Myelin and lymphocyte protein serves as a prognostic biomarker and is closely associated with the tumor microenvironment in the nephroblastoma

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Abstract
Nephroblastoma, also known as Wilms’ tumor (WT), is the most common renal tumor that occurs in children. Although the efficacy of treatment has been significantly improved by a series of comprehensive treatments, some patients still have poor prognosis. Myelin and lymphocyte (MAL) protein, a highly hydrophobic integrated membrane-bound protein, has been implicated in many tumors and is also closely linked to kidney development. However, the relationship between MAL and WT has not yet been elucidated. Therefore, we attempted to evaluate the feasibility of MAL as a promising prognosis factor for WT. The differential expression of MAL was investigated using TARGET database and was verified using the Gene Expression Omnibus database and real-time quantitative PCR. The prognostic ability of MAL was determined using Kaplan–Meier and Cox regression analyses. Pearson correlation analysis was applied to explore the relationship between MAL expression and methylation sites. The ESTIMATE and CIBERSORT algorithms showed that MAL expression was associated with the WT tumor microenvironment. Gene Set Enrichment Analysis (GSEA) indicated that multiple signaling pathways closely associated with tumorigenesis were differentially enriched between the high- and low-MAL groups. In conclusion, our study comprehensively explored the potential of MAL as a prognosis factor for WT. Meanwhile, we also demonstrated that MAL, as a prognostic factor for WT, may be closely related to the tumor microenvironment.

KEYWORDS
GSEA, MAL, methylation, tumor microenvironment, Wilms’ tumor
1 | INTRODUCTION

Nephroblastoma, also known as Wilms’ tumor (WT), is the renal tumor with the highest incidence in children, accounting for 90% of all childhood renal tumors. It has been shown that about 1 in 10,000 children have had this disease, with the highest incidence at the age of 3. At present, the overall survival (OS) rate of patients with WT has increased to more than 90% through a series of comprehensive standard therapies. However, some patients still have poor prognosis because of cancer recurrence and metastasis, and a quarter of survivors experience severe chronic diseases associated with the antitumor therapy they receive. Therefore, new and promising prognosis biomarkers are needed to explore for patients with WT and provide a reference for the implementation of clinical decision-making.

As a primitive, pluripotent embryonic renal precursors malignancy, a large number of studies have shown that the occurrence of WT was closely related to early renal dysplasia. The kidneys began to develop through a series of complex tissue interactions between mesoderm derivatives around the fifth week of pregnancy. In this process, the transformation of mesenchymal progenitor cells into epithelial cells was crucial, which was finely regulated by a gene network composed of a series of genes. Mutations of genes in this process, such as the WT1 gene and related genes in the WNT pathway, often resulted kidney dysplasia thus caused a series of kidney related diseases. Therefore, the normal development of the kidney was of great significance for the exemption of kidney-related diseases. Frank et al. found that during the whole development of kidney, the high expression level of MAL would be maintained until adulthood. And MAL played a role of structural protein in the apical membrane of specialized epithelium such as renal tubules. Its functions of differentiation and transportation may play an important role in the development of kidney. MAL had been reported to play an important role in a variety of tumors, but the role of MAL gene in nephroblastoma had not been reported yet. Therefore, we perceived that it was necessary to explore the features of MAL in WT and conduct further studies.

In this study, we tried to clarify the relationship between MAL expression and the prognosis of WT through related bioinformatics methods, and at the same time explored its relationship with tumor microenvironment and related signaling pathways. The results from our research were expected to provide new perspectives for exploring the prognosis factors for WT and guide for the implementation of clinical decision-making.

