Limma package

The limma (linear models for microarray data) package is a popular R/Bioconductor package used for the analysis of gene expression microarray data. It provides a suite of powerful tools for differential gene expression analysis, including linear models, empirical Bayes moderation, and multiple testing correction.

The limma package is particularly well-suited for the analysis of microarray data with complex experimental designs, such as those involving multiple treatments, time points, or batch effects. It can also be used for the analysis of RNA-seq data.

Some of the key functions provided by the limma package include:

lmFit: Fits linear models to gene expression data, accounting for experimental factors and covariates. eBayes: Empirical Bayes moderation of gene-wise variances to improve statistical power. topTable: Calculates moderated t-statistics and associated p-values for differential expression analysis. decideTests: Calls significant genes based on adjusted p-values and log-fold changes. plotMD: Creates diagnostic plots to assess the quality of the analysis. Overall, the limma package is a powerful and flexible tool for the analysis of gene expression data that has been widely used in both basic and clinical research.

Intracranial aneurysm (IA) is a common cerebrovascular disease. The immune mechanism of IA is more complicated, and it is unclear so far. Therefore, it is necessary to continue to explore the immune related molecular mechanism of IA.

Methods: All data were downloaded from the public database. Limma package and ssGSEA algorithm was used to identify differentially expressed mRNAs (DEmRNAs) and analyze immune cell infiltration, respectively. Machine learning and cytoscape-cytohubba plug-in was used to identify key immune types and multicentric DEmRNAs of IA, respectively. Multicentric DEmRNAs related to key immune cells were screened out as key DEmRNAs by Spearman correlation analysis. Diagnostic models, competing endogenous RNA (ceRNA) regulatory network and transcription factor regulatory network were constructed based on key DEmRNAs. Meanwhile, drugs related to key DEmRNAs were screened out based on DGIdb database. The expression of key DEmRNAs was also verified by real time-PCR.

Results: In this study, 7 key DEmRNAs (NRXN1, GRIA2, SLC1A2, SLC17A7, IL6, VEGFA and SYP) associated with key differential immune cell infiltration (CD56bright natural killer cell, Immature B cell and Type 1 T helper cell) were identified. Functional enrichment analysis showed that VEGFA and IL6 may be involved in the regulation of the PI3K-Akt signaling pathway. Moreover, IL6 was also found to be enriched in cytokine-cytokine receptor interaction signaling pathway. In the ceRNA regulatory network, a large number of miRNAs and lncRNAs were found. In the transcription factor regulatory network, the transcription factor SP1 was correlated with VEGFA, SYP and IL6. It is also predicted that drugs related to key DEmRNAs such as CARBOPLATIN, FENTANYL and CILOSTAZOL may contribute to the treatment of IA. In addition, it was also found that SVM and RF models based on key DEmRNAs may be potential markers for diagnosing IA and unruptured intracranial aneurysm (UIA), respectively. The expression trend of key DEmRNAs verified by real-time PCR was consistent with the bioinformatics analysis results.

Conclusion: The identification of molecules and pathways in this study provides a theoretical basis for

understanding the immune related molecular mechanism of IA. Meanwhile, the drug prediction and diagnosis model construction may also be helpful for clinical diagnosis and management [1].

A study of Zhao et al. aimed to identify novel tumor biomarkers with independent prognostic values in Glioblastomas. The DNA methylation profiles were downloaded from The Cancer Genome Atlas and Gene Expression Omnibus database. Differential methylated genes (DMGs) were screened from Glioblastoma recurrence samples using limma package in R software. Functional enrichment analysis was performed to identify major biological processes and signaling pathways. Furthermore, critical DMGs associated with glioblastoma outcome were screened according to univariate and multivariate cox regression analysis. A risk score-based prognostic model was constructed for these DMGs and prediction ability of this model was validated in training dataset and validation dataset. In total, 495 DMGs were identified between recurrent samples and disease-free samples, including 356 significantly hypermethylated and 139 hypomethylated genes. Functional and pathway items for these DMGs were mainly related to sensory organ development, neuroactive ligand-receptor interaction, pathways in cancer, etc. Five genes with abnormal methylation level were significantly correlated with prognosis according to survival analysis, such as ALX1, KANK1, NUDT12, SNED1, and SVOP. Finally, the risk model provided an effective ability for prognosis prediction both in training and validation data set. They constructed a novel prognostic model for survival prediction of Glioblastomas. In addition, they identified five DMGs as critical prognostic biomarkers in Glioblastoma progression [2].

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2)
Zhao J, Wang L, Kong D, Hu G, Wei B. Construction of Novel DNA Methylation-Based Prognostic Model to Predict Survival in Glioblastoma. J Comput Biol. 2019 Aug 28. doi: 10.1089/cmb.2019.0125. [Epub ahead of print] PubMed PMID: 31460783.