

Using Ultrasound, Mammography and Breast MRI Imaging in Breast Examination to Find out the Best Breast Examination Model in Early Breast Cancer

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Abstract Objective: If we detect the stage 0 breast cancer, we can cure the cancer and increase life quality of patient. We compare the 3 different diagnostic performances of mammography, direct ultrasound and contrast-enhanced Breast MRI. Regarding the sensitivity and characterization of DCIS relative to histopathologic proved, we can find out the best examination method of breast DCIS. **Materials and Methods:** During seventeen months period, Jan 2005 to Jun 2006, 832 consecutive patients with 54 breast cancer and 10 DCIS of histopathologically verified lesions were evaluated with three-dimensional rotation delivery of excitation off resonance (RODEO) breast MR imaging and in addition to mammography and direct ultrasound examination. All lesions were classified on ACR-BIRAD reporting system. The results were correlated to histopathology. The BIRAD category 0,1,2,3 were classified as negative sensitivity finding, and BIRAD category 4 or 5 are classified as positive sensitivity finding. **Results:** In our DCIS cases, the average ages of the patients was 49.8 years old (range from 35~60). We compare the 3 different diagnostic performance of sensitivity based on different tumor size group (<10 mm group, 10~15 mm group, and 16~20 mm group) with the BIRAD reporting system finding. The mean lesion dimension of tumor size is 13 mm (range from 3~20 mm). We conclude that in <10 mm tumor size group. The sensitivity of conventional mammography is 25%, direct ultrasound 33%, conventional mammography plus direct ultrasound 50%, and contrast-enhanced MRI 90%. In 10~15 mm group, the sensitivity of mammography or direct ultrasound is 40% and 50%, mammography plus direct ultrasound 60% and contrast-enhanced MRI 90%. In 16~20 mm group, the sensitivity of conventional mammography is 70%, direct ultrasound 100%, and contrast-enhanced MRI 100%. For invasive breast cancer, we compared the ultrasound and breast MRI diagnostic performance of sensitivity bases on BIRADS reporting system finding. The sensitivity of ultrasound was 98% and the sensitivity of dedicated breast MRI was 100% of all breast cancer. No any DCIS cases detect it from self-examination. **Conclusions:** Self-examination is useless for detecting early breast cancer of DCIS. The ultrasound and mammography also can easy miss the DCIS cases especially when the lesion is small than 15 mm. Dedicated RODEO breast MRI has highly sensitivity for DCIS of breast than ultrasound and mammography, especially when DCIS is smaller than 15 mm.

Keywords: DCIS; Ultrasound; Mammography; Breast MRI

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滋阴清热方对阴虚内热证SLE白细胞基因及蛋白表达谱的动态影响

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摘要 研究背景: 系统性红斑狼疮(systemic lupus erythematosus, SLE)作为一种严重危害健康的自身免疫性疾病, 其病因、发病机制尚未完全阐明, 治疗至今仍是世界难题。目前西医仍主要用糖皮质激素和免疫抑制剂治疗, 长期应用会带来明显的毒副作用。临床实践证明中西医结合治疗SLE临床疗效突出, 可减少药物的毒副作用, 提高SLE患者的生存质量。阴虚内热是SLE的基本证型, 滋阴清热法是治疗SLE基本法则。现代研究认为SLE的发生是由多基因共同作用的结果。研究SLE基因调控网络和蛋白质相互作用网络的动态变化将有助于我们对SLE发病的深入了解。目的: 观察滋阴清热方治疗阴虚内热证SLE的疗效, 研究SLE患者治疗前、中(治疗6周)、后(治疗12周)外周血单核细胞(peripheral blood mononuclear cell, PBMC)基因及蛋白表达谱的动态改变, 了解阴虚内热证SLE患者基因及蛋白表达模式与正常人之间的异同, 从基因及蛋白水平探讨中医药治疗机理, 为构建中医药治疗SLE的基因与蛋白表达谱研究方法与平台打下基础。方法: 1.研究对象: 女性阴虚内热证SLE病例来自2005年11月至2006年11月在广东省中医院门诊就诊的确诊病人, 轻、中度活动(5≤SLEDAI≤25分), 如使用了糖皮质激素, 则维持量为10~25mg/d, 3个月内不加量, 如使用过非甾体抗炎药则需停药一周以上, 如使用过其他免疫抑制剂则需停药半年以上。健康对照组来自于广州中医药大学健康大学生和健康志愿者, 共15例, 均为女性。治疗组总共纳入18例, 其中退出2例,

