会议摘要

目录

Interaction of heat shock protein Cpn10 with Cyclin E/Cdk2 substrate NPAT is involved in regulating histone transcription

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Precise modulation of histone gene transcription is critical for cell cycle progression. As a direct substrate of Cyclin E/CDK2, NPAT is a crucial factor in regulating histone transcription and cell cycle progression. Here we identified that the Cpn10/HSPE, a 10KD heat shock protein, is a novel interacting partner of NPAT. A pool of Cpn10 is colocalized with NPAT foci in nuclei. Gain- and loss-of-function experiments showed that Cpn10 was critical for histone transcription. A conserved DLFD motif within Cpn10 was critical for targeting NPAT and modulating histone transcription. Importantly, knockdown of Cpn10 disrupted the Cajal body-localization of NPAT without affecting Coilin foci formation. In addition, Cpn10 is important for S-phase progression and cell proliferation. Taken together, our finding revealed a novel role of Cpn10 in the spatial regulation of NPAT signaling and disclosed a previously unappreciated linkage between the heat shock protein and histone transcription regulation.

Advances of IDH studies in Cancers

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Abstract:

The point mutations in the active-site arginine residues of isocitrate dehydrogenase (IDH) (IDH1/R132, IDH2/R140, and IDH2/R172) occur frequently in a variety of cancers, including acute myeloid leukemia (AML),

brain tumors, and holangiocarcinomas. The IDH mutants catalyze α-ketoglutarate (α-KG) to oncometabolite 2-hydroxyglutarate (2-HG), which dysregulates a set of α-KG-dependent dioxygenases. Various studies have indicated that IDH mutations are associated with DNA hypermethylation at CpG islands which are enriched for genes implicated in stem cell maintenance/differentiation and lineage specification. We herein summarize the advances of IDH1/2 studies in clinical trials: (1) A rapid, sensitive and robust assay approach (pyrosequencing) has been used to detect all types of mutation in either IDH1 or IDH2; (2) IDH1/2 mutation status could be valuable for distinguishing intracranial chondrosarcomas from chordomas; (3) Due to the prognostic information of IDH1/2 mutations, IDH1/2 has been developed as one biomarker for tumor diagnosis; (4) IDH2 mutation plays a critical oncogenic role in proliferation, apoptosis, invasion, migration, tumorgenesis and senescence, indicating that targeting IDH mutant become a therapeutic strategy in cancers.

Keywords: IDH1, IDH2, mutation, cancers;

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Financial support from the Funding of Zhejiang Provincial Top Key Discipline of Biology, Zhejiang Public Technology Research Program (2014C33234), Science Foundation of Zhejiang Sci-Tech University (14042107-Y), China.

Biocatalytic synthesis of (5*S*)-(4-fluorophenyl)-5-hydroxypentanoic acid by *Candida parapsilosis* ZJPH1305

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Abstract (5*S*)-(4-Fluorophenyl)-5-hydroxypentanoic acid ((5*S*)-FPHPA) is a crucial chiral intermediate for the synthesis of cholesterol-lowering drug Ezetimibe (Zetia®). Enantioselective synthesis of (5*S*)-FPHPA was successfully performed *via* the bioreduction of 5-(4-fluorophenyl)-5-oxopentanoic acid (FPOPA) catalyzed by whole cells of *Candida parapsilosis* ZJPH1305. Strain ZJPH1305 was isolated from soil sample, which could asymmetrically reduce FPOPA to (5*S*)-FPHPA with high enantioselectivity. Based on its morphological and physiological characteristics, Biolog, 26S rDNA sequence and phylogenetic analysis, strain ZJPH1305 was identified and designated as *Candida parapsilosis* ZJPH1305. In order to achieve the maximum biocatalytic

activity for this isolate, the medium constituents were optimized by response surface methodology. The bioreduction of FPOPA catalyzed by *C. parapsilosis* ZJPH1305 cells was subsequently optimized in terms of different reaction parameters. The optimum conditions for the process were found to be: 47.6 mM of FPOPA, 100 g l^{-1} of glucose as co-substrate, reaction at 30 ℃ and 200 rpm for 48 h. Under the above conditions, a best yield of 95.7 % with 99.9 % enantioselectivity was obtained. The results indicated that isolate *C. parapsilosis* ZJPH1305 is a promising biocatalyst for highly enantioselective synthesis of (5*S*)-FPHPA.

Keywords: Bioreduction; *Candida parapsilosis*; Isolation; Identification; (5*S*)-(4-Fluorophenyl)-5-hydroxypentanoic acid

Acknowledgements

This work was financially supported by grants from the National Natural Science Foundation of China (No. 21076193), the Program of "Xinmiao" Talents in Zhejiang Province, China (No. 2015R403073), and the Program of Science and Technology of Zhejiang Province, China (No. 2014C33274).

HSP90 Inhibition Is a Novel Therapeutic Strategy in Gastrointestinal Stromal Tumor

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Abstract: Gastrointestinal stromal tumor (GIST) is the most common sarcoma of the gastrointestinal tract. Oncogenic KIT or PDGFRA receptor tyrosine kinase mutations are compelling therapeutic targets in GISTs, and the KIT/PDGFRA kinase inhibitor, imatinib/sunitinib, is standard of care for patients with metastatic GIST. However, most of these patients eventually develop clinical resistance to imatinib and other KIT/PDGFRA kinase inhibitors and there is an urgent need to identify novel therapeutic strategies. Heat shock protein 90 (HSP90) plays a molecular chaperone role to increase the stability and activity of its client proteins including KIT and PDGFRA receptor tyrosine kinases, which also interacts with HSP70, CDC37, mutated p53, AKT, and HIF1-α proteins to regulate cell proliferation and apoptosis through controlling oncogenic protein proper folding, function, and stability. Inhibition of HSP90 and CDC37 inactivates KIT/PDGFRA and downstream intermediates, and suppresses tumor growth in GISTs. Some HSP90 inhibitors including IPI-504, IPI-493, BIIB021, STA9090, and AT13387 are carrying out clinic trials in GISTs. HSP90 inhibition highlights a novel strategy for

imatinib-sensitive and -resistant GIST, irrespective of the types of multiple imatinib resistance mutations of KIT/PDGFRA.

Keywords: GIST, HSP90, resistance, imatinib

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Financial support from the Funding of Zhejiang Provincial Top Key Discipline of Biology, Zhejiang Public Technology Research Program (2014C33234), Science Foundation of Zhejiang Sci-Tech University (14042107-Y), China.

The Biological Function of Pyruvate Dehydrogenase in Cancers

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Abstract: Pyruvate dehydrogenase (PDH) is the multi-enzyme complex of mitochondrion. Its catalysis is of great importance in the process of energy metabolism. PDH catalyzes the decarboxylation of pyruvic acid to produce acetyl coenzyme A. And acetyl coenzyme A serves as the primary raw material to bring the glucose into the aerobic oxidation of tricarboxylic acid cycle. Due to insufficiency of PDH, human body suffers from lactic acidosis and atelencephalia. In general, the deficiency of PDH manifests dysfunction caused by the *PDH E_1A* gene mutation. The mutation of *PDH E_1A* changes the structure of PDH and reduces the enzymatic activity. PDH inhibition results in the tumor cells to mainly acquire energy through glycolysis. Activation of glycolysis promotes proliferation and inhibits apoptosis in cancer cells. Besides, due to glycolysis, the microenvironment of tumor can protect cells from attack of the host immune system and reduce the chemotherapy drug efficiency as well as help the invasion and metastasis in cancers. The increasing evidence has shown that PDH is expressed in 256 cases of gastric cancer patients with different level, which is associated with the development and invasion of gastric cancer. Compared with adjacent tissues, PDK-3 in colon cancer tissue is upregulated, which inhibits the activity of PDH. PDK-1 knockdown restores the activity of PDH, and inhibits proliferation and invation in head and neck squamous cell carcinoma. Furthermore, inactivation of PDH promotes the development of melanoma driven by

BRAF V600E mutation. Herein, we update the crucial oncogenic roles of PDH in the metabolism and signaling transduction of cancer cells.

Keywords: PDH, Mutation, Glycolysis, Metabolism, Cancer

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Financial support from the Funding of Zhejiang Provincial Top Key Discipline of Biology, Zhejiang Public Technology Research Program (2014C33234), science foundation of Zhejiang Sci-Tech University(14042107-Y). China.

Transcriptome altered by latent human cytomegalovirus infection on THP-1 cells using RNA-seq

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Abstract: Human cytomegalovirus (HCMV) has been recognized as a cause of severe, sometimes life-threatening disease in congenitally infected newborns as well as in immunocompromised individuals. However, the molecular mechanisms of the host-virus interaction remain poorly understood. Here, we profiled the expression of mRNAs and long noncoding RNAs (lncRNAs) in host cell using the emerging RNA-seq to investigate the transcriptional changes during HCMV latent infection. At 4 days post HCMV infection, a total of 169,008,624 sequence reads and 180,616 transcripts were obtained, respectively. Of these transcripts, 1,354 noncoding genes and 12,952 protein-coding genes were observed in Refseq database. Differential gene expression analysis identified 2,153 differentially expressed genes (DEGs) between HCMV-infected THP-1 cells and mock-infected cells, including 1,099 up-regulated genes and 1,054 down-regulated genes. These regulated genes were involved in pathways of apoptosis, inflammatory response and cell cycle progression, all of which may be implicated in viral pathogenesis. In addition, 768 lncRNAs (298 known lncRNAs and 470 novel lncRNAs) were upregulated and 827 (328 known

and 499 novel) were downregulated in infected THP-1 cells. These findings have provided a dynamic scenario of DE candidate genes and lncRNAs at the virus-host interface and clearly warrant further experimental investigation associated with HCMV infection.