2 | MATERIALS AND METHODS

2.1 | Differential expression of MAL between WT and normal tissues

First, the fragments per kilobase of exon model per million mapped reads (FPKM) RNA-seq data of 124 primary WT and six adjacent normal tissues were downloaded by using TCGAbiolinks R package. Further, corresponding clinical data were acquired from UCSC Xena (https://xenabrowser.net). Next, the Ensembl database (http://asia.ensembl.org/index.html) was used to convert Ensembl IDs into gene symbols. The expression file of the MAL gene was extracted for further analysis. Differential expression of MAL between WT and adjacent normal tissues was identified using a t-test. Finally, Gene Expression Omnibus database (GEO. http://www.ncbi.nlm.nih.gov/geo/) was used to verify the accuracy of the results, including GSE2712, GSE1115, and GSE73209. Detailed information of these validation datasets is shown in Table 1.

2.2 | Prognostic value analysis

The expression profile was processed in log2 (normalized value + 1) data format. Survival analysis was performed using the survminer R package. The log-rank test was used to confirm statistical significance, and the basis of grouping was dependent on the median value of MAL expression. The comparison between MAL expression and other clinical characteristics on the survival impact of patients with WT was explored by univariate and multivariate Cox analyses (age, gender, and stage).

| GSE datasets | Platform | Annotation package | Sample |
|--------------|----------|--------------------|--------|
| GSE11151     | GPL570   | Hgu133plus2.db     | ANK 3 FNK 2 WT 4 |
| GSE2712      | GPL96    | Hgu133a.db         | FNK 3 WT 18 |
| GSE73209     | GPL10556 | IlluminaHumanv4.db | ANK 2 FNK 4 WT 32 |

Abbreviation: ANK, adult normal kidney; FNK, fetal normal kidney; WT, Wilms’ tumor.
2.3 | Correlation analysis between MAL expression and methylation

From the Target database (https://ocg.cancer.gov/programs/target), the methylation data of WT were obtained. Methylation sites of the MAL gene were extracted for further analysis. The relationship between MAL expression and methylation was explored using the Pearson correlation test. The survival impact of methylation sites on patients with WT was explored by KM analysis. The basis of grouping was dependent on the median $\beta$ value.

2.4 | Exploration of relationship between MAL expression and tumor microenvironment

We further explored the relationship between MAL expression and tumor microenvironment in order to understand the difference of tumor microenvironment between high and low-MAL groups. The tumor microenvironment was identified as the cellular environment where cancerous cells were present, which was composed of a series of cell types, including mesenchymal cells, immune cells, inflammatory mediators, endothelial cells, and extracellular matrix (ECM) molecules. The stromal score, immune score, ESTIMATE score, and tumor purity of each sample were calculated in the R platform using the ESTIMATE algorithm. The difference between high and low-MAL groups was further compared using the Wilcoxon test. KM analysis was performed to explore the survival influence of tumor microenvironment-related scores. The basis of grouping was determined by its median value.

The infiltration fraction of 22 immune cell types in each sample was estimated by performing the CIBERSORT algorithm. The Wilcoxon test was conducted to compare the difference between high and low-MAL groups.

2.5 | Differential signaling pathways demonstrate through GSEA between the high and low-MAL groups

The differentially activated signaling pathways between the high- and low-MAL groups were explored in the R platform. The ordered gene list was identified using the edgeR R package, and Gene Set Enrichment Analysis (GSEA) was performed using the gseKEGG function of clusterProfiler R package.

2.6 | Real-time quantitative PCR analysis

We performed real-time quantitative PCR (RT-qPCR) in vitro to show the expression of MAL in WT. The clinical specimens were from three adjacent normal tissues and three WT tissues who had been pathologically identified at the Department of Pathology, First Affiliated Hospital of Guangxi Medical University, from January 2019 to December 2019, and processed suitably. RT-qPCR was performed using PC33-2 in an ABI 7500 cycler (Applied Biosystems, Aidlab Biotechnologies Co., Ltd). The differential expression of MAL in WT tissues relative to the adjacent normal tissues was calculated using the $2^{-\Delta\Delta Ct}$ method, and GAPDH was identified as the internal control. The primers of MAL and GAPDH were synthesized by Aidlab Biotechnologies and the sequences were as follows: MAL: Forward 5′-CGACTTGGCTCTTCATCTTTGAG-3′ and Reverse 5′ ATGTACAG GATGATCAAGGTGG-3′; GAPDH: Forward 5′- AGAAGGCTGGGGCTCATTTG-3′ and reverse 5′- AGGGGCCATCCACAGTC TTC-3′. All experiments were repeated at least three times.

