doi: 10.13241/j.cnki.pmb.2014.30.048

·生物信息学·

利用生物信息学研究肥胖与2型糖尿病患者肝组织基因表达变化*

安 冬[1] 姜 涛[2] 张翠丽[2] 殷玉玲[2] 曹雪姣[2] 辛 毅[2] 臧师竹[2△]

（1 大连医科大学 生理教研室 辽宁 大连 116044；2 大连医科大学 生物技术系 辽宁 大连 116044）

摘要 目的：利用芯片数据分析工具对GEO基因芯片数据进行数据挖掘，系统分析肥胖与2型糖尿病患者肝组织相关基因表达的变化，探讨肥胖与2型糖尿病的联系及糖尿病早期预防和诊断的新靶点。**方法：**首先在公共芯片数据库中选择肥胖与2型糖尿病相关芯片数据（GSE15653），利用R等芯片数据分析工具分析肥胖与2型糖尿病患者肝组织基因的表达变化，并预测相关差异表达基因在血中蛋白表达。**结果：**肥胖患者与正常人肝组织比较发现412个差异表达基因，其中上调表达基因212个，下调表达基因200个，2型糖尿病患者中控制良好者与正常人肝组织比较发现486个差异表达基因，其中上调表达基因253个，下调表达基因233个，而2型糖尿病患者中控制不良者与正常人肝组织比较发现1051个差异表达基因，其中上调表达基因560个，下调表达基因491个；2型糖尿病控制良好者与肥胖患者肝组织有263个相同的表达变化基因，而2型糖尿病控制不良者与肥胖患者肝组织有131个相同的表达变化基因；结合蛋白质组学结果分析肥胖与2型糖尿病相关的差异表达基因中有30个蛋白表达产物是分泌型蛋白。**结论：**肥胖及2型糖尿病患者肝组织与正常肝组织比较基因表达均发生明显变化，其基因表达变化数目随疾病的严重性增加而增多，而且2型糖尿病的控制情况与肝组织基因表达变化有密切关系。肥胖与2型糖尿病相关的差异表达基因中表达分泌型蛋白的可进一步用于研发监测疾病发生发展的候选靶分子。

关键词：基因芯片；生物信息学；2型糖尿病；肥胖

中图分类号：Q31；R587.1 **文献标识码：**A **文章编号：**1673-6273(2014)30-5976-04

Bioinformatics Analysis of Gene Expression Alterations of Liver Tissue between the Obesity and type 2 Diabetes Patients*

AN Dong[1], JIANG Tao[1], ZHANG Cui-li[2], YIN Yu-ling[2], CAO Xue-jiao[2], XIN Yi[1], ZANG Shi-zhu[2△]

(1 Biotechnology department, Dalian Medical University, Dalian, Liaoning, 116044, China;

2 Dalian Medical University Department of biotechnology, Dalian, Liaoning, 116044, China)

ABSTRACT Objective: To investigate the gene expression alterations between obesity and type 2 diabetes by using public gene microarray experiment database- GEO and explore the new targets for the early diagnosis and therapy for type 2 diabetes. **Methods:** Liver gene microarray data in both obesity and type 2 diabetes (GSE15663) were collected. Data mining process were performed by R to analyze gene expression characteristics in obesity and type 2 diabetes and predict the serum targets. **Results:** A total of 412 differentially expressed genes were recognized in obesity patients, including 212 up-regulated and 200 down-regulated genes. While for control-well type 2 diabetes, 486 differentially expressed genes were recognized, including 253 up-regulated and 233 down-regulated genes. For control-poor type 2 diabetes, a total of 1051 differentially expressed genes were found, including 560 up-regulated and 491 down-regulated genes. In addition, a total of 263 genes had the same alterations in both obesity and type 2 diabetes, and a total of 263 genes had the same alterations in both obesity and type 2 diabetes with control-well, while a total of 131 genes had the same alterations in both obesity and type 2 diabetes with control-poor. **Conclusion:** Gene expression profiles were dramatically changed in liver tissue in both obesity and type 2 diabetes and the number of the differentially expressed genes increased with the severe degree. At the same time, the control status of type 2 diabetes was related with the gene expression alteration. Some serum targets were also predicted for further analysis.

