Prognostic Role of TLE Family in Lung Adenocarcinoma

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There are no competing interests statement.

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Abstract

Background

Lung cancer is the most commonly diagnosed cancer and the leading cause of cancer death worldwide. Lung adenocarcinoma (LUAD) is the most common type of lung cancer. Transducin-like Enhancer of split (TLE) family proteins repress transcription by multiple mechanisms. The prognostic role of TLEs in LUAD is still unclear.

Methods

We took TCGA dataset to analyze the relationship between the expression of TLEs and LUAD outcome.

Results

The expression of TLEs were different between 59 normal and 513 tumor samples. High TLE1 and low TLE2 were associated with poor PFS and OS (all $p < 0.050$). Multivariate analysis demonstrated that high TLE1 and low TLE2 were independent risk factors. Moreover, the combination of TLE1 and TLE2 was a better tool in prognostication.

Conclusions

High TLE1 and low TLE2 expressions are independent adverse prognostic factors and can be used as prognostic biomarkers in LUAD.

Key words: lung adenocarcinoma, prognosis, TlE family.

Text

Background

Lung cancer is the most commonly diagnosed cancer and the leading cause of cancer death worldwide. 2.1 million new lung cancer cases and 1.8 million deaths were predicted in 2018 [1]. Lung adenocarcinoma (LUAD) is the most common type of lung cancer [2]. The outcomes are capricious and unpredictable for patients with LUAD [3, 4]. Predictive and surrogate biomarkers are missing in spite of novel technologies and strategies that can help in the identification and stratification of patients.

Transducin-like Enhancer of split (TLE) family proteins are required for many developmental processes, including lateral inhibition, segmentation, sex determination, dorsal/ventral pattern formation, terminal pattern formation, eye development, as well as development of kidney and pancreas [5]. TLE family proteins probably repress transcription by multiple mechanisms [6]. TLE proteins do not bind to DNA directly, but rather are recruited to the template by DNA-bound repressor proteins [7, 8]. Seven homologs have been found in humans, including TLE1-7. There are four full-length TLE proteins, TLE1-4, while other two partially homologous proteins, TLE5 and TLE6, are expressed in truncated forms [9, 10]. All TLE proteins interact with Tcf1 and Lef1 downstream of the Wnt signaling pathway, suggestive of involvement in T cell development and function [11, 12, 13]. TLE corepressors predominantly partner with Tcf1/Lef1 to establish CD8+ T cell identity. And are physiologically required of for T cell development in stage-specific and gene context-dependent manner [14].

Dayyani found that Tle1 and Tle4 appeared to function as tumor suppressors in the context of myeloid leukemia [15]. In lung cancer, as a putative specific oncogene, TLE1 was found to be overexpressed in a subset of aggressive and advanced human lung tumors. TLE1 might regulate lung cancer aggressiveness [16]. Yao found that TLE1 promoted EMT in A549 lung cancer cells through suppression of E-cadherin. TLE1 inhibited anoikis and promotes tumorigenicity in human lung cancer cells through ZEB1-mediated E-cadherin repression [17, 18]. Furthermore, in the blood

lineage cells, TLE4 interacted with Pax5 and PU.1 transcription factor, suggesting a role in B cell development and function [19].

However, there are still many questions to be solved about the prognostic value of TLEs in LUAD. Here we conduct a study to investigate the correlation between the expression level of TLEs and survival in LUAD.

Methods

Patients

The Cancer Genome Atlas (TCGA) gene expression data of LUAD tissue were obtained from the Genomic Data Commons (GDC, available at: http://potal.gdc.cancer.gov/) Data Portal on November 6, 2019. Fragments per kilobase of exon per million reads mapped (FPKM) were used for expression quantification for 59 normal and 513 tumor samples in the TCGA data portal. For paired analysis, 57 normal and 57 tumor paired tissues were selected. Access to the de-identified linked dataset was obtained from TCGA in accordance with the database policy.

Clinicopathologic data for the corresponding patients, including age, gender, pathologic stage, molecular data and survival information, were also retrieved from the database. Only patients with both survival information and expression data available at that time point were included in this study.

Gene Ontology terms and Reactome pathways were enriched by the TLEs family. Enrichment analyses of Gene Ontology (GO) terms included biological process (BP), cellular component (CC), and molecular function (MF). The Reactome pathways were performed for all TLEs family. An adjusted p-value <0.05 was considered as statistically significant for GO and Reactome pathways.

Statistical analysis

Progression free survival (PFS) and overall survival (OS) were used as endpoints in this study. PFS refers to the length of time in which a patient lives with the disease but it does not get worse. OS means the time from diagnosis to death for any reason or the last follow-up time.

Numerical data were compared using Wilcoxon rank-sum test, and categorical data were compared using Fisher exact test. Kaplan-Meier methods and log-rank test were used for survival analysis. Univariate and multivariate Cox proportional hazard models were constructed for PFS and OS. The confidence interval was 95%. All statistical analyses were performed by R 3.6.0.

Results

The expression level of TLEs in normal and LUAD tumor samples

In this study, we employed TCGA to explore the expression level of TLEs in LUAD. We observed remarkable change of TLEs expression in tumor samples compared with normal tissues in TCGA (Fig. 1). The same trends were also validated in the paired samples (Fig. S1). In summary, the expression level of TLEs were related to LUAD. And the result also indicated that TLEs might be involved in the progression of LUAD.

The prognostic value of TLEs expression levels in LUAD

Based on the median expression level of each TLE member, all patients were divided into two groups. The differences between two groups were presented in Table 1. Both PFS and OS were adversely affected by high expression of TLE1 (Fig. 2A) and low expression of TLE2 (Fig. 2B) (PFS: $p<0.001$, $p=0.003$; OS: $p<0.0001$, $p=0.004$, respectively).