MiRNA-33a 通过抑制 Twist1 影响肺癌细胞发生 EMT 和体内肿瘤转移的机制及其作为肺癌预后标记物的作用

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摘要：上皮间质转化(Epithelial mesenchymal transition, EMT)是肿瘤细胞发生转移的关键步骤，可以使上皮细胞转化为运动、侵袭能力较强的间质细胞。本课题主要研究 microRNA 如何通过作用 Twist1 调节 EMT 过程从而影响肺癌的发生以及发现潜在的非小细胞肺癌（Non-small cell lung cancer，NSCLC）预后标记物。首先，我们由划痕实验筛选出高转移肺癌细胞株 NCI-H1299，低转移细胞株 SPC-A-1。用定量 PCR 分析在这两个细胞系中 miR-33a 的表达，结果显示 miR-33a 在 SPC-A-1 中显著高于 NCI-H1299，这说明 miR-33a 在转移性肺癌细胞系中低表达。其次，我们在 NCI-H1299 中过表达 miR-33a，在 SPC-A-1 中干扰 miR-33a，用 Western blot 技术分别检测上皮细胞和间质细胞标记物的表达以及用划痕和 transwell invasion 实验检测细胞的转移和侵袭能力，由正反实验发现 miR-33a 促进上皮标志物的表达且抑制间质细胞标志物和细胞的转移、侵袭能力，这说明 miR-33a 参与 EMT 依赖的转移过程。为了进一步研究 miR-33a 参与 EMT 过程的分子机制，我们用生物信息学网站筛选 miR-33a 的靶向物和诱导 EMT 的基因，筛选出了 *Twist1*。并由定量 PCR 和 Western blot 实验得到 miR-33a 负调控 Twist1 的表达。也通过构建野生型和突变型的 pGL3-Twist1 3’UTR 载体,用双荧光素酶报告实验验证得到 *Twist1* 基因是 miR-33a 的直接靶点。此外，敲除 *Twist1*,能抑制 EMT 相关的转移过程，进一步说明 miR-33a 通过 *Twist1* 参与 EMT 过程。另外，在重度联合免疫缺陷的小鼠中，通过尾静脉注射稳定表达 miR-33a 的高转移肺腺癌细胞株和只表达荧光基因的稳定细胞株，可观察到表达 miR-33a 的动物模型的肺转移瘤结节数显著低于对照组，说明 miR-33a 在体内抑制肺癌的转移过程。最后，在临床样本中检测 miR-33a 的表达发现，与肺癌患者的癌旁组织相比，癌组织中 miR-33a 的表达水平很低且低水平的 miR-33a 预示着生存时间短预后差。上述研究结果共同说明 miR-33a 通过靶向 *Twist1* 抑制 NSCLC 的侵袭和转移过程。因此，miR-33a 可能是一个潜在的预后标志物和抑制 NSCLC 转移的治疗靶点。

关键词：miRNA-33a；上皮间质转化；转移；非小细胞肺癌

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MicroRNA-128 Regulates Cancer Cell Glycolysis by Targeting Phosphofructokinase and Inhibits AKT Phosphorylation

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Abstract: MicroRNAs (miRNAs) affect cancer cell glucose metabolism through targeting mRNAs of diverse enzymes implicated in both oxidative phosphorylation (OXPHOS) and glycolytic pathways. However, the mechanism underlying miRNA regulates glycolysis by targeting phosphofructokinase (PFK), the key rate-limiting enzyme that catalyzes the irreversible step of glycolytic pathway, remains largely unknown. Here, we found that miR-128 directly targets PFK liver type (PFKL), and regulates the endogenous PFKL expression at both mRNA and protein levels. Overexpression of miR-128 decreases the glucose uptake and lactate production, as well as increases the cellular ATP content. Interestingly, miR-128 knockdown promotes lung cancer cell growth and colony formation. In addition, miR-128 expression is inversely correlated with PFKL mRNA level in clinic samples and the upregulated PFKL expression predicts poor overall survival lung cancer patients. Mechanistically, we further showed that there is a feedback loop between AKT signaling pathway and miR-128. Globally, the study suggests that miR-128 acts as a metabolic miRNA and could be used as a potential therapeutic target for lung cancer treatment.

Keywords: miR-128; phosphofructokinase; glycolysis; phosphorylation; lung cancer *Correspondence：Zhaohui Gong, Tel：0574-87600740，Email：gongzhaohui@nbu.edu.cn

Circular RNAs in cancer: novel insights into origins, properties, functions and implications

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Abstract: Circular RNAs (circRNAs) are a large class of RNA that, unlike linear RNAs, form covalently closed continuous loops and have recently shown huge capabilities as gene regulators in mammals. These circRNAs mainly arise from exons or introns, and are differentially generated by back splicing or lariat introns. Interestingly, they are found to be enormously abundant, evolutionally conserved and relatively stable in cytoplasm. These features confer numerous potential functions to circRNAs, such as acting as microRNA (miRNA) sponges, binding to RNA-associated proteins to form RNA-protein complexes and then regulating gene transcription. Importantly, circRNAs associate with cancer-related miRNAs and the circRNA-miRNA axes are involved in cancer-related pathways. Some synthetic circRNAs have shown the remarkable anti-cancer effects. Though circRNAs are ancient molecules, the huge therapeutic potentials of circRNAs are recently being discovered from the laboratory to the clinic. Here, we review the current understanding of the roles of circRNAs in cancers and the potential implications of circRNAs in cancer targeted therapy.

Keywords: circular RNA, microRNA sponge, anti-cancer, targeted therapy

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大鼠腹白线通过电子传递方式传导活性氧自由基？

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研究大鼠体内自由基的代谢和分布，通过尾静脉注射自由基荧光探针的方法对SD大鼠进行活体、全身、胞内的活性氧自由基进行荧光标记，发现大鼠腹白线上出现强烈的自由基荧光信号，推测其具有传导活性氧自由基的功能。在此基础上，为了研究大鼠腹白线传导活性氧自由基的特点和机理，我们通过手术将活体大鼠的腹壁肌层与腹腔分离，并且通过导线分别将大鼠腹腔的剑突和耻骨联合部与腹壁肌层相连，阻碍本体与腹壁肌层传导分子形式的任何物质，只允许电子传递，对比观察腹白线的活性氧自由基分布，发现

只有在离体的腹壁肌层与大鼠腹腔通过导线相连的情况下，腹白线才会显现强烈的自由基荧光信号。进一步比较本体与腹壁肌层不同的连接传导位置，发现离体的腹壁肌层只有通过腹白线上端与大鼠腹腔剑突端相连，下端与腹腔耻骨联合部相连的方式连接，才能显现强烈的自由基荧光信号。实验结果揭示了腹白线通过电子传递方式传导活性氧自由基的可能性，但需进一步从细胞水平和分子水平深入分析加以验证。它的意义在于如果证实主要由结缔组织构成的腹白线与自由基密切相关，这意味着有可能发现结缔组织新的生理功能；如果能够进一步探索研究腹白线传导自由基的现象，研究他的传导方式，证实是一种依赖于电子传递的方式，这有可能是发现生物体内可能存在的针对呼吸代谢所产生的自由基的一套收集、传递、储存和处理的系统的突破口。而这些潜在的发现和成果，可以为生命科学及医学研究的突破提供新的视角。

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转 *2mG2-epsps* 基因水稻种子蛋白质非预期变异分析

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摘要：水稻作为全球最重要的农作物，稻田杂草与水稻争光、争水、争肥，造成水稻减产，甚至绝收。草甘膦是一种高效、广谱除草剂，由于其作用的靶标酶 5-烯醇丙酮莽草酸-3-磷酸合成酶（5-enolpyruvyl-shikimate-3-phosphate synthase, EPSPS) 只存在于植物和微生物中，具有很高的毒理学和环境安全性。抗草甘膦除草剂转基因（genetically modified, GM)水稻新品种的培育对于杂草控制和提高水稻产量有重要现实意义。*2mG2-epsps* 是我国科学家林敏研究员从荧光假单胞菌(*pseudom onas fluorescens*) G2 菌株中克隆后经优化改造获得的耐草甘膦新基因，具有自主知识产权，本课题组前期培育成功的转 *2mG2-epsps* 基因 GM 水稻新品系应用前景广阔。然而，国际社会对 GM 作物及食品的安全性存在广泛争议，公众对 GM 作物非预期变异效应影响人类健康的担忧是 GM 作物商品化应用的主要障碍。本研究以转 *2mG2-epsps* 基因抗草甘膦水稻为材料，根据实质等同性原则，分析转基因（genetically modified, GM)水稻种子可溶性蛋白含量和组分的非预期变异。蛋白含量测定采用 Bradford 法，蛋白组分分析采用 SDS-PAGE 法。结果表明：（1）与非转基因亲本相比，GM 水稻种子的总可溶性蛋白含量极显著降低，但清、球、醇、

谷四种蛋白的最高变异幅度（9.12%）低于 P1 和 P2 两亲本之间的最大差异幅度（14.67%）。这说明 GM 水稻种子的可溶性蛋白含量非预期变异在安全范围内，但营养品质可能与亲本品系存在差异。（2）在蛋白组分方面，GM 水稻株系 T12 和 T22 分别有 12 和 14 个蛋白谱带发生了非预期变异，涉及四种可溶性蛋白，多数表现为含量变化。突出的是，GM 水稻新增加了 41 ku 和 56 ku 谷蛋白组分，需要进一步进行毒理学分析，评价其食品安全性。14-16ku 清蛋白和球蛋白组分包含水稻主要的过敏原蛋白，GM 水稻株系 T22 的 14-16ku 清蛋白含量比亲本品系 P2 增加了 32%，需要进一步严格评估其过敏原性。

关键词：转基因水稻; 种子可溶性蛋白; 实质等同性; 非预期变异

基金项目：国家自然科学基金（项目编号：30871511）;农业部转基因新品种培育重大专项项目（编号：2011ZX08010-003）

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Wnt signaling pathway and GRP78 heat shock protein: targeted therapy of cancer

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Abstract: Glucose regulated protein 78 (GRP78) is a molecular chaperone localized in the endoplasmic reticulum (ER), involved in correct protein folding and assembly, proteasomal degradation of misfolded protein, and ER Ca^{2+} binding. Induction of GRP78 has been recognized as a marker for ER stress and the onset of the unfolded protein response. Besides its location in the ER, GRP78 has been found to be present in cell plasma membrane of some cancer cells, acting as a receptor for a wide variety of ligands. However, its presence on colorectal cancer (CRC) cell surface and its role in CRC metastatic progression remain elusive. Here, we found that GRP78 presented a special clustering distribution on CRC cell surface, and its abundance was strongly associated with CRC differentiation degree. Importantly, we demonstrated the evidence that cell-surface GRP78 could directly facilitate cell migration and invasion through interacting with β1-integrin and the uPA–uPAR protease system, and these effects were independent of its signal-receptor function. Thus these findings provide a novel understanding of surface GRP78's role in CRC metastatic progression and are of considerable therapeutic significance for malignant CRC.