失访1例,有效病例共15例,平均年龄:31.60±9.37岁;健康对照组平均年龄:28.4±6.50岁。两组年龄经两样本t检验,方差齐性($P=0.098$),差异无统计学意义($P=0.289$)。2.治疗与疗效观察:入选病例用滋阴清热方制成胶囊,每次5粒,每日3次口服治疗3个月,每两周进行中医证候积分评估,并在中药治疗前、治疗6周、治疗12周时评定活动度积分,检测患者血清补体C3、C4水平、抗核抗体(ANA)、抗双链DNA抗体(Ds-DNA)、血常规、尿常规等指标,提取PBMC总RNA和总蛋白质,-70℃保存备用,将完成3次观察的合格病例的样品进行基因芯片与蛋白芯片的检测。所有病例均按病例资料收集要求与规范详细填写《病例观察报告表》。3.基因芯片实验与分析方法:人类基因表达谱芯片由微芯生物公司采用cDNA直接点样生产,共有8064个基因点,采用Cy3单荧光标记探针,数据经标准化校正,计算基因表达量在实验组和对照组间的比值(Ratio),按Ratio大于2或小于0.5作为表达差异的显著性标准。运用国际通用的GeneCluster 2软件对标准化后数据进行SOMs(self-organizing maps;自组图)分析、功能分类分析和聚类分析。4.蛋白芯片实验与分析方法:采用表面加强激光解吸电离-飞行时间质谱(SELDI)方法建立SLE患者治疗前、治疗6周、治疗12周以及正常对照组PBMC蛋白质表达数据,利用浙江大学肿瘤研究所余捷凯等研究设计的ZUCIPDAS软件包找出蛋白质荷峰,建立判别模型,采用基因芯片表达数据库与ExPasy蛋白数据库交连比分析方法初步确定蛋白质荷峰的可能名称与功能。结果:1.糖皮质激素配合滋阴清热方治疗可以较快缓解阴虚内热证SLE患者病情,改善自觉症状,SLEDAI积分、中医证候积分治疗后显著下降,C3、C4水平上升,患者病情有明显改善。中医证候积分与SLEDAI积分呈正相关,提示中医证候评分可以较客观地综合反映患者的病情。2.经归一化等数据处理后得到2946个基因表达数据。对其中至少三次独立实验处理过程中存在明显的表达差异的数据进行自组图分析分为25类共167个基因,3次表达经重复测量数据的方差分析,差异有显著性意义($P=0.000$),其中有20个基因前后比较差异有统计学意义($P<0.05$):其中人免疫球蛋白K链c区(IGKC)基因,治疗后下调,低于正常人,其余19个基因(Arp2、ARCNI、CRY2、DAF、DPYSL2、FMR1、FYN、GRO3、LFNG、IVNS1ABP、P125、PBEF、PRKCB1、PSCDBP、PTPRE、SCP2、TMEM2、TNF、ZFP103)尽管治疗前与正常对照组比较正常、下调或上调各不相同,但治疗后较治疗前均表现为上调。3.对87个前、中、后三次样品具有相似性表达变化的基因数据进行了聚类分析,下调的有21个,上调的有66个。下调基因主要有免疫应答相关基因(DXS1357E、LY117、IGHG3、IGKC、IGL@、PTPRCAP)及炎症反应相关基因(LY117、ADORA3、MIF、EDG6、CTSH),以及一些细胞凋亡增殖、信号通道及代谢相关等基因(H2AFO、RPL28、FEZ1、EMP3、CAPN4、MIF、DXS1357E、APRT、COX6A1、PLEC1、RPS5、DGKA、NMBR、PTPRCAP)。