Expression of TLE family members in LUAD

We analyzed the correlations between the expression levels of TLE members. As shown in Fig. 3, the expression levels of TLE family members were related with each other (all $|R_{Pearson}|>0.1$, Fig. 3A), especially TLE3/TLE1, TLE4/TLE1, TLE6/TLE1, TLE3/TLE2, TLE5/TLE2, TLE6/TLE2, TLE6/TLE4, TLE6/TLE5 (all $|R_{Pearson}|>0.1$, Fig. 3B-I). These results indicated that the TLEs might be transcriptionally regulated together. Based on previous research, TLE1, TLE2, TLE5 were known to work as a complex to regulate DNA transcription. PPI network was done by using the TLE family members. TLE1-6 were interactional in the PPI network (Fig. 3J)

Clinical and molecular characteristics

The clinical and molecular characteristics were shown in Table 2. Comparing to the $TLE1^{low}$ group, the $TLE1^{high}$ group was more likely to be related to radiation therapy ($p=0.021$). Moreover, high TLE1 expresser had a trend of more pathologic T1 stage ($p=0.060$). And $TLE1^{high}$ group had less percentage of high RET (48.28% vs. 51.95%, $p=0.029$).

Comparing to the $TLE2^{low}$ group, the $TLE2^{high}$ group were more likely to be related to stage (p=0.011), pathologic T (p=0.001). Moreover, high TLE2 had a trend of more pharmaceutical therapy (p=0.057). And $TLE2^{high}$ group had less percentage of high PIK3CA (46.3% vs. 53.91%, p＜0.001), high percentage of high BRAF (54.86% vs. 45.31%, p＜0.001), and less percentage of high KRAS (46.69% vs. 53.52%, p=0.011).

Univariate and multivariate analysis of PFS and OS

To further assess the prognostic significance of TLE1/2, expression levels of TLE1-7 (high vs. low), age (≥60 vs. ＜60), gender, and common genetic mutations (ALK, BRAF, EGFR, ERBB2, KRAS, NRAS, PIK3CA, RET, ROS1) were enrolled in univariate and multivariate analysis (Table 3). For univariate analysis, three independent risk factors for PFS and five independent risk factors for OS were identified, including high TLE1, TLE2 and TLE5 expression, ALK, and NRAS (PFS: p＜0.001, p=0.004, p=0.035; OS: p＜0.0001, p=0.003, p=0.024, p=0.036, p=0.045, respectively). For multivariate analysis, two independent risk factors for PFS and OS were identified, including high TLE1, TLE2 expression (PFS: p＜0.001, p=0.020; OS: p＜0.0001, p=0.047, respectively).

The combined prognostic effect of TLE1 and TLE2 in LUAD

In multivariate analysis, TLE1 and TLE2 were independent risk factors for PFS and OS. The results showed that the $TLE1^{high}$ and $TLE2^{low}$ subgroup had significant shorter PFS and OS than others (p＜0.0001, p＜0.0001, respectively, Fig. 4).

Gene Ontology terms and Reactome pathways enriched by the TLEs family

Reactome pathways enriched by the TLEs family were related to the repression of WNT target genes, TCF dependent signaling in response to WNT and deactivation of beta-catenin transactivating complex (Fig. 5). Gene Ontology (GO) terms enrichment analyses found that the TLEs family were related to repressing transcription factor binding, beta-catenin-TCF-complex, transcription factor complex, negative regulation of canonical WNT signaling pathway, cell-cell signaling by WNT (Fig. 5). A model illustrating of TLE family members and Wnt pathway was shown in Fig S2.

Discussion

In this study, we found that the expression level of TLE1 showed an increasing trend, TLE2 showed decreasing trends in the LUAD. High TLE1, and low TLE2 were poor prognostic factors in LUAD patients. Co-expression analysis substantiated that TLE1 and TLE2 were strongly correlated and co-regulated in LUAD. In multivariate analysis, we also found that TLE1 and TLE2 were independent risk factors for DFS and OS in LUAD patients. It suggested that composite application of biomarkers might be more valuable than single-marker application in assessing tumor prognosis.

In recent years, the relationship between TLEs and cancers has been widely studied. TLE1 is shown to bind Runx1, which is essential for the generation and maintenance of hematopoietic stem/progenitor cells (HSPCs) [20, 21]. TLE1 may interact with chromatin through its interactions with the amino-terminal tail of histone H3 [22]. TLE1 gene silencing function appears to depend in part on recruitment of the histone deacetylase (HDAC) protein to target genes and the subsequent removal of acetyl groups from nearby DNA bound histones [23]. TLE1 is highly expressed in proliferative epithelial tissues as well as in diseased metaplastic and neoplastic transformed states [24]. TLE1 has been implicated in the pathogenesis of cancer. TLE1 is aberrantly expressed or upregulated in various types of human cancer including synovial sarcoma [25], breast [26] and lung cancer [16]. Allen found a convincing evidence that transgenic mice carrying the human TLE1 homologue, Grg1, develop lung adenocarcinoma [16]. Consistent with its lung oncogenic function, TLE1 was found to be upregulated in a significant fraction of human lung carcinomas. Yao found that ZEB1/TLE1/E-cadherin transcriptional mechanism was a pathway that promoted anoikis resistance and oncogenicity of lung cancer cells [18]. TLE1 was an effector of EMT in lung cancer cells through transcriptional silencing of the epithelial marker E-cadherin [17].

As a negative regulator of apoptosis, TLE1 inhibited low potassium-induced apoptosis in neurons [27]. TLE1 exhibited anti-neurogenic activity in mammalian forebrain development. Forced TLE1 expression in transgenic mice inhibited neuronal development in the forebrain in vivo [28]. Ectopic TLE1 expression in neural progenitor cells in culture promoted their un-differentiation status with

concomitant increased proliferative ability [29]. TLE1 in conjunction with Fork head box protein G1 (FoxG1) promoted survival in post-mitotic neurons [27]. TLE1 specifically inhibited the caspase-independent cell death induced by Bit1 (Bcl2-inhibitor of transcription 1) [30]. And TLE1 was shown to positively regulates Bcl2 expression and ErbB1 and ErbB2 signaling, two survival pathways that impact cancer progression [16]. Ablation of TLE1, exhibited grossly normal hematopoiesis, but resulted in excess production of inflammatory cytokines by macrophages [31]. Exogenous TLE1 expression stimulated anchorage-independent growth in chicken embryo fibroblast [32].