Key words: Gene microarray; Bioinformatics; Type 2 diabetes; Obesity

Chinese Library Classification(CLC): Q-31; R587.1 Document code: A

Article ID: 1673-6273(2014)30-5976-04

前言

肥胖、2型糖尿病等代谢紊乱性疾病已日益成为危害人类健康的重要疾病。研究发现，肥胖是导致2型糖尿病发病最重要的因素之一。Colditz等人发现，体重指数>28 kg/m^{2}的个体2型糖尿病的发生率有呈直线上升的趋势[1]，而以内脏脂肪积聚

* 基金项目：辽宁省教育厅自然科学一般项目（L2011159）

作者简介：安冬（1992-），女，硕士研究生，研究方向：神经生理，E-mail: andong999@gmail.com

△通讯作者：臧师竹，电话：0411-86110296，E-mail: szdl2001@163.com

（收稿日期：2013-12-10 接受日期：2013-12-30）

为特征的中心型肥胖比全身性肥胖更易导致 2 型糖尿病[2]。研究表明,肥胖和 2 型糖尿病这两种病理改变很可能有着共同的分子基础。目前生物芯片、双向电泳等高通量技术的应用已经揭示了 2 型糖尿病发生发展过程中多种器官组织细胞的基因组和蛋白质组水平的变化,为 2 型糖尿病发病分子机制及新的诊断和治疗靶点研究提供了成千上百的候选基因和蛋白。本研究在基因芯片公共数据库 GEO 中收集了 18 个肥胖与糖尿病相关芯片实验数据,利用多种芯片数据发掘工具,结合芯片实验样本的临床生物学特征,对芯片数据进行生物信息学分析,探索肥胖与 2 型糖尿病患者肝组织基因表达变化特征及临床生物学意义。

1 材料与方法

1.1 数据库及芯片数据的选择

利用 NCBI 中的 GEO 基因芯片公共数据库进行芯片数据搜索[3],搜索关键词为肥胖(Obesity)、2 型糖尿病(Type 2 diabetes),肝组织(Liver)。登陆网址:http://www.ncbi.nlm.gov/geo/,经过筛选,选择由 Pihlajam ki J 等提交的 18 个样品芯片数据(GSE15653)作为分析对象,该芯片实验采用 Affymetrix Human Genome U133A Array 平台系统对正常人和肥胖以及 2 型糖尿病患者(包括控制良好和不良者)肝组织基因的表达进行了系统分析。

1.2 芯片数据分析软件

芯片质量评估及标准化处理采用 R[4], Bioconductor[5]中相应软件包进行分析,利用 BAMarray[6]软件对肥胖与 2 型糖尿病患者肝组织中差异表达基因分析。使用 PANTHER[7]和Gominer[8] 等软件进行基因注释分析。

2 结果

2.1 芯片数据质量分析和标准化处理

对基因芯片公共数据库 GEO 中有关肥胖与 2 型糖尿病临床肝组织基因芯片数据(GSE15653)进行质量分析,主要根据 QC stat plot、RNA degradation plot 等分析结果进行判断,最后选择 17 个样本,其中正常肝组织 5 例,肥胖患者肝组织 3 例,2 型糖尿病患者(控制良好)5 例,2 型糖尿病患者(控制不良)4 例,选用 RMA 方法对芯片数据进行标准化处理。标准化后的数据用 Boxplots of the log2(Intensities) , Heatmap of between array distances 以及 MA plots 显示了芯片整体数据特征(图 1)。

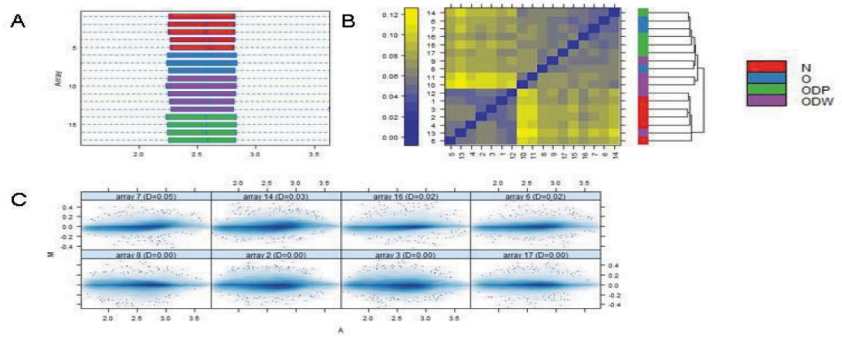

图 1 标准化后芯片数据特征 A:Boxplots of the log2(Intensities),B:Heatmap of between array distances,C:MA-plot