上调的基因中主要有信号转导相关基因(SHOC2、SEL1L、JAK2、NGB、BIG2、DPYSL2、RGS19、PTGS2、BAI3),免疫炎症相关基因(ILF3、B4-2、PTPRC、KLRC1、CSF2RB、PBEF、GRO3、DAF、SDCBP、TNF、TRA1、SSFA2、HSPA9B、JAK2),转录调控修饰相关基因(B4-2、ZNF9、RYBP、TFEC、ZNF217、SSX2、ATF3、HUMHOXY1、MAF、SMARCA5、LFNG、PTGS2、NS1-BP、EGR2、SF3B1、prpf4、GRSF1),细胞增殖、凋亡与分化相关基因(CLK1、BTG3、IF116、RYBP、TNF、CCNG2、ERCC5、IDN3、CLK1、CDC23),以及生物合成、膜蛋白等(TBCC、DLEU2、ZNF217、PLSCR1、TMEM2、SCP2、CD47、CGGBP1、TACC1、DNM1、EBI2、PIGA、SLC7A6、KIAA1382、PTPRC、IPLA2、FOSB)。4.研究发现阴虚内热证SLE患者治疗前后均有凋亡相关基因的异常表达。治疗前促进细胞凋亡的许多基因明显上调,抑制细胞凋亡的基因则多数表达下调,而治疗3个月后则出现相反的情况,证实细胞凋亡紊乱在SLE的发病中起着重要的作用,糖皮质激素配合滋阴清热方治疗可能可以从凋亡的多个环节对细胞凋亡进行调控,从而改善病情。治疗前后均下调,前后变化不大的有8个凋亡相关基因,其中抗凋亡的基因有:AIM、BLC2、BY55、HSPD1;促进细胞凋亡的有:DR6、PA26;而HUS1与ITK在细胞凋亡中起着重要的调节作用。治疗后较治疗前上调的凋亡相关基因有21个,其中抗细胞凋亡的基因有:SGK、AD022、API5、BAG1、BAG3、BCL6、SGK、VCAM1、BCL2A1、BNIP2、GG2-1、HSPA1B、IL1B、TNFRSF6、IL2RB;促进细胞凋亡的基因有:CASP3、TNF、TNFSF10,以及NKTR、HLA-DQA1、HLA-G、CD83参与体液免疫应答和信号传导等途径参与调节细胞凋亡。5.HLA基因家族等基因有异常表达:HLA-C、HLA-DQB1、HLA-G与HLA-DPB1在SLE中以上调为主,虽然治疗后表达有所下调,但总体仍高于正常人,而HLA-B、HLA-DQA1为下调,治疗后无明显改变,HLA-E治疗后下调,但与治疗前差异无统计学意义($P=0.069$)。HLA-DQB1基因可能是治疗相关基因,治疗后其对应表达的HLA-DQB1蛋白片段量减少。6.治疗前阴虚内热证SLE患者与正常人蛋白表达存在差异:治疗前SLE患者与正常人比较,找出152个蛋白峰,其中73个峰差异有统计学意义($P<0.05$),建立判别中药治疗前SLE患者与正常人的10542 Da m/z和2554 Da m/z蛋白质质谱检测模型,其判别率为100%,可以通过以上两个峰将SLE与正常人区别开来。10542标志物最可能是HLA基因家族相关的多个蛋白片段的复合物。2554Da m/z可能与SLE的发病密切相关,可能是一个红斑狼疮患者发病有关的未知蛋白或多肽片段,有待进一步研究证实。8.阴虚内热证SLE患者治疗前、后蛋白表达存在差异:治疗3个月后与治疗前比较,共发现蛋白峰167个,其中20个差异有统计学意义($P<0.05$)。结论:1.治疗后中医证候积分显著低于治疗前,滋阴清热方可以改善SLE患者的阴虚内热症状。2.阴虚内热证SLE患者细胞凋亡相关、免疫相关等基因表达紊乱,治疗前、中、后许多基因表达有显著改变,提示滋阴清热方可能通过调节患者基因表达紊乱而达到阴平阳秘的治疗作用。对SLE及其他免疫相关疾病的进一步大样本基因聚类分析研究,可能可以建立SLE早期诊断、鉴别诊断及中医不同证候的基因表达模式。3.阴虚内热证患者PBMC蛋白表达模式与正常人有差异,可以通过蛋白表达模式区别正常人和SLE样本,提示可以用蛋白芯片的方法实现SLE的早期诊断,有待进一步研究。4.治疗前、中、后许多蛋白有差异表达,提示滋阴清热方可能通过调节患者PBMC蛋白表达而达到治疗作用。对发现的蛋白质荷峰标志物进一步深入研究,有可能找到治疗相关靶蛋白,值得深入研究。