Transducing-like enhancer protein 2 (TLE2) in transcriptional repressed and defined the structural elements that mediate transcriptional and protein-protein interaction functions of Groucho/TLE proteins [33]. TLE2 could form a complex with replication and transcription activator (RTA) to access the cognate DNA sequence of the RTA-responsive element at different promoters and then inhibited replication and transactivation [34]. TLE2 and β-catenin physically associated with N-myc down-regulated gene 1 (NDRG1) to affect the Wnt pathway in esophageal cancer cells [35]. TLE2 was associated with bladder cancer patient survival and progression. High TLE2 expression was associated with favorable OS. The TCGA cohort confirmed that low TLE2 expression was associated with shorter OS and DFS [36]. Qian TT found that TLE3 was highly expressed in slow proliferation and migration breast cancer cells like MCF-7. However, it was down-regulated in more malignant stromal cells like ZR-75-30. These results revealed that TLE3 might be the key factor in the proliferation and migration of breast cancer cells [37].

The transcriptional pathways of TLEs family included Wnt, Notch, Pax2 and Runx2 [19]. Wnt growth factors mediated cell fate determination during embryogenesis and in the renewal of tissues in the adult. Co-repressors of the TLEs were known to contribute to the repression of Wnt targets in the absence of signaling, but how they were inactivated or displaced by Wnt signaling was poorly understood. Aravinda-Bharathi Ramakrishnan reviewed several reports that addressed the prevalence and molecular mechanisms of the Wnt transcription switch, including the finding of Wnt-dependent ubiquitination/inactivation of TLEs. Together, these

findings highlighted the growing complexity of the regulation of gene expression by the Wnt pathway [38]. Li et al revealed that a previously unrecognized TLE /YAP/TAZ-Groucho interaction was mobilized by loss of Lats1/2 to suppress Wnt/TCF-mediated transcription, thereby resulting in intestinal stem cell depletion and Wnt-uncoupled progenitor expansion [39]. Wnts acted by stabilizing cellular levels of the transcriptional coactivator beta-catenin, which formed complexes with sequence-specific DNA-binding Tcf/Lef transcription factors. In the absence of nuclear beta-catenin, Tcf/Lefs acted as transcriptional repressors by binding to Groucho/TLE proteins. Hanson AJ found that transcriptional switch involving XIAP-mediated ubiquitylation of Gro/TLE that facilitated its removal from TCF/Lef, thus allowing β-catenin-TCF/ Lef complex assembly and initiation of a Wnt-specific transcriptional program [40]. Daniels DL demonstrated that beta-catenin displaced Groucho/TLE from Tcf/Lef by binding to a previously unidentified second, low-affinity binding site on Lef-1 that included sequences just N-terminal to the DNA-binding domain, and that overlapped the Groucho/TLE-binding site [41]. Wu J reported that removal of O-GlcNAc from Wnt-responsive gene promoters was critical for gene activation from Wnt-responsive promoters. And identified a molecular mechanism by which Groucho/TLEs repressed gene transcription and provided a model whereby O-GlcNAc might control distinct intracellular signaling pathways [42].

In conclusion, our findings have shown that the expression level of TLEs presented a significant different trend with the progression of LUAD. High expression TLE1 and low expression of TLE2 indicated bad prognosis. Moreover, TLE1 and TLE2 were independent prognostic indicators in LUAD, and TLE1 and TLE2 might be potential therapeutic targets for treatment. And the combination of TLE1 and TLE2 was a better prognostic biomarker than single one. However, the mechanism of the correlation of TLE1/2 and further molecular mechanism of TLEs in LUAD are needed.

List of abbreviations

LUAD: Lung adenocarcinoma

TLE: Transducin-like Enhancer of split

TCGA: The cancer genome atlas

PFS: Progression free survival

OS: Overall survival

EMT: Epithelial mesenchymal transition

GDC: Genomic data commons

FPKM: Fragments per kilobase of exon per million reads mapped

GO: Gene ontology

BP: Biological process

CC: Cellular component

MF: Molecular function

PPI: Protein protein interaction

TCF: T cell factor

HSPCs: Hematopoietic stem/progenitor cells

HDAC: Histone deacetylase

FoxG1: Fork head box protein G1

Bit1: Bcl2-inhibitor of transcription 1

RTA: Replication and transcription activator

NDRG1: N-myc down-regulated gene 1

Declarations

No potential conflicts of interest were disclosed by all authors.

Consent for publication

All authors agree the publication of this research.

Availability of data and materials

Publicly available datasets are analyzed in this study. These data can be found here: TCGA: https://portal.gdc.cancer.gov/.

Competing interests

No potential conflicts of interest were disclosed by all authors.

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Authors' contributions

Qianli Ma and Chaozeng Si guarantee the integrity of the manuscript and contributed to the concept and design. Jin Zhang contribute to the data collection, data analysis, and interpretation. All authors contribute to writing and revising the manuscript. All authors read and approve the final manuscript.