Key words: Wnt signaling pathway , GRP78, cancer

创伤弧菌溶细胞素诱导小鼠原代巨噬细胞 TNF-α 表达和 ROS 产生的机制研究

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摘要：目的 创伤弧菌溶细胞素(VVC)是创伤弧菌重要的毒力。该菌感染起病急，机体抗感染以天然免疫应答为主。为深入了解 VVC 对原代巨噬细胞的影响，本文以重组表达的 VVC（rVVC）体外作用小鼠原代巨噬细胞为模型，研究 VVC 对诱导 TNF-α 表达和细胞氧化应激的作用和机制。**方法** 1）rVVC 经多粘菌素 B（PMB）脱内毒素处理作用小鼠骨髓诱导巨噬细胞(*BMMΦ*)、脾脏和肝脏单核细胞悬液，流式细胞术分析 rVVC 对上述组织中巨噬细胞存活率的影响；2）流式细胞术检测 rVVC 对 *BMMΦ* TNF-α 和 ROS 产生的影响；3）免疫印迹检测 rVVC 对 *BMMΦ* 中 p38、Erk1/2、AKT 和 NFκB p65 等磷酸化的影响；4）Real Time-PCR 检测上述细胞中 TNF-α 等 mRNA 转录水平表达情况；5）信号通路抑制剂对 rVVC 诱导 *BMMΦ*

ROS 升高的影响；6）rVVC 对 TNF-α 敲除小鼠 *BMMΦ* ROS 水平的影响。**结果** 1）rVVC 作用小鼠脾脏、肝脏、骨髓诱导的巨噬细胞生存率均明显下降，呈剂量依赖效应；2）低剂量 rVVC 对 *BMMΦ* 无显著杀伤作用，但能诱导 *BMMΦ* 胞内 ROS 和 TNF-α 表达，并促进 TNF-α mRNA 转录；3）rVVC 上调 *BMMΦ* 胞内 p38 和 NFκB p65 磷酸化水平，其诱导的 ROS 升高可被 p38 和 NFκB 抑制剂阻断；4）TNF-α 敲除 *BMMΦ* 经 rVVC 刺激后，ROS 水平与野生型 *BMMΦ* 比较无显著差异。**结论** **r**VVC 对小鼠原代巨噬细胞具有一定杀伤作用，低剂量 rVVC 以致炎为主。rVVC 能通过 p38-MAPKs 和 NFκB 信号的协同作用介导 *BMMΦ* 内 TNF-α 非依赖的 ROS 升高。本研究将进一步拓展 VVC 的分子致病机理，为进一步阐明其损伤和激活天然免疫细胞的机制提供实验依据。

关键词： 创伤弧菌溶细胞素；巨噬细胞；肿瘤坏死因子-α；活性氧簇

基金项目：浙江省自然科学基金（LY13H190007）

丹参酮ⅡA 调控 CLIC1 保护血管内皮细胞损伤的研究

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摘　要： **目的：**研究丹参酮ⅡA 对 H_2O_2 诱导的人脐静脉内皮细胞（HUVEC）损伤中细胞内氯离子通道蛋白 1（CLIC1）的影响，探讨丹参酮ⅡA 对内皮细胞的保护作用及其机制。**方法：**体外培养 HUVEC 细胞，用 ROS 法确定造成氧化损伤的最适 H_2O_2 处理浓度，分成 6 组：正常组（HUVEC 细胞正常培养 12h）、氧化组（1.1 mM H_2O_2 处理 12h）、siRNA 组（CLIC1 siRNA 转染 24h 后用 1.1 mM H_2O_2 处理 12h）、丹参酮ⅡA 低浓度组（1.1 mM H_2O_2 处理 12h 后用 1.25mg/ml 丹参酮ⅡA 处理 24h）、丹参酮ⅡA 中浓度组（1.1 mM H_2O_2 处理 12h 后用 2.5mg/ml 丹参酮ⅡA 处理 24h）、丹参酮ⅡA 高浓度组（1.1 mM H_2O_2 处理 12h 后用 5mg/ml 丹参酮ⅡA 处理 24h）。检测各组 HUVEC 细胞内丙二醛（Malondialdehyde，MDA）和活性氧（Reactive oxygen species，ROS）的含量、超氧化物歧酶（Superoxide dismutase，SOD）活性的变化，采用荧光定量 PCR 法和 Western Blot 法检测 CLIC1 的 mRNA 和蛋白质表达。**结果：**1.1 mM 的 H_2O_2 孵育 12 h 建立内皮细胞损伤模型，氧化组 ROS 较正常组显著增加（P＜0.01），而 siRNA 组 ROS 较氧化组明显减少（P＜0.01），不同浓度丹参酮ⅡA 作用后，ROS 较氧化组显著减少（P＜0.01）。与正常组比较，氧化组 SOD 活性显著下降，而 MDA 含量显著上升（P＜0.01），siRNA 组 SOD 活性上升，MDA 含量下降（P＜0.05），而经丹参酮作用后，与氧化组对比，SOD 活性上升，MDA 含量下降（P＜0.05）。与正常组比较，氧化组 CLIC1 mRNA 和蛋白质表达增加，siRNA 组表达下降，而用丹参酮处理后，CLIC1 mRNA 和蛋白质表达随丹参酮浓度升

高而降低。**结论：**HUVEC 细胞氧化损伤过程中 CLIC1 表达上调，siRNA 干预 CLIC1 表达和丹参酮ⅡA 处理后均能显著降低受损内皮细胞内 ROS 和 MDA 含量，并提高 SOD 活性，表明抑制 CLIC1 表达和丹参酮ⅡA 均能显著增强 HUVEC 抵御氧化损伤的能力，而且丹参酮ⅡA 处理后细胞内 CLIC1 mRNA 和蛋白质表达下调，表明丹参酮ⅡA 可能通过下调 CLIC1 减少内皮细胞损伤。

关键词：丹参酮ⅡA；CLIC1；内皮细胞；氧化损伤

$PM_{2.5}$ 对 HUVEC 细胞蛋白质组的影响

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摘　要：目的：应用双向电泳及质谱技术研究 $PM_{2.5}$ 对人脐静脉内皮细胞蛋白质组的影响。**方法：**双向电泳技术分离 HUVEC 细胞和经过 50μg/mL $PM_{2.5}$、100μg/mL $PM_{2.5}$ 处理的 HUVEC 细胞蛋白质，经银染后扫描得到蛋白质组图谱，用 Image Master2D 6.0 软件进行差异蛋白质组分析，质谱鉴定差异表达的蛋白质，采用流式细胞术检测各组 HUVEC 细胞中 ROS 含量和细胞凋亡情况，Western Blot 方法检测 DNA 损伤修复相关基因 Mer11A、R50 和 R51 表达水平。**结果：**相比于正常组，经 $PM_{2.5}$ 处理后的两组内皮细胞中 CAPNS1、ACTBL2、TPD52、RAB14、UPRT、TRMT112、HNRNPH1、CRK、FILIP1、TTLL8、EXOC6B、ENO1 的表达量上升；ARHGDIA、DYNC1H1、FGG、ABHD、HSPA4、FBX4、ZSCAN5A、MPP2、MYL12A、PSMA3、NCAM2、PSMB2、ZC3H7B、ST20、ZNF812、DUT、RCN1、MDH1B 表达量下降。Western Blot 法特异性检测显示，ENO1 和 HNRNPH1 蛋白质表达量均上升，与蛋白质组分析的结果一致。50 μg/mL PM2.5 和 100 μg/mL PM2.5 暴露下 HUVEC 中 ROS 含量显著增加，早期凋亡和晚期凋亡细胞占总细胞数比例提高，Mer11A、R50 和 R51 等 DNA 损伤修复相关蛋白等表达也分别有所增加。**结论：**$PM_{2.5}$ 通过影响内皮细胞中相关蛋白的表达，引起内皮细胞的损伤，进而促进心血管疾病的发生发展。

关键词：$PM_{2.5}$；HUVEC；蛋白质组

Tespa1 regulates TCR-induced calcium signal through recruiting inositol 1,4,5-triphosphate (IP3) receptors

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T cell antigen receptor (TCR) ligation induced the formation of TCR proximal LAT signalosome complexes,

recruiting PLC-γ1, GRB2, Themis, SLP76, subsequently resulting in signal transduction, including mitogen-activated protein kinases, protein kinase C pathways and calcium flux. Tespa1, a newly- identified adaptor protein, is also recruited to the LAT signalosome and interacts with PLC--γ1 and GRB2. Here we proved that Tespa1directly interacted with the X-catalytic domain of PLC--γ1. We also determined that Tespa1 was a direct partner of IP3R1, the vital calcium channel on ER, this direct interaction brought IP3R1 closely to the TCR complexes. Interestingly, we found that Tespa1 improved the tyrosine phosphorylation of IP3R1 on the tyrosine 353 by Fyn, which preferred locates on the plasma membrane close to the TCR complexes. It has been reported that IP3R1-Y353 phosphorylation enhanced Ca^{2+} entry in B lymphocytes, which indicated the key role of Tespa1 in regulating the calcium signaling in T cells. Our findings demonstrate how Tespa1 involves in the LAT signalosome formation as well as the calcium signaling induced by the TCR engagement.

创伤弧菌溶细胞素对小鼠肝脏免疫细胞的细胞毒性差异

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摘要：目的 研究重组创伤弧菌溶细胞素(rVVC)对小鼠肝脏自然杀伤细胞（NK）、自然杀伤 T 细胞（NKT）、$CD4^+$T 细胞、$CD8^+$T 细胞和 B 细胞存活的影响，比较 rVVC 对肝脏中不同免疫细胞的毒性差异。**方法** 利用不同密度 Percoll 分离液提取小鼠肝脏单个核细胞，再经重组表达的 rVVC 体外作用 6h，采用流式细胞术结合 TCRβ、NK1.1、CD1d-tetramer、CD4、CD8、B220 和 live/dead 等相应的荧光抗体（或染料）染色分析 rVVC 对肝脏 NK、NKT 细胞、T 细胞和 B 细胞存活的影响。 **结果** PBS、0.25HU/ml、0.5HU/ml 或 0.75HU/ml rVVC 体外作用 6h 后，NK 细胞的存活率为 65.3%、64.8%、63.4%和 59.3%，NKT 细胞存活率为 50.0%、40.5%、32.8%和 20.2%，肝脏单核细胞中 $CD4^+$T 细胞存活率为 80.9%、80.2%、62.1.0%和 16.1%，$CD8^+$T 细胞存活率为 88.8%、88.5%、86.2%和 43.3%。相比之下，B 细胞对 rVVC 的细胞毒性较为敏感，0.5HU/ml 作用时的存活率仅为 34.5%。**结论** rVVC 的细胞毒性对肝脏 NK 细胞、NKT 细胞、$CD4^+$T 细胞、$CD8^+$T 细胞和 B 细胞具有一定选择性差异，NK 细胞、NKT 细胞和 $CD8^+$T 细胞对 rVVC 的细胞毒活性耐受度明显高于 $CD4^+$T 细胞和 B 细胞，其中以 NK 细胞对该毒素细胞毒杀伤作用最不敏感。

关键词：创伤弧菌溶细胞素；细胞毒；肝脏；免疫细胞

基金项目：浙江省自然科学基金（LY13H190007）

Progranulin A9D mutation functionally leads to the cytoplasm retardation of angiogenin

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Abstract: Neurodegenerative diseases are progressive neurological disorders with selective neuron loss in particular regions of brain, including frontotemporal lobar degeneration (FTLD), Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), and many others. Gene defects have been recognized as a prominent factor in the etiology and pathogenesis of neurodegenerative diseases. To date, hundreds of genetic variants that located in dozens of genes have been associated with susceptibility to various neurodegenerative diseases. Among them, more than 60 mutations in *progranulin* (*PGRN*) have been reported in FTLD, AD, ALS and PD. One of the mutations in *PGRN* gene exon 1 introduces a charged amino acid in the hydrophobic core of the signal peptide at residue 9 (PGRN A9D), and results in cytoplasmic mis-sorting of this protein. However, the mechanism of actions of this mis-sorting remains elusive. To address this issue, we first examined the subcellular distribution of PGRN with A9D mutation in human neuronal-like cells (SH-SY5Y). The results have shown that PGRN A9D mutation failed to undergo maturation and accumulated in the stress granules. Interestingly, this mutation also induces redistribution of cellular angiogenin (ANG), a stress response factor and neurodegenerative diseases related protein, from nuclear to cytoplasm. PGRN was also directly interacted with ANG in stress granules. Furthermore, the cytoplasmic retardation of ANG by PGRN A9D mutation abolished ANG-enhanced rRNA transcription and cell survival. Taken together, these results indicate that PGRN with A9D mutation changed localization of ANG as well as cell growth and survival, suggesting that PGRN and ANG act in concert to regulate the progress of neurodegenerative diseases.