Fig.1 The characteristics of normalized array data A:Boxplots of the log2(Intensities),B:Heatmap of between array distances,C:MA-plot

2.2 肥胖与 2 型糖尿病患者肝组织分别与正常肝组织比较基因的差异表达

肥胖患者与正常人肝组织比较发现 412 个差异表达基因,其中上调表达基因 212 个,下调表达基因 200 个(图 2A)。2 型糖尿病患者中控制良好者与正常人肝组织比较发现 486 个差异表达基因,其中上调表达基因 253 个,下调表达基因 233 个(图 2B),而 2 型糖尿病患者中控制不良者与正常人肝组织比较发现 1051 个差异表达基因,其中上调表达基因 560 个,下调表达基因 491 个(图 2C)。由此可以看出,基因表达的变化数目随疾病的严重性增加(表 1)。

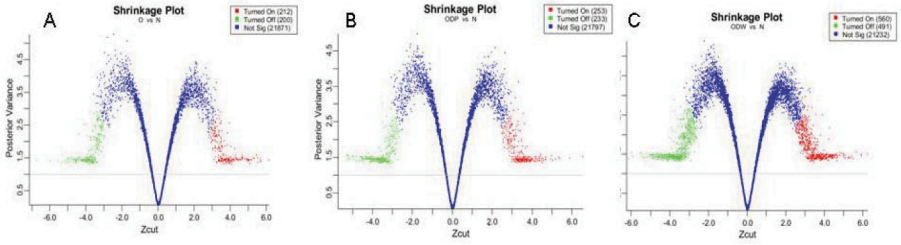

图 2 肥胖与 2 型糖尿病患者肝组织分别与正常肝组织比较基因的差异表达: A:肥胖患者; B: 2 型糖尿病控制良好; C:2 型糖尿病控制不良。蓝色点代表无表达变化的基因,红色点代表表达上调的基因,绿色点代表表达下调的基因。

Fig.2 Differential expression genes between obesity and type 2 diabetes compared with normal liver:A:Obesity; B: Type 2 diabetes (control well); C: Type 2 diabetes (control poor)。Blue point: genes without changes Red point: Up-regulated genes Green point: Down-regulated genes.

2.3 肥胖与 2 型糖尿病患者肝组织中相同的表达变化基因

2 型糖尿病控制良好者与肥胖患者肝组织有 263 个相同的表达变化基因(图 3A),而 2 型糖尿病控制不良者与肥胖患者肝组织有 131 个相同的表达变化基因(图 3B)。由此说明肥胖患者肝组织中某些基因表达变化可能与 2 型糖尿病的发生是相关的,而控制良好者,肝组织的特征更接近肥胖状态,但控

Table 1 Differentially expressed genes in liver between obesity and type 2 diabetes

	Obesity	Type 2 diabetes (control well)	Type 2 diabetes (control poor)
Up-regulated genes	212	253	560
Down-regulated genes	200	233	491
Total	412	486	1051

制不良者,肝组织的基因表达就更偏离正常及肥胖状态,因此疾病的有效控制对保护肝组织非常重要。

2.4 肥胖与 2 型糖尿病患者肝组织相关差异表达基因的分泌型蛋白表达

利用 Gominer 分析发现在肥胖与 2 型糖尿病相关的差异表达基因中有 30 个是分泌型蛋白(表 2),这些分子可通过血清学检测,研究其在肥胖、2 型糖尿病早期诊断、肝组织功能检测等方面的临床应用价值。