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Reference

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394-424.
2. Travis WD. Pathology of Lung cancer. Clin Chest Med. 2011;32:669-92.
3. Z Li, S Yamada, Y Wu, KY Wang, YP Liu, H Uramoto, et al. Polypeptide N-acetylgalactosaminytransferse-6 expression independently predicts poor overall survival in patients with lung adenocarcinoma after curative resection. Oncotarget. 2016;7:54463-54473.
4. Greenhalgh J, Dwan K, Boland A, Bates V, Vecchio F, Dundar Y, et al. First-line treatment of advanced epidermal growth factor receptor (EGFR) mutation positive non-squamous non-small cell lung cancer. Cochrane Database Syst Rev. 2013;5:CD010383.
5. Agarwal, M, P. Kumar, and SJ Mathew. The Groucho/Transducin-like enhancer of split protein family in animal development. IUB MB Life. 2015;67:472-481.
6. G Chen, A J Courey. Groucho/TLE family proteins and transcriptional repression. Gene. 2000;249:1-16.
7. Jennings, BH, and D Ish-Horowicz. The Groucho/TLE/Grg family of transcriptional co-repressors. Genome Biol. 2008;9:205.
8. Turki-Judeh, W, and AJ Courey. Groucho: a corepressor with instructive roles in development. Curr. Top. Dev. Biol. 2012;98:65-96.
9. Gasperowicz, M, and F Otto. Mammalian Groucho homologs: redundancy or specificity? J. Cell. Biochem. 2005;95:670-687.
10. Buscarlet, M, and S Stifani. The ‘Marx’ of Groucho on development and disease. Trends Cell Biol. 2007;17:353-361.
11. Brantjes, H, J Roose, M van De Wetering, and H Clevers. All Tcf HMG box transcription factors interact with Groucho-related co-repressors. Nucleic Acids Res. 2001;29:1410-419.
12. Xue, HH, and DM Zhao. Regulation of mature T cell responses by the Wnt signaling pathway. Ann. N. Y. Acad. Sci. 2012;1247:16-33.
13. Steinke, FC, and HH Xue. From inception to output, Tcf1 and Lef1 safeguard development of T cells and innate immune cells. Immunol. Res. 2014;59:45-55.

14. Xing S, Shao P, Li F, Zhao X, Seo W, Wheat JC, Ramasamy S, Wang J, Li X, Peng W, Yu S, Liu C, Taniuchi I, Sweetser DA, Xue HH. Tle corepressors are differentially partitioned to instruct CD8+ T cell lineage choice and identity. J Exp Med. 2018;215:2211-2226.
15. Dayyani, F, J. Wang, JR Yeh, EY Ahn, E Tobey, DE Zhang, ID Bernstein, RT Peterson, and DA Sweetser. Loss of TLE1 and TLE4 from the del(9q) commonly deleted region in AML cooperates with AML1-ETO to affect myeloid cell proliferation and survival. Blood. 2008;111:4338-4347.
16. T. Allen, M. van Tuyl, P. Iyengar, S. Jothy, M. Post, M.S. Tsao, C.G. Lobe, Grg1 acts as a lung-specific oncogene in a transgenic mouse model, Cancer Res. 2006;66:1294–1301.
17. Yao X, Ireland SK, Pham T, Temple B, Chen R, Raj MH, Biliran H. TLE1 promotes EMT in A549 lung cancer cells through suppression of E-cadherin. Biochem Biophys Res Commun. 2014;455:277-84.
18. Yao X, Pham T, Temple B, Gray S, Cannon C, Hardy C, Fletcher K, Ireland SK, Hossain A, Chen R, Abdel-Mageed AB, Biliran H. TLE1 inhibits anoikis and promotes tumorigenicity in human lung cancer cells through ZEB1-mediated E-cadherin repression. Oncotarget. 2017;8:72235-72249.
19. Eberhard, D, G. Jiménez, B Heavey, and M Busslinger. Transcriptional repression by Pax5 (BSAP) through interaction with corepressors of the Groucho family. EMBO J. 2000;19:2292-2303.
20. D Levanon, RE Goldstein, Y Bernstein, H Tang, D Goldenberg, S Stifani, Z Paroush, and Y Groner. Transcriptional repression by AML1 and LEF-1 is mediated by the TLE/Groucho corepressors. Proc Natl Acad Sci USA. 1998;95:11590-11595.
21. Cai, X, L Gao, L Teng, J Ge, ZM Oo, AR Kumar, DG Gilliland, PJ Mason, K Tan, and NA Speck. Runx1 Deficiency Decreases Ribosome Biogenesis and Confers Stress Resistance to Hematopoietic Stem and Progenitor Cells. Cell Stem Cell. 2015;17:165-177.
22. A Palaparti, A Baratz, S Stifani, The Groucho/transducin-like enhancer of split transcriptional repressors interact with the genetically defined aminoterminal silencing domain of histone H3. J Biol Chem. 1997;27226604-26610.