Functional analysis of ALS- and PD- associated mutations in the signal peptide of angiogenin

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Abstract: Gene defects have been recognized as a prominent factor in the etiology and pathogenesis of neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS; sporadic and familial) and Parkinson's disease (PD). Loss-of-function mutations in angiogenin (ANG), a member of the secreted and vertebrate-specific ribonuclease superfamily, have been identified in ALS and PD. The majority of mutations locate in the coding region of ANG that affect its structure of the catalytic sites and thus impair its neuroprotection function. A

minority of mutations in the signal peptide (SP) domain of ANG have also been reported in both groups, but with unknown pathophysiologic relevance. Currently, it is known ANG is a neuron-secreted factor that is endocytosed by astroglia and then plays a neuroprotection role. Here, we studied the differences of translation efficiency and secretion level of ANG between wild-type ANG and nine reported SP mutations in human neuronal-like cell lines. The expression level of ANG was significantly increased in four variants (P-4S, P-4Q, G-8D and G-10D) compared with wild-type, but declined in other four variants (V-12A, F-13S, F-13L and M-24I). Interestingly, the A-1P mutant affected the ANG signal peptide cleavage and produced three different types of mature peptide. Functional assays showed that these ANG SP mutations resulted in deficiency of neuron cell proliferation, survival or migration. Our findings imply that the expression level and the signal peptide cleavage of ANG may play a role in the pathogenesis of ALS and PD.

Tumorigenic activity of human ribnuclease-4 in gliomas

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Abstract: Malignant gliomas are the most common and aggressive primary brain tumors with an extremely poor prognosis and limited treatment options. Although substantial efforts have been devoted into glioma management, effective treatment is still lacking. Therefore, it is urgent to investigate the pathogenesis of malignant gliomas and seek for novel therapeutic targets. Through analysis of 430 cases of human gliomas with different disease stages, we found that the expression of human ribonuclease-4 (RNASE4) was positively correlated with disease progression, with higher level of RNASE4 in higher degree of gliomas and poorer survival rates. RNASE4 is the fourth member of the secreted and vertebrate-specific ribonuclease superfamily. It has been revealed that RNASE4 played a role in neuroprotective activity through promoting angiogenesis, neurogenesis, and neuronal survival under stress. However, the molecular mechanism of RNASE4 in glioma progress remains to be elucidated. Here we have identified that RNASE4 was necessary for the proliferation of glioma cells *in vitro* and xenograft tumor growth, angiogenesis and metastasis in nude mice. We also found that the tumor-promoting effect of RNASE4 depended on its ribonuclease activity, suggesting the importance of the ribonucleolytic activity in the progression of gliomas. Since RNASE4 is a secreted protein and can be easily targeted, our finding would shed light on the development of novel therapeutic strategy of malignant gliomas.

miR-141 acts as an anti-angiomiR through multiple targets in angiogenin induced angiogenesis

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Abstract: Angiogenesis is a key event in tumor growth and metastasis. Angiogenin (RNase-5, ANG), one of angiogenic factors, can induce angiogenesis and promote tumor growth. However, its underlying mechanism of action is poorly understood. MicroRNAs (miRNAs), a class of highly conserved noncoding small RNA molecules that repress target gene expression post-transcriptionally, are believed playing regulatory roles in angiogenesis and tumorigenesis. To investigate ANG-responsive and angiogenesis-related miRNAs, we did a miRNA array screening and found miR-141 was down-regulated by ANG. Functional analysis showed miR-141 could inhibit HUVEC proliferation, migration and tube formation *in vitro*, as well as angiogenesis *in vivo*. Rescue assay indicated that ANG enhanced tube formation *in vitro* in miR-141 overexpressed HUVECs, suggesting that ANG could induce angiogenesis through down-regulation of miR-141. To explore the molecular mechanism of miR-141 in inhibition of angiogenesis, six target genes of miR-141, which related to angiogenesis, were screened out by *in silico* analyses. Further RT-qPCR, western blot and dual-luciferase report assays identified that four out of the six genes were targets of miR-141. Moreover, we found ANG could regulate the expression of these target genes by down-regulation of miR-141. Thus, our study elucidate a novel mechanism of ANG in regulation of angiogenesis through down regulating a miRNA – miR-141.

MicroRNA-409-3p suppresses colorectal cancer invasion and metastasis partly by targeting GAB1 expression

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Abstract: Colorectal cancer (CRC) is one of the most common cancers worldwide and its metastasis accounts for the majority of deaths. However, the molecular mechanisms underlying CRC progression are not well characterized. In this study, we identified miR-409-3p as a tumor suppressor of CRC. MiR-409-3p expression was significantly down-regulated in CRC tissue compared to adjacent non-tumor tissue, and reduced miR-409-3p expression was correlated with CRC metastasis. *In vitro* and *in vivo* studies revealed that miR-409-3p negatively regulated CRC metastatic capacities, including suppressing cancer cell migration, invasion, and metastasis. To

explore the mechanism of action of miR-409-3p, we adopted a pathway and pathophysiological event-based target screening and validation approach, and found 9 known metastasis-related genes as potential targets. The 3'-UTR binding assays between the candidates and miR-409-3p suggested that only *GAB1*, *NR4A2*, and *LMO4* were directly regulated by the miRNA. However, endogenous expression analysis revealed that only *GAB1* was modulated by miR-409-3p in CRC cells at both the mRNA and protein levels. Furthermore, we provided evidence to conclude that *GAB1* was partially responsible for miR-409-3p-mediated metastasis. Taken together, our data demonstrate that miR-409-3p is a metastatic suppressor, and post-transcriptional inhibition of the oncoprotein GAB1 is one of the mechanisms of action of this miRNA. Our finding suggests miR-409-3p might be a novel target for CRC metastasis treatment.

Identification of translational initiation changes during nutrient starvation

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Abstract: Cells have evolved exquisite mechanisms to fine-tune the rate of protein synthesis in response to nutrient starvation, including alternative translation initiation. However, we do not know exactly how cells select alternative initiators during different growth conditions. Using our recently developed global translation initiation sequencing (GTI-seq) approach, we have observed a group of transcripts exhibiting altered aTIS initiation relative to upstream TIS (uTIS) under starvation. The majority of genes with reduced aTIS were involved in the process of transcription. Most of genes with increased aTIS were involved in the protein catabolic process, in particular autophagy system and ubiquitin/proteasome system. The favored production of these gene products under acute starvation condition apparently helps recycling of intracellular amino acids by promoting protein breakdown. Our results suggest a rapid translational control via TIS selection during starvation.

Ultrafine particles induced pulmonary fibrosis by affect autophagic flux and targeting lysosome.

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Abstract: Recently,concerns about the inhalation risks of ultrafine particles (UFP) have increased with the continued development of new nanotechnologies and air pollution. Limited evidences have shown UFP could induce pulmonary fibrosis, the corresponding mechanism is frequently contributed to ROS and inflammation. However, accumulating evidence indicated that the extent of alveolar epithelial cell (AEC) injury and lack of sufficient repair are critical determinants of pulmonary fibrosis. Whether and how UFP impairs AEC and induces pulmonary fibrosis remains elusive. Here, firstly, we found UFP triggered pulmonary fibrosis in vivo, meanwhile, we showed that UFP induced autophagosome accumulation was accompanied by unchanged MTOR. Furthermore, we investigated the cellular and molecular mechanism of UFP-induced autophagy in AEC by focusing on autophagic flux. Autophagic flux inhibition which is proved by increasing p62 is due to decreased lysosomal degradative capacity rather than affecting autophagosome-lysosome fusion. Further morphological and functional analysis indicated that UFP significantly impaired lysosomal acidification leading to the decreased degradative capacity, the conclusion also is supported by rescuing lysosomal acidification could enhancing autophagic flux. Finally, rescuing lysosomal acidification might protect AEC from apoptosis, a key process of ACE in pulmonary fibrosis. These date suggested that UFP inhibited autophagic flux via targeting lysosomal acidification, and thus regulated apoptosis of AEC, and this may be critical to the development of pulmonary fibrosis associated with UFP.

Mobile phone signal exposure induces transient DNA damage in mouse embryonic fibroblasts

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Abstract: Radio frequency electromagnetic fields (RF-EMF) have been classified as possible carcinogen to human, however, this conclusion of IARC is based on limited epidemiological findings and lack of solid supports from experimental studies. Especially, there are no consistent data regarding the RF-EMF-induced DNA damage. To systematically address the debate on whether RF-EMF exposure results in DNA damage, here we investigate the effects of 4 W/kg 1800 MHz RF-EMF exposure on DNA damage in wide-type (Atm+/+) and ataxia telangiectasia mutated (Atm-/-) mouse embryonic fibroblasts (MEFs) by comet assay. In Atm+/+ MEFs, we found that RF-EMF exposure can induce DNA single-strand strand breaks (SSBs) at 1 hour exposure, but, instead of increased, the DNA damage were quickly repaired and reduced by activated single-strand repair as the exposure time extended. In Atm-/- MEFs, we found that RF-EMF exposure can induce both DNA SSBs and double-strand

breaks (DSBs) at 12 hours exposure, but also, the induced DNA damage were repaired and reduced by the activated DNA repair. Our results suggest that, under our conditions, 1800 MHz RF-EMF exposure is not able to induce persistent DNA damage in Atm+/+ or Atm-/- MEFs.

The effects of RF-EMF on genome stability of nervous system

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Abstract: Currently, there are about 7 billion mobile subscriptions around the world which accounts for 96% of the population, and the people are exposed to the Radio Frequency Electromagnetic Fields (RF-EMF) emited by the mobile phone day and night. Therefore, public concerns are dramatically raised during the past decade. Based on the epidemiological finding about the association between mobile phone use and brain tumors, the International Agency for Research on Cancer (IARC) has classified RF-EMF as human 2B carcinogens. However, the data available from laboratory studies neither provide consistent corroborating evidence nor suggest mechanisms of action to support or clarify this epidemiological finding. We propose to investigate the weak biological effects induced by low-intensity of RF-EMF exposure using systemic strategies with more sensitive methods. In this study, we adopted γH2AX foci formation, the most sensitive method currently available for detecting DNA double stand breaks (DSB) in cells, to systematically observe the effect of GSM 1800 MHz RF-EMF at SAR of 4.0 W/kg on the main experimental cell models of nervous system, including neuroblastoma cells (SH-SY5Y), human glioma cells (A172 and U251), primary cerebral cortex neurons, and microglia cells. After exposed for 1 h, 6h or 24 h, the cells were subjected to γH2AX foci formation analyses. The data analyses revealed there were no significant differences between the sham and RF-EMF exposure groups in all the examined cells, suggesting the RF-EMF exposure under current condition does not induce significant DNA DSB damage.