2.7 | Statistical analysis

All statistical analyses were carried out using the R platform (version: 3.61, http://www.r-project.org/). The relationship between MAL expression and pathological features was analyzed using the Kruskal–Wallis and Wilcoxon rank tests. The minifl R package was used to process the original data and calculate the $\beta$ value. The IlluminaHumanMethylation450kanno.ilmn12.hg19 R package was used for the conversion of gene symbols. Two-tailed $p$ value $<$ 0.05 was considered statistically significant in all statistical analysis: $^* p < 0.05$, $^{**} p < 0.01$, $^{***} p < 0.001$, and $^{****} p < 0.0001$.

3 | RESULTS

3.1 | Differential expression in MAL between WT and normal tissues

The results of RT-qPCR analysis are shown in Figure 1, and the expression of MAL was lower in WT than in adjacent normal tissues ($p < 0.05$, Figure 1), which showed the differential expression of MAL in adjacent normal tissues and WT in vitro.

The expression of MAL in the TARGET, GSE2712, GSE11151, and GSE73209 datasets also showed significant statistical differences (Figure 2A–D, $p < 0.05$). MAL was inadequately expressed in WT, and it was not significantly...
correlated with age, gender, stage, and histologic type (Figure 3A–D).

### 3.2 Survival outcomes and univariate COX analysis

KM analysis showed that the low-MAL group had worse prognosis than the high-MAL group in WT \((p = 8.81e-03, \text{HR} = 0.471, 95\% \text{CI}: 0.27–0.822, \text{Figure 4})\). In addition, univariate and multivariate Cox analyses proved that MAL was an independent prognostic factor for overall survival (OS), with an HR of 0.582 \((p = 2.35E-02, \text{Table 2})\).

### 3.3 Correlation analysis between MAL expression and methylation

We found that there were 17 methylation sites in the MAL gene. The \(\beta\) value of the methylation site in the MAL gene is shown in Figure 5A. The correlation analysis showed that MAL expression was related to cg03566174 \((R = 0.21, p = 2.04E-02, \text{Figure 5B, Figure S1})\) and cg05314420 \((R = −0.265, p = 3.17e-03, \text{Figure 5C, Figure S1})\) methylation sites. Kaplan–Meier analysis revealed that the cg05314420 methylation site of MAL was associated with OS \((p = 2.29e-02, \text{HR} = 2.188, 95\% \text{CI}: 1.225–3.907, \text{Figure 5D,E})\).

### 3.4 MAL expression and tumor microenvironment

The results of the immune microenvironment score indicated that the immune score, stromal score, and ESTIMATE score in the high-MAL group were
statistically higher than those in the low-MAL group, while the tumor purity was opposite ($p < 0.05$, Figure 6A–D). KM analysis showed that the stromal score and ESTIMATE score presented protective factors, while tumor purity showed a risk factor, which indirectly reflected the low-MAL group with a worse prognosis (Figure 7A–D).

Additionally, the infiltration analysis of 22 immune cell components of each WT sample revealed that CD8 T cells, resting CD4 memory T cells, monocytes, M2 macrophages, resting dendritic cells were significantly different between the high- and low-MAL groups via Wilcoxon analysis ($p < 0.05$, Figure 8), which indicated that there were certain differences in immune cell infiltration in tumor microenvironment between high- and low-MAL groups, which may affect the prognosis of WT patients to a certain extent.