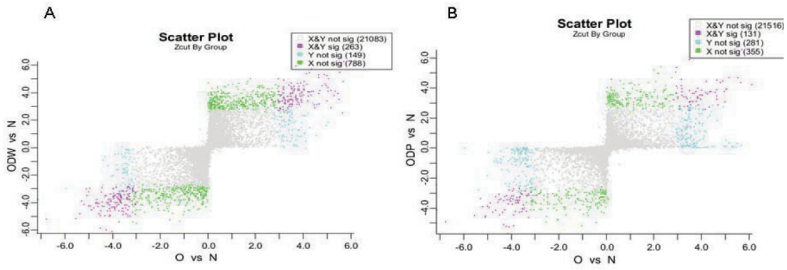

图 3 肥胖与 2 型糖尿病患者肝组织中相同的表达变化基因: A:2 型糖尿病控制良好; B:2 型糖尿病控制不良。紫色点是代表肥胖与 2 型糖尿病患者肝组织中相同的表达变化基因。

Fig.3 Consistent differential expression genes in obesity and type 2 diabetes:A: Type 2 diabetes (control well); B: Type 2 diabetes (control poor)。Purple point: Consistent differential expression genes in obesity and type 2 diabetes

3 讨论

现今发现的与 2 型糖尿病发病相关联的基因越来越多,它们或影响胰岛素发挥作用的正常途径,或通过其基因的多态性使个体对 2 型糖尿病具有一定的易感性(如 Ca1pain-10 基因),或通过影响 β 细胞的正常代谢及影响正常的信号传导通路等,引起了机体一系列的代谢和功能的紊乱,最终导致 2 型糖尿病的发生[9-11]。目前研究证实肥胖导致糖尿病发生的主要病理生理学变化是胰岛素抵抗和胰岛 β 细胞损伤[12-14]。胰岛素抵抗发生时由于胰岛素信号传导通路发生异常,导致胰岛素对血糖的调节能力下降。但当胰岛 β 细胞功能正常时,可通过增加胰岛素释放来有效代偿胰岛素抵抗引发的胰岛素效能降低,此时血糖仍可维持正常水平。然而当胰岛 β 细胞发生损伤时,无法释放足够的胰岛素来缓解胰岛素抵抗,因此血糖调控出现失代偿[15-18]。但是肥胖及 2 型糖尿病发生时,胰岛素靶器官的整体分子水平变化还尚不完全清楚,本文利用生物信息学分析工具对肥胖及 2 型糖尿病肝组织基因芯片数据进行系统分析,结果发现肥胖和 2 型糖尿病患者其肝组织基因表达均发生明显变化,基因表达的变化数目随疾病的严重性增加而增多。与正常人相比,单纯性肥胖患者与 2 型糖尿病控制良好者其肝组织基因表达谱改变有一定的相似性,而控制不良者,肝组织的基因表达就更偏离正常及肥胖状态,因此肥胖和 2 型糖尿病疾病的有效治疗和控制对保护肝组织功能具有极其重要的意义。这些基因表达的改变很可能是肥胖与 2 型糖尿病发生的共同分子基础,进一步证实了肥胖与 2 型糖尿病具有直接或间接的联系,对这些基因的进一步研究将有助于更好地了解肥胖及 2 型糖尿病的发生机制。在肥胖与 2 型糖尿病相关的差异表达基因中有 30 个表达分泌型蛋白,通过检测血清中相关蛋白,可以进一步研究对糖尿病早期诊断及肝组织功能检测具有一定临床应用价值的靶分子。

参 考 文 献 (References)

[1] Colditz GA,Willett WC, Rotnitzky A, et al. Weight gain as a risk factor for clinica1 diabetes mellitus in women [J]. Ann Intern Med, 1995, 122:481-486

[2] Ohlson LO, Larsson B, Svardsud K, et al. The influence of body fat distribution on the incidence of diabetes mellitus.13.5 years of follow-up of the participants in the study of men born in 1913 [J]. Diabetes, 1998, 34:1055-1058

[3] Edgar R, Domrachev M, Lash AE. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository [J]. Nucleic Acids Res, 2002, 30:207-210

[4] http://www.r-project.org/

[5] Gentleman RC, Carey VJ, Bates DM, et al. Bioconductor: open software development for computational biology and bioinformatics [J]. Genome Biol, 2004, 5:R80

[6] Ishwaran H, Rao J S. Detecting differentially expressed genes in microarrays using Bayesian model selection [J]. Journal of the American Statistical Association, 2003, 98:438-455