Transcriptional activation of follistatin protects pulmonary epithelial cells against silica nanoparticle-induced oxidative stress

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Abstract: The wide use of silica nanoparticles (SiNPs) has raised serious concerns about their safety for human health. Evidence has shown that SiNP exposure causes toxicity in respiratory system. Meanwhile, human cells launch adaptive responses to overcome SiNP toxicity. However, besides a few examples, the regulation of SiNP-responsive proteins and their functions in SiNP response remain largely unknown. In this study, we demonstrated that SiNP induced the expression of follistatin (FST), a stress responsive gene, in mouse lung tissue as well as in human epithelial cells (A549). The induction of FST by SiNP was due to the transcriptional activation of FST gene, as evidenced by the increase of active gene markers, Ac-H3(K9/18) and H3K4me2 at FST promoter region. Down-regulation of FST promoted SiNP-induced apoptosis both in cultured cells and in mouse lung tissue. Furthermore, knockdown of FST increased while overexpression of FST decreased the expression level of NADPH oxidase 1 (NOX1) and NOX5 as well as the production of cellular reactive oxygen species (ROS). Taken together, these findings demonstrated a protective role of FST in SiNP-induced oxidative stress and shed light on the interaction between SiNPs and biological systems.

Genetic diversity and relationships of medicinal *Chrysanthemum morifolium* revealed by Start Codon Targeted (SCoT) markers

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Abstract: *Chrysanthemum morifolium* plants are important commercial herbs in China, widely used in ornamental horticulture and traditional medicine. In this study, start codon targeted (SCoT) polymorphic markers were applied to assess genetic relationships among 32 populations of *Chrysanthemum morifolium*. Thirty-two SCoT primers produced 872 loci, 90% (785) of which showed Polymorphism. Average polymorphism information content of the SCoT primers was 0.987 (0.950–0.993), showing that plenty of genetic diversity exists among Chinese *Chrysanthemum morifolium* populations. Cluster analysis of SCoT markers (based on UPGMA and PCoA method) grouped the 32 populations of *Chrysanthemum morifolium* into two clusters. The partition of clusters in

the dendrogram and PCoA plot was similar and large degree of grouping according to their origin and ecological distribution. Our results will provide much more useful information for resource protection and will also useful to improve the current plant breeding programs. Our results also indicated that SCoT markers are informative and could be used to evaluate genetic diversity of Chinese medicinal *Chrysanthemum morifolium* varieties.

Keywords: *Chrysanthemum morifolium*; Genetic diversity; SCoT markers

Acknowledgments

This study was supported in part by the National Natural Science Foundation of China (No. 31470407), the Zhejiang Provincial Natural Science Foundation of China (LQ13H28006), the Zhejiang Provincial Public Welfare Technology Applied Research Foundation of China (2014C32090).

铁皮石斛器官特异转录组测序及分析

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摘要: 兰科石斛属植物，特别是铁皮石斛（*Dendrobium officinale*），是我国及东南亚部分国家十分有名的民间传统中草药。其干燥、加工、炮制后的茎是主要的入药部位，被称为“枫斗”。迄今为止，石斛中的许多药用活性成份，例如石斛多糖、石斛碱、黄酮类物质，被研究人员分离发现，并被认为可能是“枫斗”具有药用滋补功效的主要生化活性成份，而这些活性成份往往与石斛属植物的次生代谢过程相关。分子水平的遗传学研究是解析石斛有效药用成份产生、积累过程中的调控机制的有效途径之一。然而，缺乏石斛“组学”数据成为该项研究的阻碍。高通量测序技术的广泛应用使得我们有能力对石斛的基因组、转录组进行高效、低成本的大规模探测分析。前不久，国内相关研究团队结合二代、三代测序，对铁皮石斛的基因组进行了测序、拼接。然而遗憾的是，拼接结果并不尽如人意，得到的是数以万计的骨架（scaffolds）；而且根据其发表论文中提供的链接，读者无法批量下载这些骨架序列用于后续相关研究。

在本研究中，我们将 RNA、小分子 RNA（small RNA，sRNA）、降解组（degradome）三种测序技术结合起来，对铁皮石斛根、茎、叶、花 4 种不同器官进行了系统的转录组学研究。基于 RNA 测序数据，我们拼接得到了 536,558 个转录本（可以归类到 299,107 个 unigenes）。随后，我们对所有转录本的器官特异表达模式进行了分析，发现有 2,645 条转录本在花器官中高表达，256 条转录本在根中高表达，42 条转录本在叶中高表达，以及有 54 条转录本在茎中高表达。基于 sRNA 测序，我们发现 2,038、2、21 和 24 个

小分子 RNAs 分别在花、根、叶和茎中特异表达。此外，累计 1,047 个候选 microRNAs 被高通量测序所探测到。基于测序数据以及二级结构预测，我们从拼接得到的转录本中发现了数十个 microRNA 前体。有趣的是，在其中两个前体的“茎”结构上发现了连续相位分布的小分子 RNAs；这些小分子 RNAs 的加工过程得到了 degradome 测序数据的支持。我们对 1,047 个候选 microRNAs 进行了全转录组范围的靶基因预测；基于 degradome 测序数据的验证，我们获得了 1,257 对可靠的 microRNA--target 调控关系（其中包含了 147 个 microRNAs 和 276 条靶转录本）。我们从中发现了一些器官特异的调控关系。基于上述调控关系，我们建立了 4 个具有生物学研究意义的调控子网络，它们分别涉及激素信号转导、植物生长发育、次生代谢以及 microRNA 产生作用途径中的重要因子 Argonaute 1 相关的调控。总结来说，我们的转录组学分析结果为铁皮石斛的遗传学、分子生物学及有效药用成份深入发掘研究提供了极具价值的基础数据。

酸浆属植物苦蘵 P450 家族基因克隆及功能研究

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苦蘵（*Physalis angulata* L.），为茄科（Solanaceae）酸浆属（Physalis）一年生草本植物，广泛分布与海拔 500-1500 米的我国南方地区。作为一种有着悠久历史的药用植物，许多中药地方志都对苦蘵的药用性状及价值有着详细的描述。利用现代医学的研究手段，人们发现苦蘵的酸浆苦素和谷甾醇等化学成分，具有显著的抗宫颈癌和皮肤癌活性，这为大规模利用及开发苦蘵奠定了结实的理论基础。分析苦蘵的次生代谢途径，克隆代谢途径的关键酶编码基因，可加速改良苦蘵的遗传性状，提高苦蘵的药用价值。

Physagulin R 是新近发现的一种高效抗癌物质，它的生物合成途径和代谢网络成为当前酸浆属植物苦蘵研究的热点之一。通过对 Physagulin R 代谢途径的分析，我们解析了三个重要的代谢合成途径关键步骤：1) 3β-hydroxysteroid dehydrogenase (3β HSD); 2) P450 supfamily; 3) Epoxide hydrolase families。其中，P450 基因超级家族，成员众多，功能多样，结构复杂，并广泛参与植物次生代谢的各个分支，是研究苦蘵药用成分合成的突破口。我们利用前期高通量转录组测序数据，分析筛选得到了 85 个苦蘵 P450 编码基因的候选 Unigene。通过分子生物学技术，成功了分离并克隆得到了两个 P450 编码基因 CYP1A-1 (comp101263_c0) 和 CYP1A-2 (comp127702_c0)的全长序列，并初步研究了它们的生物学功能。烟草叶肉细胞瞬时表达研究表明，苦蘵 CYP1A-1 和 CYP1A-2 蛋白定位于内质网和细胞质内，具有典型的生物酶催化活性。在进化上，CYP1A-1 和 CYP1A-2 分属两个不同的分支，序列差异较大，可能具有不同的功能。

通过进一步的遗传转化实验，将为我们深入研究 CYP1A-1 和 CYP1A-2 的功能提供线索，同时为苦蘵

次生代谢产物及其代谢网络的遗传解析提供实验证据。我们的工作将有助于抗癌活性产物 Physagulin R 生物合成途径人工调控和药用优良品种选育等相关研究工作的开展。

红豆杉琥珀酰化蛋白组学研究

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曼地亚红豆杉 (Taxus X media)，是一种天然杂交品种，其母本为日本红豆杉(T.cuspidata)，父本为欧洲红豆杉(T.baccata)，在美国、加拿大生长发展已有近 100 年的历史。曼地亚红豆杉的植株各部分均含有紫杉醇，枝叶含量达 0.03%以上，根系部分可达 0.06%，全株都可用于提取紫杉醇，植株利用率高，其深度加工后综合经济价值更高。然而，曼地亚红豆杉种子萌发困难，植株再生与扩繁率低，这些都极大的限制了曼地亚红豆杉规模化种植，造成了紫杉醇价格居高不下。近年来的研究表明，琥珀酰化通过调控代谢相关酶的活性，进而影响植物代谢产物的合成和降解。研究红豆杉蛋白组琥珀酰化，有助于我们揭示紫杉醇合成代谢途径的调控机制，为更好的开发利用红豆杉奠定基础。

我们提取了曼迪亚红豆杉树皮的总蛋白，纯化后进行了高分辨率 LC-MS/MS 分析。分析结果表明，在 193 个红豆杉蛋白中鉴定得到了 325 个赖氨酸琥珀酰化位点。通过生物信息学手段，我们详细的进行了红豆杉琥珀酰化蛋白组的基本注释，功能分类，亚细胞定位和聚类分析。所有的琥珀酰化蛋白分别属于不同的 GO 功能组，其中 138 个蛋白具有代谢功能，120 个蛋白具有催化活性，95 个蛋白可能参与胞内代谢过程。亚细胞定位为我们提供了更多的蛋白功能研究辅助信息。分布最多的三类细胞器：92 个琥珀酰化蛋白定位于叶绿体，51 个琥珀酰化蛋白定位于细胞质中，23 个琥珀酰化蛋白定位于线粒体中。我们还特别分析了琥珀酰化对四种重要的代谢途径的影响。研究表明，有 4 个糖酵解途径，5 个丙酮酸代谢途径，9 个 TCA 循环和 14 个碳固定代谢途径相关蛋白酶具有的琥珀酰化位点，是琥珀酰化调控潜在的靶蛋白。这些结果为我们深入研究曼迪亚红豆杉紫杉醇代谢途径提供了分子生物学基础。

酸浆属植物苦藠转录组测序研究

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苦蘵（*Physalis angulata* L.），为茄科（Solanaceae）酸浆属（Physalis）一年生草本植物，别名炮仔灯、灯笼草、天泡子、天泡草、黄姑娘、小酸浆、朴朴草、打额泡等。广布于全世界，常生于海拔 500～1500 米。在我国广泛分布于华东、华中、华南及西南等地。苦蘵以果、根或全草入药，早在秦汉时期的《尔雅》中就有记载，很多中药材地方志中均有对其描述，其性味苦、寒，主治咽喉肿痛、腮腺炎、急慢性气管炎、肺脓疡、痢疾、小便不利和外用治脓泡疮等。现代医学研究表明苦蘵主要有醉茄内酯、酸浆苦素、黄酮、生物碱及甾醇等化学成分，其中酸浆苦素和谷甾醇具有多种体内外的抗癌活性，对于宫颈癌和皮肤癌都具有明显疗效。苦蘵果实中的多糖还对超氧阴离子自由基和二苯代苦味酰基自由基起抗氧化作用，其提取液具有抗菌消炎、降血糖、降血脂等疗效。除了药用功效外，苦蘵还被用作高档的营养保健“本草水果”，在美容养颜、提高人体肌体免疫力、延缓衰老、预防心脑血管病等许多方面都有良好的功效；也可以做罐头和蜜饯，国外常用来制作沙拉酱汁，风味独特，是深受广大消费者喜爱的绿色珍品。