### 3.5 | Differential signaling pathways demonstrate through GSEA between the high- and low-MAL groups

We performed GSEA between low- and high-MAL groups and the result showed that some important signaling pathways existed differentially enriched between the two groups, such as Wnt signaling pathways and PPAR signaling pathways (NES > 1, Adjust <0.05, Figure 9A–C).
**DISCUSSION**

As an integrated-membrane protein with multiple biological functions and playing a significant role in cell differentiation and proliferation, MAL has been extensively studied in relation to numerous tumors.\(^{20,21}\) MAL not only inhibited the progression of cancer,\(^{15,22,23}\) but in some cases, it also promoted the development of cancer.\(^{17,24–26}\) Moreover, the role of MAL in the kidney has been mentioned in relevant studies.\(^{21,27,28}\) However, there have been few studies on the role of MAL in WT. In our study, we found that MAL was under-expressed in WT, and it was not significantly correlated with age, gender, or stage. Meanwhile, RT-qPCR in vitro also confirmed the low expression of MAL in WT, which provided us with a preliminary understanding of the role of MAL in WT.

Considering the difference of MAL expression between WT and adjacent normal tissues, the survival status difference of WT was further analyzed based on the grouping of MAL expression values. Meanwhile, the univariate and multivariate Cox analyses were also used to explore the influence of MAL expression on prognosis of WT and it was shown to be an independent prognostic factor in our analysis. Therefore, in the subsequent analysis, we conducted a series of explorations based on the expression difference of MAL in WT to understand the potential impact of MAL as a prognostic factor.

The level of gene methylation can affect the expression of genes, and methylation of MAL has been extensively demonstrated in a variety of cancers.\(^{15,16,22,23,29}\) Therefore, in order to explore the methylation level of MAL in WT, we used Pearson correlation analysis to investigate the relationship between MAL expression and methylation sites in WT. The results showed that the expression of MAL was closely related to the methylation sites of cg05314420 and cg03566174. It was worth noting that cg05314420 was positively correlated with the expression of MAL, while cg03566174 was negatively correlated with the expression of MAL. Therefore, the decrease of MAL expression may be the result of the combined effects of multiple methylation sites. Further, our analysis showed that methylation sites of cg05314420 could affect the OS of patients with WT, which indicated that the degree of methylation of MAL may be one of the reasons why MAL is a prognostic factor of WT. Unfortunately, the related methylation sites of MAL in WT had not been reported, but which would provide us with a new idea for the study of WT.

Not only the characteristics of the tumor itself, but also the microenvironment of the tumor affects the progression of the tumor. There is growing evidence showing that the tumor microenvironment is critical to the occurrence and prognosis of tumors.\(^{30,31}\) A large number of studies have highlighted the presence of immunosuppressive microenvironments in WT\(^{32–34}\) and immune infiltration microenvironment may play an important role in the development of WT.\(^{35}\) Therefore, we further explored the difference of tumor microenvironment

| Characteristics | Univariate Cox analysis | Multivariate Cox analysis |
|-----------------|-------------------------|---------------------------|
|                 | HR (95% CI) | p value | HR (95% CI) | p value |
| Age | ≤6 | Reference | 0.57 (0.33–1) | 4.86E-02 | Reference | 0.467 (0.26–0.82) | 8.39E-03 |
| >6 | Reference | 0.559 (0.26–1.19) | 1.33E-01 | Reference | 3.151 (1.74–5.72) | 1.60E-04 | Reference | 3.462 (1.89–6.35) | 6.06E-05 |

**TABLE 2** The results of univariate and multivariable Cox regression analyses
between high- and low-MAL group by the ESTIMATE algorithm and CIBERSORT algorithm. The results of ESTIMATE algorithm showed that patients with low-MAL expression turned out to have lower stromal score, immune, and ESTIMATE scores, while purity of tumor was the opposite. The patients with higher stromal score or lower tumor purity had more favorable prognostic. This is consistent with previous studies. Meanwhile, the results of CIBERSORT algorithm also indicated that the expression of MAL was closely correlated with the infiltration of CD8 T cells, resting CD4 memory T cells, monocytes, M2 macrophages, and resting dendritic cells, which suggested to some extent that MAL, as a prognostic factor of WT, may be closely related to tumor microenvironment. Due to the low mutation load and immunogenicity of WT, it was not sensitive to most immunotherapy methods. However, because of the low toxicity of immunotherapy, the exploration of immunotherapy approaches for WT has never stopped. Therefore, it was hoped that our study could bring some ideas for the follow-up research of WT.

