·论 著·

胶质母细胞瘤驱动基因相关的竞争性内源RNA调控网络

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【摘要】**目的** 探讨胶质母细胞瘤(GBM)驱动基因相关的竞争性内源RNA(ceRNA)调控网络。**方法** 从癌症基因组图谱(TCGA)中下载169例GBM及5例正常组织长链非编码RNA(lncRNA)表达数据,从UCSC Xena数据库下载509例GBM及10例正常脑组织微小RNA(miRNA)表达数据。对获取的lncRNA及miRNA表达数据进行差异表达分析。GBM的17个驱动基因是从文献(PMID: 30096302)中获得。miRcode,TargetScan,miRTarBase和miRDB数据库预测lncRNA、miRNA和GBM驱动基因之间的相互作用。**结果** GBM组织TP53及PTEN突变率最高,达30%,且TP53错义突变最常见。筛选出差异表达lncRNA共2 445个,表达上调1 052个,下调1 393个;差异表达miRNA共56个,表达上调28个,下调28个。共有5个GBM驱动基因、6个miRNA及297个lncRNA筛选出用于构建ceRNA网络,包括HOX转录反义RNA在内的8个lncRNA与GBM病人的生存相关。**结论** 采用生信分析方法构建ceRNA网络有助于深化GBM发生、发展机制的认识。

【关键词】胶质母细胞瘤;竞争性内源RNA;癌症基因组图谱;生信分析

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Competing endogenous RNA regulatory network related to glioblastoma driver genes

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【**Abstract**】 **Objective** To explore the competing endogenous RNA (ceRNA) regulatory network related to glioblastoma driver genes. **Methods** The long non-coding RNA (lncRNA) expression data of 169 patients with glioblastoma and 5 normal brain tissues were downloaded from the Cancer Genome Atlas (TCGA), and the microRNA (miRNA) expression data of 509 patients with glioblastoma tissues and 10 normal brain tissues were downloaded from the UCSC Xena database. Differential expression analysis was performed on the lncRNA and miRNA expression data. The 17 driver genes of glioblastoma were obtained from the literature (PMID: 30096302). The miRcode, TargetScan, miRTarBase and miRDB databases were used to predict the interactions among lncRNA, miRNA and glioblastoma driver genes. **Results** The TP53 and PTEN mutation rates of glioblastoma tissues were the highest, which were up to 30%, and TP53 missense mutation was the most common. A total of 2 445 differentially expressed lncRNA was screened, with 1 052 up-regulation and 1 393 down-regulation. A total of 56 differentially expressed miRNA was screened, with 28 up-regulation and 28 down-regulation. A total of 5 driver genes of glioblastoma, 6 miRNA and 297 lncRNA was screened to construct the ceRNA network. Eight lncRNAs, including HOX transcrit antisense RNA, were related to the survival of glioblastoma patients. **Conclusion** The construction of ceRNA network with bio-information analysis method helps to further elucidate the mechanism of glioblastoma development.

【**Key words**】Glioblastoma; Competing endogenous RNA; Long non-coding RNA; MicroRNA; Bio-information analysis

胶质母细胞瘤(glioblastoma,GBM)是成人最常见原发性恶性脑肿瘤[1],预后差,5年平均生存率仅为5%[2]。竞争性内源性RNA(competitive endogenous RNA,ceRNA)假说认为,长链非编码核糖核酸(long non-coding RNA,lncRNA)等充当ceRNA,可以通过与mRNA竞争性结合微小RNA(microRNA,miRNA)反应元件(miRNA response elements,MREs),参与肿瘤的发生和发展[3]。本文采用生信分析方法构建ceRNA网络,深化GBM发生、发展机制的认识。