[7] Thomas PD, Kejariwal A, Campbell MJ, et al. PANTHER: a browsable database of gene products organized by biological function, using curated protein family and subfamily classification[J]. Nucleic Acids Res, 2003, 31:334-341

[8] Zeeberg BR, Feng W, Wang G, et al GoMiner: a resource for biological interpretation of genomic and proteomic data [J]. Genome Biol, 2003, 4:R28

[9] Wild S, Roglic G, Green A, et al. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030[J]. Diabetes Care, 2004, 27:1047-1053

表 2 肥胖与 2 型糖尿病患者肝组织中相关差异表达基因的分泌型蛋白

Table 2 The predicted secreted proteins in obesity and type 2 diabetes

Gene Symol	Gene Description
IGF2	Insulin-like growth factor II Ala-25 Del;IGF2;ortholog
PPIA	Peptidyl-prolyl cis-trans isomerase A;PPIA;ortholog
PCYOX1	Prenylcysteine oxidase 1;PCYOX1;ortholog
BMP8A	Bone morphogenetic protein 8A;BMP8A;ortholog
CCL11	Eotaxin;CCL11;ortholog
PTPRG	Receptor-type tyrosine-protein phosphatase gamma;PTPRG;ortholog
PLXNB1	Plexin-B1;PLXNB1;ortholog
HLA-C	HLA class I histocompatibility antigen, Cw-17 alpha chain;HLA-C;ortholog
IGHG1	Ig gamma-1 chain C region;IGHG1;ortholog
LEPR	Leptin receptor gene-related protein;LEPROT;ortholog
ST3GAL2	CMP-N-acetylneuraminate-beta-galactosamide-alpha-2,3-sialyltransferase;ST3GAL2;ortholog
PPIA	Peptidyl-prolyl cis-trans isomerase A;PPIA;ortholog
P4HB	Protein disulfide-isomerase;P4HB;ortholog
LTBP3	Latent-transforming growth factor beta-binding protein 3;LTBP3;ortholog
HMOX1	Heme oxygenase 1;HMOX1;ortholog
SERPING1	Plasma protease C1 inhibitor;SERPING1;ortholog
SPINT3	Kunitz-type protease inhibitor 3;SPINT3;ortholog
CRP	Cysteine and glycine-rich protein 1;CSRP1;ortholog
TNFRSF25	Tumor necrosis factor receptor superfamily member 25;TNFRSF25;ortholog
NENF	Neudesin;NENF;ortholog
IGHM	Ig mu chain C region;IGHM;ortholog
LEPR	Leptin receptor;LEPR;ortholog
COL4A5	Collagen alpha-5(IV) chain;COL4A5;ortholog
SAA3P	Putative serum amyloid A-3 protein;SAA3P;ortholog
CXADR	Coxsackievirus and adenovirus receptor;CXADR;ortholog
LTBP3	Latent-transforming growth factor beta-binding protein 2;LTBP2;ortholog
INS	Insulin A chain;INS;ortholog
CRP	C-reactive protein(1-205);CRP;ortholog
TNXB	Tenascin-X;TNXB;ortholog
IGFBP1	Insulin-like growth factor-binding protein 1;IGFBP1;ortholog
FAS	Tumor necrosis factor receptor superfamily member 6;FAS;ortholog
OGN	Mimecan;OGN;ortholog
TNXB	Tenascin-X;TNXB;ortholog
IGHG3	Ig gamma-3 chain C region;IGHG3;ortholog
HLA-C	HLA class I histocompatibility antigen, Cw-7 alpha chain;HLA-C;ortholog
IL32	Interleukin-32;IL32;ortholog

[10] World Health Organization. The world health report 2006: working together for health[R]. Geneva: World Health Organization, 2006

[11] Hossain P, Kawar B, El Nahas M. Obesity and diabetes in the developing world--a growing challenge[J]. N Engl J Med, 2007, 356: 213-215

[12] Cao HNShorey S, Rpbinson J, et al. GCK and HNF1A mutations in canadian families with maturity onset diabetes of the young (MODY) [J]. Human Mutation, 2002, 20:478-479

(下转第 5933 页)

[14] Alneab ME, Crabtree VP, Boutin A. Prospective assessment of lower-extremity peripheral artery disease in diabetic patients using anovel automated optical device[J]. Angiology, 2007, 58(5): 579-585