To investigate the differential activated signaling pathways between the high- and low-MAL groups, we performed GSEA. The results showed that the differential activated signaling pathways between the high- and low-MAL groups were important in cancer development, such as the Wnt signaling pathway and PPAP signaling pathway. The Wnt signaling pathway
is a highly conserved pathway throughout evolution, and the complexity and function of the pathway is of vital importance in human embryonic development and growth. Activation of the Wnt signaling pathway is very common in tumors and is abundant in other diseases. In terms of cancer, Fan et al. demonstrated that RTL1 could promote melanoma proliferation by regulating the Wnt/β-catenin signaling pathway and Korbut et al. also revealed the Wnt/β-catenin signaling pathway regulated by Tiam1 could contribute to the metastasis of thyroid cancer. In breast carcinoma, ALX4 has been shown to be a downregulated epigenetic tumor suppressor gene that could also inhibit the progression of breast cancer via the Wnt/β-catenin pathway. Moreover, the mutations of related genes in the Wnt signaling pathway play a critical role in WT, which suggests the importance of the Wnt signaling pathway in WT. Peroxisome proliferator-activated receptors (PPARs) are nuclear transcription factors that are related to cellular growth and differentiation, energy metabolism, insulin sensitization, and tumor regulation. PPARs include three major members: PPARα, PPAR β/δ, and PPARγ, which have different tissue distribution and functions. In the kidney, PPARα regulates the homeostasis of energy metabolism and is important for the control of renal function. Although little research has been done on the effect of the PPAR signaling pathway in the WT, our study would provide a novel view for the study of the WT and PPAR signaling pathways. The results of GSEA showed that multiple signaling pathways closely associated with tumorigenesis were differentially enriched between the high- and low-MAL groups, which greatly demonstrated the different biological status between the high- and low-MAL groups, and further indicated the feasibility of MAL as a potential prognostic factor for WT.

This study comprehensively explored the potential of MAL as a prognosis factor for WT through complex bioinformatics analysis. Meanwhile, we also demonstrated that MAL, as a prognostic factor for WT, may be closely related to the tumor microenvironment. However, some limitations remain to be considered in our research. First, due to a lack of support from more in vitro and in vivo experiments, the credibility of tumor microenvironment-related analysis is a challenge. Second, the data in our study comes from public databases, so there are unknown risks in grouping, blinding, and hidden bias. Therefore, although we have demonstrated the potential of MAL as a prognostic factor for WT in various ways, a prospective, well-designed clinical trial is awaiting further validation of our findings.
FIGURE 8  Differential infiltration analysis of 22 immune cell components in patients with WT. The red represents high-MAL group, and blue represents low-MAL group. The X axis represents the type of immune cells, and the Y axis represents the average level of immune cell infiltration in different MAL groups. *means $p < 0.05$, **means $p < 0.01$, and *** means $p < 0.001$. An ns means not statistically significant.

FIGURE 7  KM survival analysis of immune score, stromal score, ESTIMATE score, and tumor purity in patients with WT.
5 | CONCLUSION

In this study, we explored the potential of MAL as a prognosis factor for WT through related bioinformatics analysis. Meanwhile, we also demonstrated that MAL, as a prognostic factor for WT, may be closely related to the tumor microenvironment. Our results were expected to provide a new perspective for exploring prognostic factors of WT.

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CONFLICT OF INTEREST

All authors had read and approved to submit it to your journal. There were no conflicts of interest of any author in relation to the submission.

AUTHOR CONTRIBUTIONS

All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All procedures were approved by Institutional Review Boards of the First Affiliated Hospital of Guangxi Medical University. Written informed consents were obtained from patients involved in the study.