转录组作为连接基因组和蛋白组的纽带，是基因功能、结构和表达差异研究的基础，可以在不依赖于基因组参考序列的基础上，以高通量测序技术为平台进行全基因组转录水平研究。与传统构建 cDNA 文库再利用 Sanger 法测序获得 EST 序列相比，可以获得通量更大、覆盖范围更广、精度更高的全基因组转录本信息。我们对苦蘵根、茎、叶、花、果进行了高通量转录组测序（测序平台：Illumina GAIIX；测序方法：Paired-end RNA-seq，每个样本包含 3 次生物学重复），有效 reads 混合后使用软件 Trinity 进行 *de novo* 拼接，得到 506, 412 个转录本（平均长度 810 bp，N50 为 1, 406 bp）和 176, 834 个 Unigene（平均长度 618 bp，N50 为 918 bp）。基于 GO（Gene Ontology）注释，我们筛选得到了 2, 371 个 Unigene 参与次生代谢过程，其中的 538 个 Unigene 参与次生代谢产物生物合成。

通过转录组测序，为苦蘵次生代谢产物相关基因及其代谢网络的遗传解析奠定了基础，有助于活性产物生物合成途径解析、分子标记开发和辅助育种等相关研究工作的开展。

Development of perfect microsatellite markers for medicinal chrysanthemum (“Hang-ju”) (*Chrysanthemum morifolium*)

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Abstract

• ***Premise of the study:*** Microsatellite markers were developed in medicinal chrysanthemum ("Hang-ju") (*Chrysanthemum morifolium*), which is widely used for treating common cold (wind-heat), headache, and dizziness; however, the origin and effectiveness of these microsatellite markers were not subject to quality control. ***Methods and Results:*** Using the protocol for the development of expressed sequence tag (EST)-derived simple sequence repeat (SSR) markers, twelve primer pairs were developed for microsatellite loci and the rate of polymorphism was tested in 32 individuals of "Hang-ju" (Xiangyiju) germplasm from Tongxiang, Zhejiang province, China. Results showed the mean number was 6.25 alleles per locus were studied, and the expected heterozygosity (H_e) per locus ranged from 0.5448 to 0.7639. • ***Conclusions:*** These markers will be useful in various applications such as construction of linkage maps, identification of markers linked to different traits, and assessment of genetic variability, which would aid medicinal chrysanthemum crop improvement.

Keywords: *Chrysanthemum morifolium*, "Hang-ju", microsatellite, expressed sequence tag (EST)

Acknowledgements

This study was supported in part by the National Natural Science Foundation of China (No. 31470407), the Zhejiang Provincial Natural Science Foundation of China (LQ13H28006), the Zhejiang Provincial Public Welfare Technology Applied Research Foundation of China (2014C32090).

RNA-mediated Suppression of RNA Silencing

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植物可利用RNA沉默来抵抗病毒侵染。病毒侵染植物后，会诱导RNA沉默抗病毒机制，降解病毒RNA，从而产生大量病毒来源的小RNA分子（virus-derived small interfering RNAs,vsiRNA），vsiRNA可进一步介导病毒RNA的降解和/或抑制病毒基因的表达。而在与植物长期共进化过程中，病毒通过编码一个或多个RNA沉默抑制子（viral suppressor of RNA silencing,VSR）来抑制植物的基因沉默，从而逃脱植物的这种防卫反应。近年来，针对VSRs作用机制的研究表明，VSRs通常是以蛋白质的形式通过与植物基因沉默通路中的RNA或者关键蛋白分子相互作用，干扰植物正常的基因表达调控，来抑制植物对病毒的抵抗。而我们发现了一个VSR蛋白，它与马铃薯Y病毒编码的RNA沉默抑制子HC-Pro具有相似性，同样能够抑制RNA沉默；

但其无义突变体不能翻译成蛋白质，只能以RNA的形式发挥作用，仍然可以抑制RNA沉默，打破了我们对RNA沉默抑制子的常规认识。

SNX10 plays an essential role in macrophage bacteria clearance via regulation of phagosome maturation

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SNX10 belongs to the sorting nexin (SNX) family, which contains the PX domain and involved in protein trafficking. SNX10 does not have other reported domain except the PX domain, which is a phosphoinositides (Pls) binding domain. Previous reports suggested that SNX10 is localized to the Rab5 positive organelles, and the overexpression of SNX10 induces the formation of giant vacuoles in mammalian cells. Since most giant vacuoles induced by SNX10 overexpression are Rab7 positive, SNX10 might promote late endosome maturation. Further studies showed that SNX10 interacts with the V1D subunit of V-ATPase, involved in V-ATPase assembly and transport and regulate the function of V-ATPase. In addition, SNX10 is required for osteoclast formation and bone resorption, which raises the possibility that SNX10 could interact with the osteoclast ATPase. As an ATP drive enzyme, V-ATPase plays an important role in the innate immune system. It regulates the pH of organelles such as phagosomes, endosomes and lysosomes and influences the recycling of receptors after endocytosis as well as the digestion of lysosomes. During the infection of bacteria, the acidification and maturation of phagosomes plays a key role in the killing and elimination of bacteria. We have discovered that the expression of SNX10 in macrophages was upregulated after the stimulation of gram positive or negative bacterias. SNX10-deficient mice are more susceptible to the infection of bacterias. Further studies revealed that SNX10 does not affect the phagocytosis of macrophages while it exerts great influence on the killing of bacteria via the regulation of the acidification and maturation of phagosomes.

蛋白磷酸酶 PP2A 在 T 细胞发育中的功能及作用机制研究

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T 淋巴细胞是机体抵抗外界病原体的重要免疫细胞，其发育过程也一直是国际免疫学界所关注的热点

之一。T 细胞的发育受到了 TCR 信号的调节，其中蛋白磷酸酶能够去磷酸化 TCR 下游的信号蛋白，发挥了重要的开关作用。PP2A 亚家族属于丝苏氨酸（Ser/Thr）蛋白磷酸酶，主要成员有 PP2A、PP4 和 PP6。已有研究表明，在 T 细胞中特异性缺失 PP4 可以导致小鼠的胸腺 T 细胞阳性选择受阻，同时下游的 PLC-γ1 和 Erk 信号也都减弱。PP6 在 T 细胞中能通过抑制 TCR 信号从而负向调节其发育过程。而 PP2A 在 T 细胞发育中的作用目前尚未有报道。PP2A 是一类广泛表达且非常重要的异质三聚体丝苏氨酸磷酸酶，为了进一步研究 PP2A 在 T 细胞发育分化和活化中作用，构建了 PP2A 条件性缺陷小鼠，对小鼠的表型分析显示，T 细胞中特异性缺失 PP2A 则导致其早期发育阶段明显受阻，胸腺双阳性 T 细胞、CD4 和 CD8 T 细胞比例和数目都明显下降，外周的 T 细胞比例和数目也同步降低。提示 PP2A 在 T 细胞的发育过程中发挥重要的作用。本课题组将继续深入研究 PP2A 影响 T 细胞发育的具体阶段及其如何调控 T 细胞早期发育的机制，希望进一步完善我们对 T 细胞发育中分子事件的认识。

基于 96 孔过滤板的高通量方法及人血白蛋白分离应用

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传统的蛋白层析分离过程开发主要依靠手动或者全自动的层析系统，通过一系列的优化实验来确定合适的分离条件，开发过程需要消耗大量的料液、介质和缓冲溶液，同时过程繁杂，时间成本高，因此构建更加高效的高通量方法已成为过程开发的关键热点。本文以 Q Sepharose FF 介质分离人血白蛋白（HSA）为模型，采用 96 孔过滤板建立高通量过程开发（High-Throughput Process Development，HTPD）方法，评价和优化分离条件，实现酵母发酵液中分离 HSA。

首先，比较了蛋白吸附等温线测定的不同方法，在相比（液相体积/固相介质体积）保持不变的前提下，验证了 **200**μl 过滤板、2mL 离心管、25mL 三角瓶三种操作模式的一致性，表明 96 孔过滤板模式的可靠性，确定了合适的操作条件：介质 14μl，溶液 200μl，相比 15，搅拌转速 1500rpm，平衡时间 3h。其次，按照 DOE 设计理念，采用 96 孔过滤板考察了不同 pH 和 NaCl 浓度对 HSA 吸附的影响，结果表明在 pH 5.5 醋酸盐缓冲液和 pH 8.5 Tris-HCl 缓冲液中，Sepharose FF 介质的 HSA 吸附容量高，饱和吸附量大于 140 mg g^{-1} 湿介质，可作为上样吸附条件；在磷酸盐缓冲液（pH 6.5，0.2 M NaCl）和醋酸盐缓冲液（pH 4.0, 0.1 M NaCl）中，介质对 HSA 的吸附量仅 40 mg g^{-1} 湿介质，可作为洗脱条件。最后，采用 96 孔过滤板实现了高通量的吸附-洗脱实验，用于酵母发酵液液中分离 HSA，优化了上样和洗脱条件的影响，SDS-PAGE 分析表明可实现 HSA 的高效分离，进一步比较了填充柱分离效果，验证了基于 96 孔过滤板的高通量层析分离过程开

发方法的可靠性。

结果表明，采用基于 96 孔过滤板的吸附条件筛选和层析分离过程优化，显著提高了过程通量，降低了蛋白料液和实验耗材（介质和溶液），减小了过程优化时间，提高了过程开发效率。若进一步引进自动配液和加样处理的操作平台，可显著提高过程通量，实现快速高效的过程开发。

关键词：高通量过程开发，吸附，层析，分离，人血白蛋白

疏水性电荷诱导型扩张床吸附介质制备及抗体分离研究

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扩张床吸附（Expanded Bed Adsorption，EBA）是一种集成化的生物分离新技术，可以直接从含有生物质等固体颗粒的料液中捕获目标物，避免了固液分离等前处理，简化步骤，提高分离效率。疏水性电荷诱导层析（Hydrophobic Charge-Induction Chromatography，HCIC）是一种新型的混合模式层析分离方法，在中性 pH 条件下通过疏水作用吸附蛋白，改变 pH 以静电排斥作用实现蛋白洗脱，具有吸附容量高、选择性好、耐盐性强等特色，可减少料液稀释或加盐等处理，适合于生物初分离。本文将 HCIC 和 EBA 两种技术相结合，实现疏水性电荷诱导的扩张床吸附分离，以人 IgG（hIgG）和牛 IgG（bIgG）为分离目标，探讨新技术在抗体分离中的应用潜力。