23. G Chen, J Fernandez, S Mische, AJ Courey. A functional interaction between the histone deacetylase Rpd3 and the corepressor groucho in Drosophila development. Genes Dev. 1999;13:2218-2230.
24. Liu Y, Dehni F, Purcell KJ, Sokolow J, Carcangiu ML, Artavanis-Tsakonas S, Stifani S. Epithelial expression and chromosomal location of human TLE genes: implications for notch signaling and neoplasia. Genomics. 1996;31:58-64.
25. Seo SW, Lee H, Lee HI, Kim HS. The role of TLE1 in synovial sarcoma. J Orthop Res. 2011;29:1131-1136.
26. Brunquell C, Biliran H, Jennings S, Ireland SK, Chen R, Ruoslahti E. TLE1 is an anoikis regulator and is downregulated by Bit1 in breast cancer cells. Mol Cancer Res. 2012;10:1482-1495.
27. X Zhang, HM Chen, E Jaramillo, L Wang, SR D'Mello, Histone deacetylase related protein inhibits AES-mediated neuronal cell death by direct interaction, J Neurosci Res. 2008;86:2423-2431.
28. Yao J, Liu Y, Lo R, Tretjakoff I, Peterson A, Stifani S. Disrupted development of the cerebral hemispheres in transgenic mice expressing the mammalian Groucho homologue transducin-like-enhancer of split 1 in postmitotic neurons. Mech Dev. 2000;93:105-115.
29. Nuthall HN, Joachim K, Stifani S. Phosphorylation of serine 239 of Groucho/TLE1 by protein kinase CK2 is important for inhibition of neuronal differentiation. Mol Cell Biol. 2004;24:8395-8407.
30. Y Jan, M Matter, JT Pai, YL Chen, J Pilch, M Komatsu, E Ong, M Fukuda, E Ruoslahti. A mitochondrial protein, Bit1, mediates apoptosis regulated by integrins and Groucho/TLE corepressors. Cell. 2004;116:751-762.
31. Ramasamy, S, B Saez, S Mukhopadhyay, D Ding, AM Ahmed, X Chen, F Pucci, R Yamin, J Wang, MJ Pittet, et al. Tle1 tumor suppressor negatively regulates inflammation in vivo and modulates NF-κB inflammatory pathway. Proc Natl Acad Sci USA. 2016;113:1871-1876.
32. CK Sonderegger, PK Vogt, Binding of the corepressor TLE1 to Qin enhances Qin-mediated transformation of chicken embryo fibroblasts, Oncogene. 2003;22:1749-1757.
33. Grbavec D, Lo R, Liu Y, Stifani S. Transducin-like Enhancer of split 2, a mammalian homologue of Drosophila Groucho, acts as a transcriptional repressor,

interacts with Hairy/Enhancer of split proteins, and is expressed during neuronal development. European journal of biochemistry.1998;258:339-49.

34. ZH He, YH Liu, DG Liang, Z Wang, Erle S Robertson, K Lan. Cellular corepressor TLE2 inhibits replication-and-transcription-activator-mediated transactivation and lytic reactivation of Kaposi's sarcoma-associated herpesvirus. Journal of virology. 2010;84:2047-62.
35. Runna Ai, YL Sun, ZM Guo, Wei Wei, LP Zhou, F Liu, Denver T Hendricks, Y Xu, XH Zhao. NDRG1 overexpression promotes the progression of esophageal squamous cell carcinoma through modulating Wnt signaling pathway. Cancer biology & therapy. 2016;17:943-54.
36. S Wu, Katja Nitschke, Jakob Heinkele, Cleo-Aron Weis, Thomas Stefan Worst, Markus Eckstein, Stefan Porubsky, Philipp Erben. ANLN and TLE2 in Muscle Invasive Bladder Cancer: A Functional and Clinical Evaluation Based on In Silico and In Vitro Data. Cancers. 2019,11:1840.
37. Qian TT. The effects of Tle3/Grg3 Expression on the proliferation and Migration of Human Breat Cancer cells. Hunan University. 2014:1-68.
38. Ramakrishnan AB, Abhishek Sinha, Vinson B Fan, Ken M Cadigan. The Wnt Transcriptional Switch: TLE Removal or Inactivation? Bioessays. 2018;40(2).
39. Fu Vivian, Guan Kun-Liang. Tales from the Cryptkeeper: New Roles for Lats1/2 in Wnt-driven Homeostasis. Cell Stem Cell. 2020;26:612-614.
40. Hanson AJ, Wallace HA, Freeman TJ, Beauchamp RD, Lee LA, Lee E. XIAP monoubiquitylates Groucho/TLE to promote canonical Wnt signaling Mol Cell. 2012;45(5):619-28.
41. Daniels DL, Weis WI. Beta-catenin directly displaces Groucho/TLE repressors from Tcf/Lef in Wnt-mediated transcription activation. Nat Struct Mol Biol. 2005;12:364-71.
42. J Wu, DB Bowe, A Sadlonova, TR Whisenhunt, Y Hu, AK Rustgi, Y Nie, AJ Paterson, X Yang. O-GlcNAc transferase is critical for transducin-like enhancer of split (TLE)-mediated repression of canonical Wnt signaling. J Biol Chem. 2014;289:12168-76.

Figure legends

Fig. 1 The expression level of TLEs in TCGA of LUAD patients in normal and tumor tissues. X-axis represents the sample type; Y-axis represents the TLEs expression level. The different expression levels of TLE1-7 in normal (n=59) and tumor (n=513).

Fig. 2 Kaplan-Meier curves of progression-free survival (PFS) and overall survival (OS) in TCGA. A. High TLE1 expression had shorter PFS and OS than the low expressers. B. High TLE2 expression had longer PFS and OS than the low expressers.

Fig. 3 The correlations between the expression levels and PPI network of TLE family members.

A. Co-expression heat map of TLE genes in TCGA.
B. Co-expression relationship between TLE3 and TLE1 genes in TCGA.
C. Co-expression relationship between TLE4 and TLE1 genes in TCGA.
D. Co-expression relationship between TLE6 and TLE1 genes in TCGA.
E. Co-expression relationship between TLE3 and TLE2 genes in TCGA.
F. Co-expression relationship between TLE5 and TLE2 genes in TCGA.
G. Co-expression relationship between TLE6 and TLE2 genes in TCGA.
H. Co-expression relationship between TLE6 and TLE4 genes in TCGA.
I. Co-expression relationship between TLE6 and TLE5 genes in TCGA.
J. PPI network of TLE family members.

Fig. 4 Kaplan-Meier curves of progression-free survival (PFS) and overall survival (OS) in TCGA. (LL: $TLE1^{low}/TLE2^{low}$, LH: $TLE1^{low}/TLE2^{high}$, HL: $TLE1^{high}/TLE2^{low}$, HH: $TLE1^{high}/TLE2^{high}$)

A. $TLE1^{high}/TLE2^{low}$ expression had shorter PFS.
B. $TLE1^{high}/TLE2^{low}$ expression had shorter OS.

Fig. 5 Gene Ontology terms and Reactome pathways enriched by the TLEs family.