FIGURE 9 Parts of GSEA analysis results. Various signaling pathways associated with MAL are shown, including (B) Wnt signaling pathway and (C) PPAR signaling pathway.
DATA AVAILABILITY STATEMENT

R 3.6.1 (http://www.r-project.org/) was an open-source software. The RNA-FPKM data and clinical data of WT samples came from UCSC Xena (https://xenabrowser.net). GSE2712, GSE1115, and GSE73209 were downloaded from Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/). The methylation data of WT were downloaded from the Target database (https://ogc.cancer.gov/programs/target). The c5.bp.v6.2.entrez.gmt file came from Molecular Signatures Database (MSigDB, http://software.broadinstitute.org/gsea/index.jsp).

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REFERENCES

1. Parkin DM, Stiller CA, Draper GJ, Bieber CA. The international incidence of childhood cancer. Int J Cancer. 1988;42(4):511-520.
2. Breslow N, Olshan A, Beckwith JB, Green DM. Epidemiology of Wilms tumor. Med Pediatr Oncol. 1993;21(3):172-181.
3. Gatta G, Bottia L, Rossi S, et al. Childhood cancer survival in Europe 1999–2007: results of EUROCare-5—a population-based study. Lancet Oncol. 2014;15(1):35-47.
4. Green DM, Breslow NE, D’Angio GJ, et al. Outcome of patients with Stage II/favorable histology Wilms tumor with and without local tumor spill: a report from the National Wilms Tumor Study Group. Pediatr Blood Cancer. 2014;61(1):134-139.
5. Spreafico F, Pritchard Jones K, Malogolowkin MH, et al. Treatment of relapsed Wilms tumors: lessons learned. Expert Rev Anticancer Ther. 2009;9(12):1807-1815.
6. Termuhlen AM, Tersak JM, Liu QI, et al. Twenty-five year follow-up of childhood Wilms tumor: a report from the Childhood Cancer Survivor Study. Pediatr Blood Cancer. 2011;57(7):1210-1216.
7. Rivera MN, Haber DA. Wilms’ tumour: connecting tumorigenesis and organ development in the kidney. Nat Rev Cancer. 2005;5(9):699-712.
8. Kreidberg JA, Sariola H, Loring JM, et al. WT-1 is required for early kidney development. Cell. 1993;74(4):679-691.
9. Essafi A, Webb A, Berry R, et al. A wt1-controlled chromatin switching mechanism underpins tissue-specific wt14 activation and repression. Dev Cell. 2011;21(3):559-574.
10. Kispert A, Vainio S, McMahon AP. Wnt-4 is a mesenchymal signal for epithelial transformation of metanephric mesenchyme in the developing kidney. Development. 1998;125(1):4225-4234.
11. Vainio S, Lin Y. Coordinating early kidney development: lessons from gene targeting. Nat Rev Genet. 2002;3(7):533-543.
12. Frank M, van der Haar ME, Schaeren-Wiemers N, Schwab ME. rMAL is a glycosphingolipid-associated protein of myelin and apical membranes of epithelial cells in kidney and stomach. J Neurosci. 1998;18(13):4901-4913.
13. Pérez P, Puertollano R, Alonso MA, Pérez P, Puertollano R, Alonso MA. Structural and biochemical similarities reveal a family of proteins related to the MAL proteolipid, a component of detergent-insoluble membrane microdomains. Biochem. Biophys. Res. Commun. 1997;232(3):618–621. doi:10.1006/bbrc.1997.6338
14. Mimori K, Shiraishi T, Mashino K, et al. MAL gene expression in esophageal cancer suppresses motility, invasion and tumorigenicity and enhances apoptosis through the Fas pathway. Oncogene. 2003;22(22):3463-3471.
15. Overmeer RM, Henken FE, Bierkens M, et al. Repression of MAL tumour suppressor activity by promoter methylation during cervical carcinogenesis. J Pathol. 2009;219(3):327-336.