首先，将两种 HCIC 配基，4-巯乙基吡啶（MEP）和 5-氨基苯并咪唑（ABI），偶联于高密度的碳化钨/琼脂糖复合微球（扩张床基质）上，制备了两种 HCIC 扩张床介质，T-MEP 和 T-ABI。考察了两种介质对 hIgG 和 bIgG 的静态和及动态吸附能力，结果表明 IgG 吸附容量高，pH 影响显著，最佳吸附范围为 pH 7.0~9.0，酸性条件可实现解吸，且具有较好的耐盐吸附特点。层析柱蛋白穿透实验结果表明，T-ABI 比 T-MEP 具有更高的动态载量，适合用于高流速的扩张床吸附。以混合蛋白为模型料液（2 mg/ml IgG 和 10 mg/ml BSA），考察了上样 pH、洗脱 pH、膨胀率和上样量等因素对 T-ABI 扩张床分离抗体的影响。结果显示，对于 hIgG， pH 7.0 上样，pH 4.0 洗脱，2 倍膨胀率为合适的分离条件；对 bIgG，pH 8.0 上样，pH 3.5 洗脱，2 倍膨胀率更为合适。最后，考察了两个实际分离体系，牛乳清（含 0.62 mg/ml bIgG，约占总蛋白 4.7%）中分离 bIgG，以及动物细胞培养液单抗发酵液（含 0.65 mg/ml hIgG，约占总蛋白 13.1%）分离单克隆抗体，优化分离条件，均取得良好的分离效率。对于牛乳清分离，bIgG 纯度 90.6%，收率 78.2%，纯化因子为 19.3；对于细胞培养液分离，单抗纯度 93.7%，收率 79.4%，纯化因子为 7.2。

结果表明，HCIC 和 EBA 相结合，形成疏水性电荷诱导型扩张床吸附分离新技术，充分集成了两种分离方法的优势，显著提高了分离效率，体现出抗体分离应用的良好前景。

关键词：扩张床吸附，疏水性电荷诱导层析，5-氨基苯并咪唑，单抗，牛乳清，IgG

混合模式介质对人 IgG 及 Fab 和 Fc 片段的吸附选择性研究

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混合模式层析（Mixed-mode Chromatography，MMC）是一种新型的生物分离技术，其配基兼有两种或两种以上的功能基团，可以与目标物产生多种相互作用。通过调节洗脱缓冲液 pH，可改变蛋白和/或介质的电荷特性，利用静电排斥作用协助洗脱，提高洗脱效率。混合模式介质的配基密度较高，吸附容量大，且具有非盐依赖特性，已在抗体分离纯化中得到应用，不过对于抗体及其片段的分离选择性仍有待深入研究。本文选择典型的商业化混合模式介质 MEP HyperCel，以及三种实验室自主设计的新型介质为考察对象，以人 IgG 及其 Fab 和 Fc 片段为分离对象，分析不同 pH 条件下不同介质的吸附选择性。

首先，考察了不同 pH 条件下 MEPHyperCel 和实验室自制 MMI、ABI、WABI 四种混合模式介质对 IgG 的吸附行为。发现 pH 影响显著，不同介质体现一定的差异。当 6.0≤pH≤8.9 时，MEP、MMI 和 ABI 三种介质可结合 90%以上的 IgG，WABI 介质达到同样效果的 pH 范围为 5.0~7.0。当 pH4.0 时，除了 WABI 介质仍有 12%IgG 吸附，其它三种介质均基本不吸附 IgG。然后，考察了不同 pH 条件下四种介质对 IgG 片段 Fab 和 Fc 的结合情况，分析不同介质的吸附差异。结果表明，对于 ABI 介质，在 pH 4.0~8.9 范围内，对 Fab 和 Fc 都吸附；对于 MMI 介质，在 pH 5.0 时吸附 20% Fab，不吸附 Fc；对于 MEP 介质，在 pH 4.0~8.0 之间对 Fab 和 Fc 均有吸附，而 pH 8.9 时仅 Fab 吸附 90%以上，对 Fc 几乎不吸附；对于 WABI 介质，在 pH 8.0~8.9 时，对 Fc 不结合，但能结合 80%以上的 Fab。因此，选择 MEP 和 WABI 介质，优化吸附和洗脱 pH，可实现 Fab 和 Fc 的有效分离。

结果表明，不同的混合模式配基具有不同的分子结构，提供了抗体及其片段分离的选择性差异，优化选择合适的混合模式介质和 pH 条件，可以实现人 IgG 及其 Fab、Fc 片段混合物的分离。

关键词：混合模式层析，IgG，抗体，抗体片段，分离

新型混合模式层析介质制备及人血白蛋白分离研究

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混合模式层析（Mixed-mode Chromatography，MMC）是一种新型的生物下游分离技术，配基兼有多种功能基团，可以通过疏水相互作用吸附蛋白，静电排斥作用协助洗脱，已在抗体等蛋白的分离纯化中得到应用。同时，混合模式介质具有显著的耐盐吸附特性，可以从较宽离子强度的料液中直接捕获目标蛋白，避免对料液的预处理，如稀释、加盐等步骤，提高分离效率，减少分离步骤。本文以色胺为 MMC 配基，制备一种新型的混合模式层析介质，以人血白蛋白（HSA）为分离对象，考察 pH 和盐浓度对蛋白影响，并应用于毕赤酵母发酵液中分离重组人血白蛋白(rHSA)。

首先，考察了介质制备过程中反应 pH 和配基添加量的影响，发现配基密度随反应 pH 升高而增大；配基添加量为 3 倍双键密度时，配基密度达最高；优化制备条件，得到了高配基密度的 MMC 介质，配基密度达到 150 μmol/g。其次，考察了不同 pH 和盐浓度对 HSA 吸附的影响，结果表明 pH 对 HSA 吸附影响较大，pH5 时饱和吸附容量最高，达到 141.3 mg/g；pH4 时，吸附容量显著下降；pH 大于 5 时，随 pH 增大吸附容量逐步下降。添加 NaCl，饱和吸附容量随盐浓度升高逐渐下降，但体现一定的耐盐吸附特性，当 NaCl 达 1.0 M 时吸附量为 70.5mg/g；添加$(NH_4)_2SO_4$的影响略有不同，随盐浓度升高，饱和吸附容量呈先下降后升高的趋势。最后，将色胺介质用于分离毕赤酵母发酵液中的 rHSA，发现 pH 影响与 HSA 类似，料液稀释对 rHSA 吸附量影响较小，优化分离过程，设计了两步阶跃洗脱，pH7.0 洗脱去除小分子杂质（如色素），pH4.0 洗脱得到 rHSA，电泳纯度 87.0%，收率 90.8%。

结果表明，以色胺为配基的新型 MMC 介质具有良好的 HSA 分离能力，具有较高的吸附容量，较好的耐盐特性，发酵液无需稀释和 pH 调节，即可直接层析分离，分离效率高，体现出良好的应用前景。

关键词：混合模式层析，生物分离，人血白蛋白，色胺

共聚焦激光扫描显微镜辅助混合模式介质内蛋白吸附研究

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蛋白质的吸附行为是吸附性层析的关键之一，主要研究方法集中于间歇吸附和柱穿透实验，均是通过分析液相中蛋白浓度的变化，以表征介质中的蛋白吸附情况，因此所获得的信息是宏观吸附的结果，难以

反映介质颗粒内部蛋白质传递和吸附的真实情况。近年来，共聚焦激光扫描显微镜（Confocal Laser Scanning Microscopy，CLSM）为代表的微分析技术，已成功应用于介质内蛋白吸附行为研究。本文采用 CLSM 分析，以人免疫球蛋白（hIgG）和牛血清白蛋白（BSA）为模型蛋白，考察混合模式介质（Nuvia cPrime 和 KB-ABI）的吸附和解析过程，分析介质内蛋白分布和变化，探讨 pH 影响和不同蛋白的竞争性吸附。

首先，对蛋白进行了荧光标记，考察了不同 pH 下单组分蛋白的吸附行为，实现了荧光强度和宏观吸附容量的定量关联。对于 Nuvia cPrime 介质，在结合作用较强的条件下（hIgG@pH 6.0；BSA@pH 5.0），表现出“收缩核”吸附现象，介质中蛋白吸附区和非吸附区具有明显的界限，呈现以孔扩散控制为主的传质行为；在相对较弱的吸附条件下，介质中蛋白吸附区和非吸附区界限不明显，存在孔扩散和表面扩散共同作用的复杂过程。对于 KB-ABI 介质，hIgG 和 BSA 都是以孔扩散为主，表面扩散为辅，但 BSA 的表面扩散更为明显。进一步采用双荧光标记，考察了双组分蛋白的共吸附行为。对于 KB-ABI 介质，发现 40 min 时 BSA 的绿色荧光明显增强，反映两种蛋白之间存在竞争性取代。最后，考察了蛋白质的解吸和顺序吸附，发现两种介质中蛋白分子都是首先从介质内部开始解吸，可能介质表面与蛋白具有更强的结合力。顺序吸附结果表明，Nuvia cPrime 介质中两种蛋白均呈现出“层层递进”的吸附方式，而 KB-ABI 介质中 BSA 基本类似，但 hIgG 表现为“跨越式吸附”，即后吸附的 hIgG 分子会跨越先前与介质结合的 hIgG，再与功能配基结合。

结果表明，借助 CLSM，可以直观地观测荧光标记蛋白在介质中的传质和吸附过程，分析液相条件的影响，评价不同蛋白的竞争性吸附过程，深入了解层析介质的吸附和解吸机制，促进分离过程优化和新型介质开发。

关键词：共聚焦激光扫描显微镜，混合模式介质，吸附机制，人免疫球蛋白，牛血清白蛋白

等温滴定量热法测定 4-巯乙基吡啶与蛋白的分子相互作用

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疏水性电荷诱导层析（Hydrophobic Charge-Induction Chromatography，HCIC）是一种新型的生物分离技术，已在抗体分离中得到应用。HCIC 配基兼有离子交换和疏水基团，可以与目标蛋白产生复杂的相互作用，提高选择性；且配基所带电荷随 pH 变化而变化，可通过调节溶液 pH 值，实现蛋白的高效洗脱。此外，HCIC 吸附具有耐盐特性，适用于较宽的离子强度范围。本文以 4-巯乙基吡啶（MEP）为典型的 HCIC 配基，采用等温滴定量热法（Isothermal Titration Calorimetry，ITC）测定配基—蛋白结合过程的热变化，

分析焓变、熵变和自由能变化等热力学参数，探讨 HCIC 的分子相互作用本质。

考察了 MEP 与三种免疫球蛋白（人免疫球蛋白 hIgG，牛免疫球蛋白 bIgG 和单克隆抗体 mAb）和两种白蛋白（人血清白蛋白 HSA 和牛血清白 BSA）的相互作用。ITC 结果表明，MEP 与五种蛋白间的相互作用包含了静电作用和疏水性相互作用，其中疏水相互作用占主导地位。MEP 与免疫球蛋白的相互作用要强于白蛋白，这与 MEP HyperCel 介质对 IgG 的饱和吸附量较高、解离常数较小的实验结果一致。进一步考察了 pH 和盐浓度变化对 MEP-IgG 相互作用的影响。ITC 实验表明，随着 pH 降低，MEP 与 IgG 相互作用的焓变值变大，说明 MEP 与 IgG 间的静电相互作用显著增强，这种静电排斥作用导致了在酸性 pH 条件下 MEP 介质对 IgG 的解吸。随着盐浓度的增加，MEP 与 IgG 间的静电作用先有所增加然后下降，而疏水相互作用则先下降后上升，因此 MEP-IgG 总相互作用的变化趋势较小，体现了 MEP 介质吸附蛋白的耐盐性本质。