Fig. S1 The expression level of TLEs in TCGA of LUAD patients in paired tissues of normal and tumor. X-axis represents the sample type; Y-axis represents the TLE

expression level. The different expression levels of TLE1-7 in normal (n=57) and tumor (n=57).

Fig. S2 A model illustrating of TLE family members and Wnt pathway.

Figures

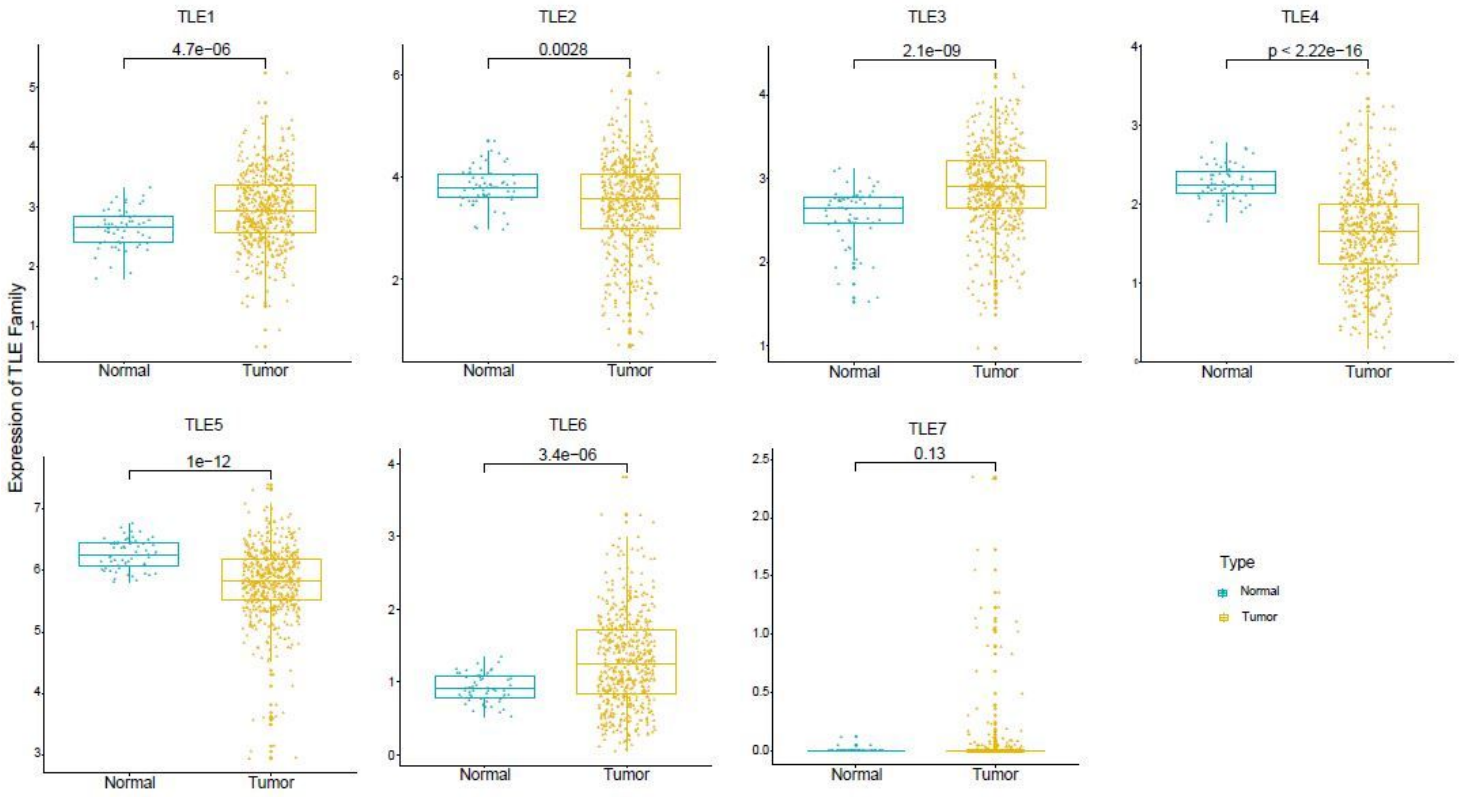

Figure 1

The expression level of TLEs in TCGA of LUAD patients in normal and tumor tissues. X-axis represents the sample type; Y-axis represents the TLEs expression level. The different expression levels of TLE1-7 in normal (n=59) and tumor (n=513).