16. Buffart TE, Overmeer RM, Steenbergen RDM, et al. MAL promoter hypermethylation as a novel prognostic marker in gastric cancer. Br J Cancer. 2008;99(11):1802-1807.
17. Hsi ED, Sup SI, Alemany C, et al. MAL is expressed in a subset of Hodgkin lymphoma and identifies a population of patients with poor prognosis. Am J Clin Pathol. 2006;125(5):776-782.
18. Runa F, Hamalian S, Meade K, Shisgal P, Gray PC, Kelber JA. Tumor microenvironment heterogeneity: challenges and opportunities. Curr Mol Biol Rep. 2017;3(4):218-229.
19. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS. 2012;16(5):284-287.
20. Alonso MA, Weissman SM. cDNA cloning and sequence of MAL, a hydrophobic protein associated with human T-cell differentiation. Proc Natl Acad Sci U S A. 1987;84(7):1997-2001.
21. Frank M, Atanasoski S, Sancho S, et al. Progressive segregation of unmyelinated axons in peripheral nerves, myelin alterations in the CNS, and cyst formation in the kidneys of myelin and lymphocyte protein-overexpressing mice. J Neurochem. 2000;73(5):1927-1939.
22. Kalmár A, Péterfia B, Hollósi P, et al. DNA hypermethylation and decreased mRNA expression of MAL, PRIMA1, PTGDR and SFRP1 in colorectal adenoma and cancer. BMC Cancer. 2015;15:736.
23. Horne HN, Lee PS, Murphy SK, Alonso MA, Olson JA Jr, Marks JR. Inactivation of the MAL gene in breast cancer is a common event that predicts benefit from adjuvant chemotherapy. Mol Cancer Res. 2009;7(2):199-209.
24. Jacobs G, Sandmann W. Surgical aspects in arterial occlusion (author’s transl). Zentralbl Chir. 1977;102(18):1097-1101.
25. Kohno T, Moriuchi R, Katamine S, Yamada Y, Tomonaga M, Matsuyama T. Identification of genes associated with the progression of adult T cell leukemia (ATL). Jpn J Cancer Res. 2000;91(11):1103-1110.
26. Schwartz DR, Kardia SL, Shedden KA, et al. Gene expression in ovarian cancer reflects both morphology and biological behavior, distinguishing clear cell from other poor-prognosis ovarian carcinomas. Cancer Res. 2002;62(16):4722-4729.
27. Kim T, Fiedler K, Madison DL, Krueger WH, Pfeiffer SE. Cloning and characterization of MYPV17: a developmentally regulated myelin protein in oligodendrocytes. J Neurosci Res. 1995;42(3):413-422.
28. Lebrero MA, Monica A, Agustin B. Use Of The Mal Protein As Tumour Marker. Patent No. WO2003076948A1.
29. Beder LB, Gunduz M, Hotomi M, et al. T-lymphocyte maturation-associated protein gene as a candidate metastasis suppressor for head and neck squamous cell carcinomas. Cancer Sci. 2009;100(5):873-880.
30. Zeng D, Li M, Zhou R, et al. Tumor microenvironment characterization in gastric cancer identifies prognostic and immunotherapeutically relevant gene signatures. Cancer Immunol Res. 2019;7(5):737-750.
31. Zhang C, Cheng W, Ren X, et al. Tumor purity as an underlying key factor in glioma. Clin Cancer Res. 2017;23(20):6279-6291.

32. Maturu P, Jones D, Ruteshouser EC, et al. Role of cyclooxygenase-2 pathway in creating an immunosuppressive microenvironment and in initiation and progression of wilms’ tumor. Neoplasia. 2017;19(3):237-249.

33. Maturu P, Overwijk WW, Hicks J, Ekmeckioglu S, Grimm EA, Huff V. Characterization of the inflammatory microenvironment and identification of potential therapeutic targets in wilms tumors. Transl Oncol. 2014;7(4):484-492.