1 资料与方法

1.1 数据获取与差异分析 GBM驱动基因文献检索下载(PMID:30096302169),R包maftools可视化基因

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表1 胶质母细胞瘤差异表达最显著的20个miRNA和20个lncRNA（正常vs.肿瘤）

miRNA	LogFC	p	lncRNA	LogFC	p
hsa-miR-21	−4.3245	2.31E-28	AP000924.1	−9.1782	3.00E-14
hsa-miR-27a	−2.4815	6.48E-27	HOTAIR	−9.1125	3.63E-10
hsa-miR-210	−2.2010	3.83E-11	HOXD-AS2	−8.8869	2.17E-13
hsa-miR-23a	−2.1137	8.82E-23	HOXA-AS2	−8.7383	2.13E-11
hsa-miR-10b	−1.7489	0.0002	HOXA-AS3	−8.1714	2.13E-11
hsa-miR-155	−1.7411	1.13E-10	AC093895.1	−7.7817	2.01E-08
hsa-miR-106b	−1.7378	4.49E-16	HOXC-AS1	−7.7656	7.19E-08
hsa-miR-25	−1.7226	4.85E-16	HOXC13-AS	−7.7523	3.70E-06
hsa-miR-15b	−1.5910	2.99E-12	HOXC-AS2	−7.7112	7.59E-09
hsa-miR-148a	−1.5814	8.45E-06	FOXD3-AS1	−7.6074	1.68E-17
hsa-miR-128a	2.3438	1.06E-27	AC073525.1	5.7517	3.50E-07
hsa-miR-128b	2.7157	2.07E-26	AP001993.1	5.8217	5.20E-05
hsa-miR-218	2.9625	1.14E-37	AC008568.1	5.8303	9.87E-08
hsa-miR-219	2.9663	7.08E-08	AL031429.2	5.9428	0.0002
hsa-miR-129	3.0050	8.88E-52	AC011995.2	6.1416	8.24E-10
hsa-miR-338	3.0160	1.83E-11	AC068254.1	6.1510	3.67E-05
hsa-miR-7	3.2780	1.68E-18	Z68323.1	6.1513	0.0090
hsa-miR-137	3.3971	4.09E-37	LINC01476	6.3560	0.0002
hsa-miR-139	3.6067	5.55E-43	AC007922.3	6.6651	0.0020
hsa-miR-124a	5.3180	9.59E-21	LINC01007	6.9231	5.48E-05

注：miRNA. 微小RNA；lncRNA. 长链非编码RNA；logFC. 对数差异倍数

突变信息。169例GBM及5例正常脑组织lncRNA表达谱从TCGA数据库下载（https://portal.gdc.cancer.gov/），509例GBM组织及10例正常组织miRNA表达数据从UCSC Xena数据库下载（http://xena.ucsc.edu/）。R包DEseq2分析lncRNA差异表达，limma包鉴定差异表达miRNA。相比正常脑组织，差异在2倍以上，即logFCI>1，且P<0.05认为有统计学意义。

1.2 ceRNA网络构建 利用miRcode数据库[4]预测lncRNA与miRNA相互作用关系，miRTarBase[5]、TargetScan[6]和miRDB[7]预测miRNA靶基因，并与GBM驱动基因取交集，得到lncRNA-miRNA-mRNA的ceRNA调控网络，用cytoscape v3.7.1软件可视化。

2 结 果

2.1 驱动基因及差异lncRNA、miRNA分析 GBM中TP53及PTEN突变率最高，达30%，且TP53错义突变最常见（图1）。按差异表达基因筛选标准：差异表达lncRNA共2 445个，GBM组织表达上调1 052个，下调1 393个；差异表达miRNA共56个，GBM组织表达上调28个，下调28个。差异表达最显著的20个lncRNA及20个miRNA见表1。

2.2 构建ceRNA调控网络及lncRNA生存分析 将筛选出的表达差异的2 445个lncRNA，应用miRcode数据库预测出与其配对的miRNA，应用miRNA靶基因预测数据库筛选出与驱动基因相匹配的miRNA。共有5个GBM驱动基因、6个miRNA及297个lncRNA筛选出用于构建ceRNA网络，并用cytoscape可视化（图2）。对ceRNA网络中lncRNA进行生存分析，结果显示8个lncRNA（包括HOX转录反义RNA、CYP1B1反义RNA-1、DBH反义RNA-1、MIR155宿主基因、WWTR1反义RNA-1、WWTR1内含子非编码RAN-1及淋巴细胞白血病缺失基因1）表达上调时，GBM病人的临床预后较差（图3）。