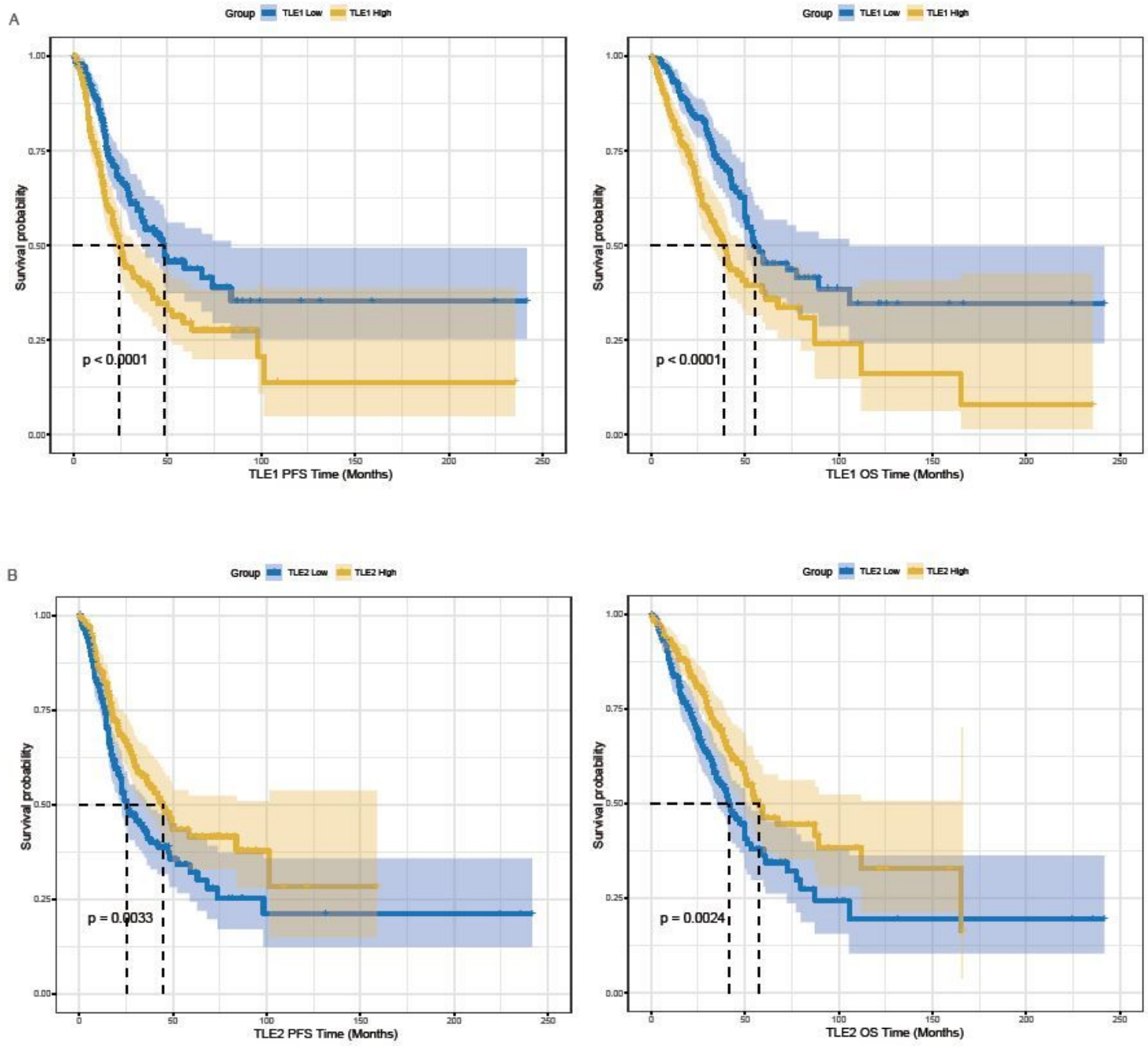

Figure 2

Kaplan-Meier curves of progression-free survival (PFS) and overall survival (OS) in TCGA. A. High TLE1 expression had shorter PFS and OS than the low expressers. B. High TLE2 expression had longer PFS and OS than the low expressers.