34. Amarante MK, de Oliveira CEC, Ariza CB, Sakaguchi AY, Ishibashi CM, Watanabe MAE. The predictive value of transforming growth factor-beta in Wilms tumor immunopathogenesis. Int Rev Immunol. 2017;36(4):233-239.

35. Palmisani F, Kovar H, Kager L, Amann G, Metzelder M, Bergmann M. Systematic review of the inflammatory landscape of Wilms tumors. Mol Ther Oncolytics. 2021;22:454-467.

36. Kalluri R, Zeisberg M. Fibroblasts in cancer. Nat Rev Cancer. 2006;6(5):392-401.

37. Aran D, Sirota M, Butte AJ. Systematic pan-cancer analysis of tumour purity. Nat Commun. 2015;6:8971.

38. Wang P, Chen Y, Wang C. Beyond tumor mutation burden: tumor neoantigen burden as a biomarker for immunotherapy and other types of therapy. Front Oncol. 2021;11:672677.

39. Tabatabai R, Linhares Y, Bolos D, Mita M, Mita A. Targeting the Wnt pathway in cancer: a review of novel therapeutics. Target Oncol. 2017;12(5):623-641.

40. Duchartre Y, Kim YM, Kahn M. The Wnt signaling pathway in cancer. Crit Rev Oncol Hematol. 2016;99:141-149.

41. Johnson ML, Rajamannan N. Diseases of Wnt signaling. Rev Endocr Metab Disord. 2006;7(1–2):41-49.

42. Taciak B, Pruszynska I, Kiraga L, Bialasek M, Krol M. Wnt signaling pathway in development and cancer. J Physiol Pharmacol. 2018;69(2).

43. Fan G, Ye D, Zhu S, et al. RTL1 promotes melanoma proliferation by regulating Wnt/beta-catenin signalling. Oncotarget. 2017;8(62):106026-106037.

44. Liu L, Wu B, Cai H, et al. Tiam1 promotes thyroid carcinoma metastasis by modulating EMT via Wnt/beta-catenin signaling. Exp Cell Res. 2018;362(2):532-540.

45. Yang J, Han F, Liu W, et al. ALX4, an epigenetically down regulated tumor suppressor, inhibits breast cancer progression by interfering Wnt/beta-catenin pathway. J Exp Clin Cancer Res. 2017;36(1):170.

46. Lee SB, Haber DA. Wilms tumor and the WT1 gene. Exp Cell Res. 2001;264(1):74-99.

47. Koesters R, Ridder R, Kopp-Schneider A, et al. Mutational activation of the beta-catenin proto-oncogene is a common event in the development of Wilms’ tumors. Cancer Res. 1999;59(16):3880-3882.

48. Maiti S, Alam R, Amos CI, Huff V. Frequent association of beta-catenin and WT1 mutations in Wilms tumors. Cancer Res. 2000;60(22):6288-6292.

49. Cheng HS, Tan WR, Low ZS, Marvalim C, Lee JYH, Tan NS. Exploration and development of PPAR modulators in health and disease: An update of clinical evidence. Int J Mol Sci. 2019;20(20):5055.

50. Vitale SG, Laganà AS, Negro A, et al. Peroxisome proliferator-activated receptor modulation during metabolic diseases and cancers: master and minions. PPAR Res. 2016;2016:6517313.

51. Tan CK, Zhuang Y, Wahl W. Synthetic and natural Peroxisome Proliferator-Activated Receptor (PPAR) agonists as candidates for the therapy of the metabolic syndrome. Expert Opin Ther Targets. 2017;21(3):333-348.

52. Tovar-Palacio C, Torres N, Diaz-Villasenor A, Tovar AR. The role of nuclear receptors in the kidney in obesity and metabolic syndrome. Genes Nutr. 2012;7(4):483-498.

53. Corrales P, Izquierdo-Lahuerta A, Medina-Gomez G. Maintenance of kidney metabolic homeostasis by PPAR gamma. Int J Mol Sci. 2018;19(7).

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