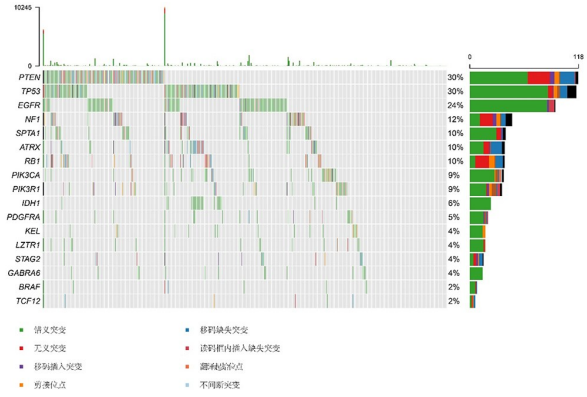

图1 TCGA数据库中胶质母细胞瘤驱动基因突变信息

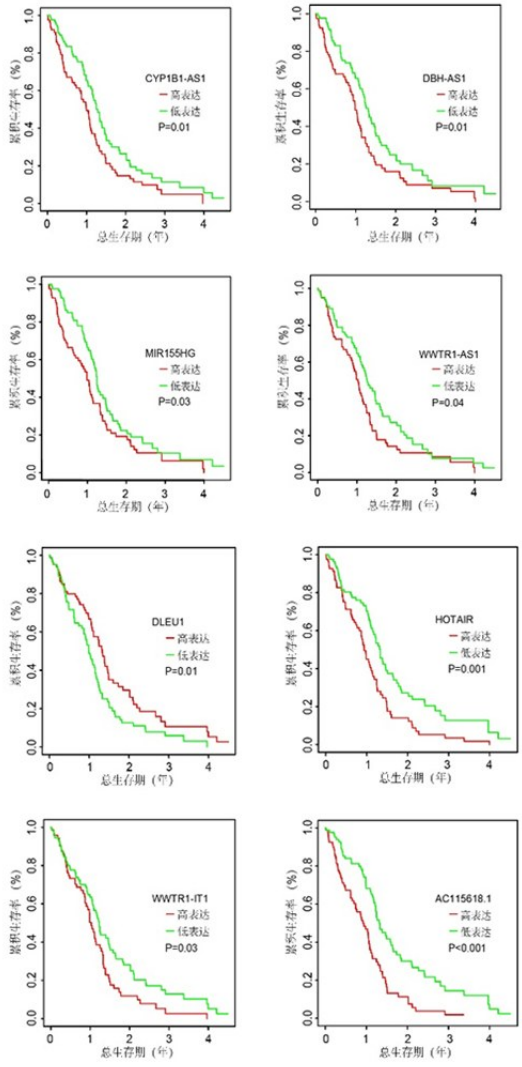

图3 竞争性内源性RNA调控网络中与胶质母细胞瘤病人生存相关的长链非编码RNA

3 讨 论

本文使用297个lncRNA、6个miRNA和5个GBM驱动基因构建一个ceRNA调控网络，其中8个lncRNA与生存相关（P<0.05），而HOX转录反义RNA、MIR155宿主基因、淋巴细胞白血病缺失基因1等3个lncRNA已经被证实是GBM的独立预后预测因子[8-10]。因此，使用生物信息学分析，构建ceRNA调控网络并筛选与预后相关的lncRNA分子，为GBM相关的ceRNA网络提供了新颖的见解，进一步深化了对GBM的发病机制的了解。

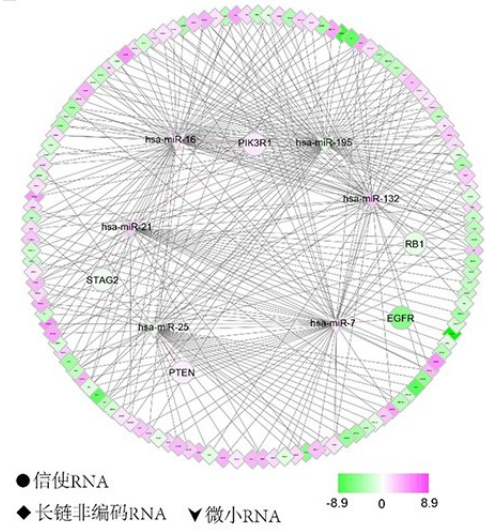

图2 胶质母细胞瘤驱动基因相关竞争性内源RNA调控网络

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