6_10

Johnston, R. (2016). Gene therapy to cure HIV: Where to from here? *AIDS Patient Care STDS*, *30*(12), 531–533. doi: 10.1089/apc.2016.0240

Kiem, H. P., Jerome, K. R., Deeks, S. G., & McCune, J. M. (2012). Hematopoietic-stem-cell-based gene therapy for HIV disease. *Cell Stem Cell*, *10*(2), 137–147. doi: 10.1016/j.stem.2011.12.015

Koneva, L. A., Zhang, Y., Virani, S., Hall, P. B., McHugh, J.B., Chepeha, D. B., Wolf, G. T., Carey, T.E., Rozek, L.S., & Sartor, M. A. (2018). HPV Integration in HNSCC correlates with survival outcomes, immune response signatures, and candidate drivers. *Molecular Cancer Research*, *16*(1), 90–102. doi: 10.1158/1541-7786.MCR-17-0153

Kanehisa, M., Sato, Y., Furumichi, M., Morishima, K., & Tanabe, M. (2019). New approach for understanding genome variations in KEGG. *Nucleic Acids Research*, *47*(D1), D590–D595. doi: 10.1093/nar/gky962

Lucas, S., & Nelson, A. M. (2015). HIV and the spectrum of human disease. *Journal of Pathology*, *235*(2), 229–241. doi: 10.1002/path.4449

Lena, A. M., Duca, S., Novelli, F., Melino, S., Annicchiarico-Petruzzelli, M., Melino, G., & Candi, E. (2015). Amino-terminal residues of DeltaNp63, mutated in ectodermal dysplasia, are required for its transcriptional activity. *Biochem Biophys Res Commun*, *467*(2), 434–440. doi: 10.1016/j.bbrc.2015.09.111

Luhovy, A.Y., Jaberi, A., Papillon, J., Guillemette, J., & Cybulsky, A. V. (2012). Regulation of the Ste20-like kinase, SLK: Involvement of activation segment phosphorylation. *Journal of Biological Chemistry*, *287*(8), 5446–5458. doi: 10.1074/jbc.M111.302018

Marchioni, M., Berardinelli, F., Nunzio, C. D., Spiess, P., Porpiglia, F., Schips, L., & Cindolo, L. (2018). New insight in penile cancer. *Minerva Urol Nefrol*, *70*(6), 559–569. doi: 10.23736/S0393-2249.18.03215-0

Meier, N., Krpic, S., Rodriguez, P., Strouboulis, J., Monti, M., Krijgsveld, J., Gering, M., Patient, R., Hostert, A., & Grosveld, F. (2006). Novel binding partners of Ldb1 are required for haematopoietic development. *Development*, *133*(24), 4913–4923. doi: 10.1242/dev.02656

Mullins, J. I., & Frenkel, L. M. (2017). Clonal expansion of human immunodeficiency virus-infected cells and human immunodeficiency virus persistence during antiretroviral therapy. *Journal of Infectious Diseases*, *215*(suppl_3), S119–S127. doi: 10.1093/infdis/jiw636

Noy, A. (2019). Optimizing treatment of HIV-associated lymphoma. *Blood*, *134*(17), 1385–1394. doi:10.1182/blood-2018-01-791400

Puronen, C. E., Ford, E. S., & Uldrick, T. S. (2019). Immunotherapy in people with HIV and cancer. *Front Immunol*, *10*, 2060. doi: 10.3389/fimmu.2019.02060

Quizi, J. L., Baron, K., Al-Zahrani, K. N., O'Reilly, P., Sriram, R. K., Conway, J., Laurin, A. A., & Sabourin, L. A. (2013). SLK-mediated phosphorylation of Paxillin is required for focal adhesion turnover and cell migration. *Oncogene*, *32*(39), 4656–63. doi: 10.1038/onc.2012.488

Simonik, E. A., Cai, Y., Kimmelshue, K. N., Brantley-Sieders, D. M., Loomans, H. A., Andl, C. D., Westlake, G. M., Youngblood, V. M., Chen, J., Yarbrough, W. G., Brown, B. T., Nagarajan, L., & Brandt, S. J. (2016). LIM-Only protein 4 (LMO4) and LIM domain binding pro-tein 1 (LDB1) promote growth and metastasis of human head and neck cancer (LMO4 and LDB1 in Head and Neck Cancer). *PLoS One*, *11*(10), e0164804. doi: 10.1371/journal.pone

Sowd, G. A., Serrao, E., Wang, H., Wang, W., Fadel, H. J., Poeschla, E. M., & Engelman, A. N. (2016). A critical role for alternative polyadenylation factor CPSF6 in targeting HIV-1 integration to transcriptionally active chromatin. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(8), E1054–E1063. doi: 10.1073/pnas.1524213113

Saladi, S. V., Ross, K., Karaayvaz, M., Tata, P. R., Mou, H., Rajagopal, J., Ramaswamy, S., & Ellisen, L. W. (2017). ACTL6A is co-amplified with p63 in squamous cell Carcinoma to drive YAP activation, regenerative proliferation, and poor prognosis. *Cancer Cell*, *31*(1), 35–49. doi: 10.1016/j.ccell.2016.12.001. Epub 2016 Dec 29

Su, X. H., Chakravarti, D., & Flores, E. R. (2013). p63 steps into the limelight: Crucial roles in the suppression of tumorigenesis and metastasis. *Nat Rev Cancer*, *13*(2), 136–143. doi: 10.1038/nrc3446

Shmakova, A., Germini, D., & Vassetzky, Y. (2020). HIV-1, HAART and cancer: A complex relationship. *International Journal of Cancer*, *146*(10), 2666–2679. doi: 10.1002/ijc.32730

Wagner, T. A., McLaughlin, S., Garg, K., Cheung, C. Y., Larsen, B. B., Styrchak, S., Huang, H. C., Edlefsen, P. T., Mullins, J. I., & Frenkel, L. M. (2014). HIV latency. Proliferation of cells with HIV integrated into cancer genes contributes to persistent infection. *Science*, *345*(6196), 570–573. doi: 10.1126/science.1256304

Wang, W. J., Wu, S. P., Guo, M. Z., & He, J. X. (2016). LMO4 is a prognostic marker involved in cell migration and invasion in non-small-cell lung cancer. *The Journal of Thoracic Disease*, *8*(12), 3682–3690. doi: 10.21037/jtd.2016.12.22

Wang, N., Dong, Q., & Zhou, X. N. (2019). LMO4 promotes the invasion and proliferation of gastric cancer by activating PI3K-Akt-mTOR signaling. *American Journal of Translational Research*, *11*(10), 6534–6543.

Zhang, D. D., Wei, Y. S., Zhou, J., Wang, G. N., Xiao, L., Xu, J. J., Wei, N., Li, W. C., & Zhang, M. Z. (2019). miR-150 might inhibit cell proliferation and promote cell apoptosis by targeting LMO4 in Burkitt lymphoma. *Journal of Cellular Physiology*, *234*(6), 9652–9662. doi: 10.1002/jcp.27652. Epub 2018 Nov 13