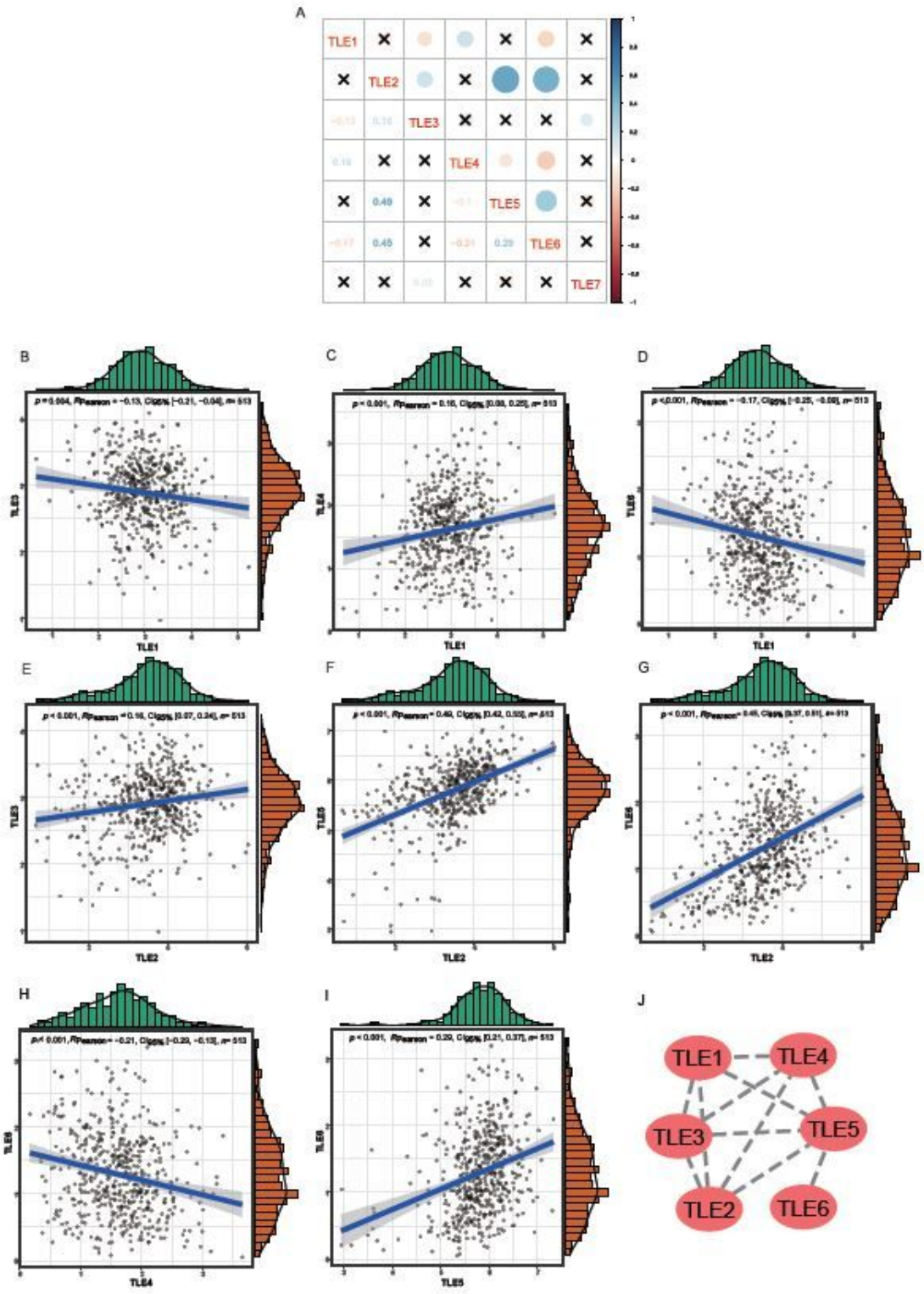

Figure 3

The correlations between the expression levels and PPI network of TLE family members. A. Co-expression heat map of TLE genes in TCGA. B. Co-expression relationship between TLE3 and TLE1 genes in TCGA. C. Co-expression relationship between TLE4 and TLE1 genes in TCGA. D. Co-expression relationship between TLE6 and TLE1 genes in TCGA. E. Co-expression relationship between TLE3 and TLE2 genes in TCGA. F. Co-expression relationship between TLE5 and TLE2 genes in TCGA. G. Co-expression

relationship between TLE6 and TLE2 genes in TCGA. H. Co-expression relationship between TLE6 and TLE4 genes in TCGA. I. Co-expression relationship between TLE6 and TLE5 genes in TCGA. J. PPI network of TLE family members.

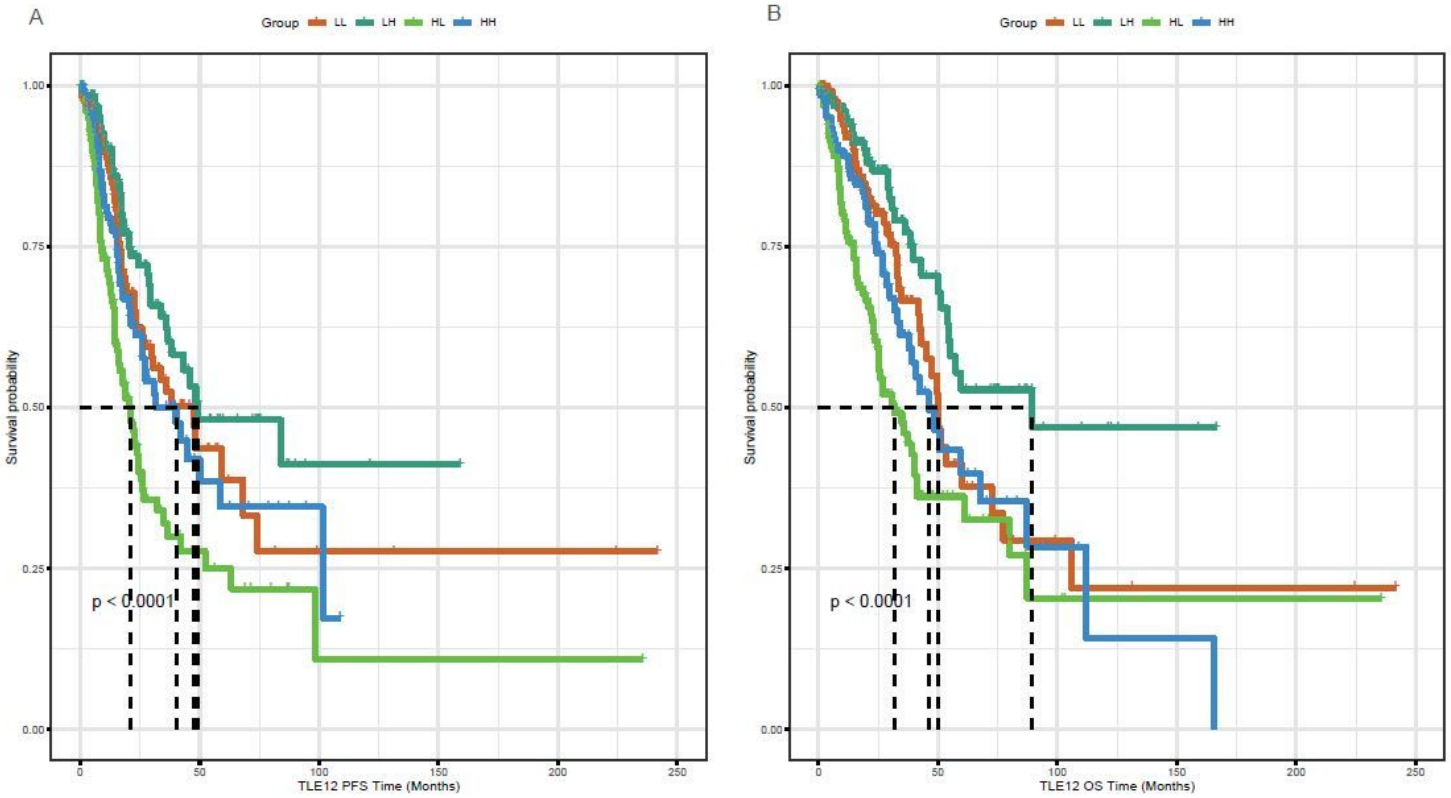

Figure 4

Kaplan-Meier curves of progression-free survival (PFS) and overall survival (OS) in TCGA. (LL: TLE1low/TLE2low, LH: TLE1low/TLE2high, HL: TLE1high/TLE2low, HH: TLE1high/TLE2high) A. TLE1high/TLE2low expression had shorter PFS. B. TLE1high/TLE2low expression had shorter OS.

Figure 5

Gene Ontology terms and Reactome pathways enriched by the TLEs family.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.JPG
- Table2.JPG
- Table2a.JPG
- Table2b.JPG
- Table3.JPG
- Fig.S1.pdf
- Fig.S2.pdf