关键词: 系统性红斑狼疮; 滋阴清热方; 阴虚内热证; 基因表达谱; 蛋白表达谱; SELDI

Study the Dynamic Influence of Nourishing Yin Clearing Heat formula on Gene and Protein Expression of PBMC of SLE with Internal Heat Due to Yin Deficiency

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Abstract Background: Systemic lupus erythematosus (SLE) as a serious autoimmune disease, the etiology, pathogenesis of this disease had not been fully explained and the treatment are still a world's problems. Currently, corticosteroids and immunosuppressants are the main treatments, but the application will bring long-term significant toxicities. Clinical Practice has proved that integrated traditional and western medicine **Methods:** can significantly reduce drug toxicity and improve life quality of SLE patients. Internal Heat due to Yin Deficiency(IHDYD) is the basic types of SLE, Nourish yin, tonify the kidney, clear away heat and suppress fire are the basic rules for the treatment of SLE. Many studies showed that many genes take part in the onset of SLE. Study the dynamic changes of gene regulation networks and protein interaction network of SLE will help us to understand the pathogenesis of SLE deeply. **Objective:** Observed the Treatment Effects of Nourishing Yin Clearing Heat formula(NYCHF) on patients of SLE with Internal Heat due to Yin Deficiency(IHDYD); Studied the dynamic changes of gene and protein expression of the peripheral blood mononuclear cell (PBMC) according to three different stages: Before cure, six weeks and 12 weeks after treatment among 3 months. Try to learn the similarities and differences of gene and protein expression patterns between patients and normal persons, explore treatment mechanism of NYCHF and prepare to establish methods and platform for Chinese medical research for SLE at the level of gene and protein expression. **Methods:** 1. Objects Fifteen IHDYD female SLE patients were 18 to 50 years old, outpatient of the TCM hospital of Guangdong province of China from November 2005 to November 2006, with diagnosis as SLE according to the American Rheumatism Association 1997 revised diagnostic criteria, in line with IHDYD certification standards, and in the mild or moderate activity with the score between 5 to 25 according to SLEDAI system of the United States and Canada SLE center. If had been using glucocorticoids, then must maintain the dosage at 10-25 mg/d within the next three months study. Must withdrawal non-steroidal anti-inflammatory drug over a week and hadn't used immunosuppressant over six months. All of the cases must finish 3 months courses of treatment and record detailed according to Case Report Form. Fifteen normal control were healthy voluntaries from Guangzhou University of Traditional Chinese Medicine with a mean age: 28.4 ±6.50 years. Treatment group had included 18 cases, two cases exodus and one lost, 15 cases were effective, with a mean age:31.60±9.37 years. The age variance of two groups were homogeneity ($P=0.098$), and there were nonsignificant statistically difference between two groups according to two sample t test ($P=0.289$). 2. Treatment and observation, Patients were given five NYCHFs three times per day during three months, and score of IHDYD were got every two weeks. C3, C4, anti-nuclear antibody (ANA), anti-dsDNA, blood routine, urine routine, and other indicators were detected before, 6 weeks and 12 weeks after cured with NYCHF, and PBMC were extracted and stored in refrigerator at -70°C. Only samples of those cases that had finished three months observation were sended to gene or protein microarray chips detect. Case information was collected detailed according to "Case Report Form." 3. DNA microarray chip experiment, cDNA were directly pointed on chips to made the human cDNA microarray chips by the Chipscreen Biosciences, with total genes as 8,064, probes were marked with Cy3 single fluorescent, data were revised standardizd, Calculated the ratio of gene expression in the experimental group and control group. If Ratio was greater than 2 or less than 0.5, there were significant differences between them. GeneCluster 2 software was use to analysis the data with self-organizing maps, functional classification and clustering analysis methods. 4. Protein microarray experiment, Using Surface Enhanced Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (SELDI) methods established the PBMC protein expression data of SLE patients before treatment, six weeks, and 12