[15] Marteles MS, Urrutia A. Acute heart failure: acute cardiogenic pulmonary edema and cardiogenic shock [J]. Med Clin (Barc), 2014, 142S1: 14-19

[16] Ratanasit N, Karaketklang K, Chirakarnjanakorn S, et al. Left atrial volume as an independent predictor of exercise capacity in patients with isolated diastolic dysfunction presented with exertional dyspnea [J]. Cardiovasc Ultrasound, 2014, 14, 12(1): 19

[17] Wang Y, Zheng A, Yan Y, et al. Association between HMW adiponectin, HMW-total adiponectin ratio and early-onset coronary artery disease in Chinese population[J]. Atherosclerosis, 2014, 2, 235 (2): 392-397

[18] 史亚娜, 王凡非, 邓捷, 等. 心尖肥厚型心肌病的临床特征及治疗 [J]. 现代生物医学进展, 2012, 12(34): 6684-6685+6646
Shi Ya-na, Wang Fan-fei, Deng Jie, et al. Clinical characteristics and treatment methods of the AHCM [J]. Progress in Modern Biomedicine, 2012, 12(34): 6684-6685+6646

[19] 顾秀莲, 樊济海, 巢胜吾, 等. 心脏室间隔起搏与右室心尖部起搏对心功能的影响[J]. 现代生物医学进展, 2013, 13(21): 4081-4084
Gu Xiu-lian, Fan Ji-hai, Chao Sheng-wu, et al. Cardiac Function Influence on Right Ventricular Septum Pacing and Right Ventricular Apex Pacing [J]. Progress in Modern Biomedicine, 2013, 13 (21): 4081-4084

[20] 刘楠楠, 侯明晓, 曹军英, 等. 应用斑点成像及组织多普勒估测左室充盈压[J]. 现代生物医学进展, 2013, 13(13): 2521-2524
Liu Nan-nan, Hou Ming-xiao, Cao Jun-ying, et al. Research on the Prediction of LV Filling Pressure by Speckle Tracking and Tissue Doppler Imaging [J]. Progress in Modern Biomedicine, 2013, 13(13): 2521-2524

（上接第 5979 页）

[13] Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes[J]. Nature, 2006, 444:840-846

[14] Nolan CJ. Failure of islet β-cell compensation for insulin resistance causes type 2 diabetes: what causes non-alcoholic fatty liver disease and non-alcoholic steatohepatitis [J]. J Gastroenterol Hepatol, 2010, 25:1594-1597

[15] Aguirre V, Uchida T, Yenush L, et al. The c-Jun NH(2)-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser (307) [J]. J Biol Chem, 2000,24:9047-9054

[16] Tfayli H, Bacha F, Gungor N, et al. Phenotypic type 2 diabetes in obese youth: insulin sensitivity and secretion in islet cell antibody-negative versus-positive patients[J]. Diabetes, 2009, 58:738-744

[17] Tiberti C, Giordano C, Locatelli M, et al. Identification of tyrosine phosphatase 2 (256-760) construct as a new, sensitive marker for the detection of islet autoimmunity in type 2 diabetic patients: the non-insulin requiring autoimmune diabetes (NIRAD) study 2 [J]. Diabetes, 2008, 57:1276-1283

[18] Diani AR, Sawada G, Wyse B, et al. Pioglitazone preserves pancreatic islet structure and insulin secretory function in three murine models of type 2 diabetes [J]. Am J Physiol Endocrinol Metab, 2004, 286: E116-122

[19] Kim CH, Kim HK, Kim EH, et al. Relative contributions of insulin resistance and β-cell dysfunction to the development of Type 2 diabetes in Koreans[J]. Diabet Med, 2013, 30(9):1075-1079

[20] Mamtani M, Kulkarni H, Dyer TD, et al. Waist circumference independently associates with the risk of insulin resistance and type 2 diabetes in mexican american families [J]. PLoS One, 2013, 8(3): e59153

[21] Mohan V, Amutha A, Ranjani H, et al. Associations of β-cell function and insulin resistance with youth-onset type 2 diabetes and prediabetes among Asian Indians [J]. Diabetes Technol Ther, 2013,15(4):315-322