weeks after treatment and the normal control group. ZUCIPDAS package designed by Jie-Kai Yu of Cancer Institute of Zhejiang University were used to identify the protein peaks discriminant model. The name and the preliminary function of the protein peaks were identified by Crosslinked comparing between Gene microarray expression database and the Expasy protein database. **Results:** 1. Glucocorticoid with NYCHF can relieve pathogenetic condition of IHDYD SLE quickly, soft self discomfortable sense, decrease scores of SLEDAI and IHDYD significantly, and increase the level of C3 and C4. Scores between IHDYD and SLEDAI was positively correlated which suggested that Scores of IHDYD can reflect the condition of the patient fairly integrally and objectively. 2. 2,946 gene expression data were acquired by using normalized data process methods. 167 genes which has marked differences expression at least one time in three independent experiments were categorized to 25 different patterns and analysis the difference among three stages used the repeat measurements analysis of variance, the differences were statistically significant ($P=0.000$). 20 different genes had statistically significant different ($P<0.05$) expression at three different test times: IGKC expression decreased after cure 12 weeks and lower than the expression of normal control, the expression of another 19 genes (Arp2, ARCN1, CRY2, DAF, DPYSL2, FMR1, FYN, GRO3, LFNG, IVNS1ABP, P125, PBEF, PRKCB1, PSCDBP, PTPRE, Carrier, TMEM2, TNF, ZFP103) were increased, decreased or normal in SLE than in the normal control, but compared with the expression of themselves before cure, they all increased after treatment. 3. Genes with similar changes in the expression of three samples (before, during and after the cure) were analyzed with cluster method, and 87 genes were clustered: 21 genes expressed decreased and 66 genes expression increased after cure than before cure. down expressed Gene mainly related to the immune response genes (DXS1357E, LY117, IGHG3, IGKC, IGL@, PTPRCAP), inflammation related genes (LY117, ADORA3, MIF, EDG6, CTSH), and some cell apoptosis and proliferation, signal pathway and metabolic associate gene (H2AFO, RPL28, FEZ1, EMP3, CAPN4, MIF, DXS1357E, APRT, COX6A1, PLEC1, RPS5, DGKA, NMBR, PTPRCAP). Up-regulated expression genes mainly related to the signal transduction genes (SHOC2, SEL1L, JAK2, NGB, BIG2, DPYSL2, RGS19, PTGS2, BAI3), immune inflammation related genes (ILF3, B4-2, PTPRC, KLRC1, CSF2RB, PBEF, GRO3, DAF, SDCBP, TNF, TRA1, SSFA2, HSPA9B, JAK2), the transcriptional regulation of gene-modified (B4-2, ZNF9, RYBP, TFEC, ZNF217, SSX2, ATF3, HUMHOXY1, MAF, SMARCA5, LFNG, PTGS2, NS1-BP, EGR2, SF3B1, prpf4, GRSF1), cell proliferation, apoptosis and differentiation-related genes (Aug, BTG3, E6, RYBP, TNF, CCNG2, ERCC5, IDN3, CLK1, CDC23), and the biosynthesis and membrane protein related genes (TBCC, DLEU2, ZNF217, PLSCR1, TMEM2, SCP2, CD47, CGGBP1, TACC1, DNM1, EBI2, PIGA, SLC7A6, KIAA1382, PTPRC, IPLA2, FOSB). **Conclusions:** 1. Score of IHDYD symptom decreased significantly along with the time of treatment, suggesting NYCHF can improve the symptoms of IHDYD SLE patients. 2. Apoptosis, immune-related gene expression disorder were found in IHDYD SLE patients before or during treatment, Gene expression patterns change significantly in the three months study. NYCHF may adjust YIN and YANG in a relative equilibrium through regulates the gene expression disorder patients and achieves treatment effect. Further study on SLE and other immune-related diseases use large sample gene clustering analysis may be able to establish early diagnosis, differential diagnosis and build different gene expression patterns of Chinese medicine syndromes. 3. Different protein expression patterns of PBMC were observed between IHDYD SLE Patients and normal samples, Suggesting protein chip can be used to build the method for early diagnosis of SLE, pending further study. 4. Many differences of protein expression observed before, during and after the treatment, suggesting NYCHF may cure IHDYD SLE patients by regulating protein expression of PBMC. Further study on the discovered protein-marker may lead to find treatment-related protein target. It is worth to depth studying.

Keywords: Systemic Lupus Erythematosus; Nourishing Yin Clearing Heat formula; Internal Heat Due to Yin Deficiency; Gene Expression; Protein Expression; SELDI

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中药穴位注射对慢性盆腔炎大鼠免疫功能影响的实验研究

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摘要 目的: 观察黄芪注射液穴位注射对慢性盆腔炎大鼠局部SIgA, 血清IL-6、TNF- α 含量的影响及其病变局部组织形态学变化, 探讨黄芪注射液穴位注射疗法对慢性盆腔炎免疫功能影响。**方法:** 将wistar大鼠随机分成7组, 除正常组和假手术组外, 其余采用20%苯酚胶浆0.1ml注入大鼠左侧子宫造成慢性盆腔炎模型, 黄芪注射液穴注组和生理盐水穴注组选用关元、足三里进行穴位注射; 黄芪注射液肌注组用等量药物在大鼠两侧臀大肌处肌注; 药物对照组用妇科千金片灌胃。观察各组大鼠子宫局部的组织形态学变化, 检测阴道冲洗液中SIgA的含量及血清中IL-6、TNF- α 的含量。**结果:** 黄芪注射液穴位注射可以