综上所述，通过等温滴定量热法测定和分析了 MEP 配基和 IgG 分子之间的分子相互作用，阐述了 HCIC 的三个典型特性：多模式相互作用提高结合的选择性，静电相互作用导致吸附的 pH 依赖性，以及静电/疏水相互作用互补产生耐盐结合，该结果为深入了解 HCIC 和分离过程优化提供了一定依据。

关键词：等温滴定量热法，疏水性电荷诱导层析，分子相互作用，免疫球蛋白 G

中国樱桃 *PpcERF* 基因克隆与表达特性分析*

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ERF类转录因子是AP2/EREBP超家族重要成员之一，广泛参与植物生长发育及非生物胁迫应答等过程。本研究从中国樱桃(*Prunuspseudocerasus*)转录组文库中获得了一个ERF类转录因子编码基因，命名为*PpcERF*。该基因开放阅读框长度为1059 bp，编码 352个氨基酸残基，分子量约为39 kD。含有单一、保守的AP2/ERF结构域。聚类分析发现该基因与碧桃*ERF*基因具有较近亲缘关系，在拟南芥基因数据库中比对后发现与*ERF5/6*相似性较高，推测*PpcERF*基因与拟南芥*ERF5/6*基因具有相似功能。

采用 Real-time PCR 技术对 *PpcERF* 基因在不同处理条件下的表达特性进行分析表明，在 H_2O_2 和 PEG2000 处理下，*PpcERF* 基因的表达量分别呈先上升后下降趋势；在 4℃处理下，*PpcERF* 基因表达下调；在低温诱导樱桃花芽休眠解除过程中，低温积累达到 236 C.U 时，表达量达到最高，随后下降；在 ABA 和 NaCl 处理下，*PpcERF* 基因的表达量变化不明显，综合以上结果，我们认为 *PpcERF* 基因可能参与氧化

* 基金资助：浙江省自然科学基金(LQ13C150001)和浙江省新品种选育重大专项(2012C12904-6)
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胁迫、干旱胁迫以及低温胁迫响应，本研究为进一步研究该基因功能奠定了基础。

Isolation and Characterization of PpcERF Transcription Factor cDNA of Chinese Cherry (Prunuspseudocerasus)

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ERF are transcription factorsand are important members AP2/EREBP super gene family, and play important roles in development and abiotic stress responding in plant. An encoding sequence of ERF was identified from transcriptome library of Chinese cherry (*Prunuspseudocerasus*), named *PpcERF*. The structure and expression of*PpcERF*be analyzed toreveal the functionof PpcERF. The ORF of *PpcERF* was 1059 bp, which encoded 352 amino acids (aa). The PpcERFcontained one conservative AP2/ERF domain. The cluster analysis revealed that PpcERF have closer genetic relationship to *Prunuspersica*. PpcERF and ERF5/6 have high similarity after aligning with Arabidopsis gene database, suggested that *PpcER*F might have similar functions with *ERF5/6* of Arabidopsis.

The expression characteristion of *PpcERF* under different processing conditions was analyzed as indicated by qRT-PCR analysis, the expression level of *PpcERF* displayed significantly up-regulated during H_2O_2 and PEG2000 treatment, and dropped off again. The results showed that was a trend that decline after rising first under treatment. The expression level of *PpcERF* was suppressed by 4℃. During transition of flower bud dormancy, the expression of *PpcERF* peaked at 236 C.U while the change of expression level of *PpcERF* was not obviously under ABA and NaCl treatment. The results shown above indicated that *PpcERF* might response to drought stress, oxidative stress and low temperature stress.

蓝莓 Lon2 蛋白酶对植物衰老的作用

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摘要：植物衰老往往伴随着过氧化物酶体功能紊乱，Lon2 蛋白酶作为定位于过氧化物酶体的 Lon 蛋白酶家族成员，对过氧化物酶体的功能和结构的维持可能具有重要作用，但目前未见相关报道。本研究在研究蓝莓 Lon2（*VcLon2*）表达模式的基础上，克隆蓝莓 Lon2 基因全长，并通过 VIGS 和烟草转基因技术对该基因功能进行研究，以揭示蓝莓 Lon2 蛋白酶对植物衰老的作用。结果表明，*VcLon2* 在幼叶、幼果等细嫩器官中的表达水平显著高于成熟器官，暗示着 *VcLon2* 与植物衰老关系密切。经克隆测序后发现，*VcLon2*

cDNA 全长 3165 bp，编码 888 个氨基酸，进行系统发育分析发现其与苹果和葡萄 Lon2 蛋白酶同源性最高。在表型方面，NbLon2 沉默本氏烟植株表现为矮小、叶片小且黄化严重，而 *VcLon2* 超表达本氏烟则生长茁壮且叶片鲜绿。同时，*NbLon2* 沉默植株各叶绿素含量仅为对照的 50%，膜脂过氧化程度显著高于对照；而 *VcLon2* 超表达植株则呈相反的变化趋势。此外，*NbLon2* 沉默植株蛋白羰基化程度显著高于对照，植物衰老标记基因 *SAG12* 表达量高达对照 15642.2 倍。以上结果表明，Lon2 蛋白酶缺失显著加速植物衰老进程，包括加剧光合色素降解、破坏细胞膜系统、羰基化蛋白大量积累等；由此可见，Lon2 蛋白酶对有效降低 ROS 对细胞膜的伤害程度、维护光合相关蛋白的功能、植物健康的维系及衰老的延缓具有重要作用。

The Function of Lon2 Protease in Plant Senescence

Abstract: Plant senescence always parallels peroxisome dysfunction, and Lon2 protease plays an important role in maintaining the function and structure of peroxisomes. In the present study, after analyzing the express pattern in different parts of blueberry (*Vacciniumcorymbosum*L.), Lon2 gene was cloned from blueberry and named *VcLon2*, then the biological functions of *VcLon2* were studied through rapid reverse (VIGS) and forward genetic techniques, which aimed to to understand its roles in plant senescence. The results showed *VcLon2* was primarilly expressed in the younger organs, such as young leaves and young fruits, which indicated it is closely related to senecence in plant. The results of sequence showed that the full-length of *VcLon2* cDNA is 3165 bp, encoding 888 amino acids which shows highest homology with apples and grapes Lon2 protease by Phylogenetic analysis. Phenotypically, *NbLon2* silence *N.benthamiana* plants showed pygmyism with small and serious yellowing leaves, while *VcLon2* induced-expression *N.benthamiana* plants grew well with bright green leaves. Meanwhile, chlorophyll content in NbLon2 silence plants was only 50% of control plants, and the membrane lipid peroxidation wasmore serious than the control; on the contrary, conversed chang trend were observed in VcLon2 induced-expression plants. In addition, protein carbonylation degree in NbLon2 silence plants were significantly higher than the control, and the expression level of *SAG12* was 15642.2 times as the level in control. Our data suggest that Lon2 protease missing significantly accelerates the pace of plant senescence, including accelerating degradation of photosynthetic pigment, destruction of membrane system and accumulating carbonylated protein etc. It may also be decuced that Lon2 protease plays an important role in preserving photosynthesis associated proteins, maintenance healthy and delaying senescence in plants.

Production of catechindimers, trimer and polymers through enzymatic oxidation

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Abstract: Tea is one of the most popular beverages worldwide. The major components of green tea include (−)-epicatechin (EC), (−)-epicatechin -3-O-gallate (ECG), (-)-epigallocatechin (EGC) and

(-)-epigallocatechin-3-gallate (EGCG). The typical pigments in black tea are theaflavins (TFs) and thearubigins (TRs), which are formed by oxidation of catechins during fermentation processing. Polyphenoloxidase (PPO) and peroxidase (POD) are two key enzymes in pigment formation during the process of black tea. Many studies have been conducted to reveal how PPO and POD catalyze the formation of dimers, trimer and polymers from tea catechins. However, there were few reports to summarize catalytic reaction of PPO and POD until now. The present review summarized the studies regarding the transformation of catechins to different kinds of dimers, trimers and polymers in various conditions.

Key words: Catechins, Enzymatic oxidation, Dimer, Trimmer, Polymer

Structure-function relationships of tea polysaccharides

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Abstract: The worldwide consumption of tea is second only to water. Tea polysaccharides have various biological activities including anti-blood coagulation, anti-radiation, antioxidant, reducing blood sugar levels, hypoglycemic activities, anti-HIV,anti-cancer, cell–cell communication, cell adhesion and molecular recognition in the immune system, which are strongly associated with their chemical structures. A variety of studies have showed that most of tea polysaccharides are glycoconjugateswith a polypeptide backbone,in which a protein carries one or more carbohydrate chains via N- or O-linkages. It is of great interestand importance to elucidate the relationship among chemical structures, chain conformations and biological activities of tea polysaccharides. However, tea polysaccharides are usually composed of various monosaccharides linked by different glucosidic bonds, and have hyperbranched structures. Moreover, tea polysaccharides often have high molecular weights, and tend to form aggregates in solution that mask the behavior of individual macromolecules. Therefore, characterization of the chemical structures and chain conformations of polysaccharides is not easy. This article attempts to review the current development on structural and conformational characterization of some tea polysaccharides, and introduce the **analytical methods** including FTIR, NMR, CD and AFM.

Keywords: Tea polysaccharides, Chemical structure, Chain conformation, Biological activity

Synergistic biological activities of tea polyphenols with othersubstances

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Abstract: Co-treatment with phytochemicals or drugs is a promising approach to enhance the chemoprevention and chemotherapy in some diseases. Combination of compounds may overcome multidrug resistantance, decrease the effective dose of individual drug, reduce side effects and achieve clinical success. Tea (*Camellia sinensis (L.) O. Kuntze*, *Theaceae*) is second only to water in popularity as a beverage in the world, and its health benefits are mostly attributed to tea polyphenols. This review has summarized the synergistic effects of tea polyphenols with other phytochemicals or drugs on antioxidation, antimicroorganism, anticancer, improvement of neurodegenerative diseases and other functions including protection from nephrotoxicity, inhibition of sporozoite survival, promotion of hematopoietic stem cell proliferation, improvement of bone mass and weight maintenance. The synergism of tea polyphenols with other compounds indicates that combination of tea polyphenols with other agents is a promising approach for treatment of some diseases. In addition, the synergistic bioactivities of tea polyphenols with other phytochemicals or nutrients may overcome the poor bioavailability of tea polyphenols *in vivo*, and provide a new explanation for the health benefits of tea.

Keywords: Synergism, Tea polyphenols, Bioactivity, Antioxidation, Anti microorganism, Anticancer, Neurodegenerative diseases

Essential Role of mTORC1 in Self-renewal of Murine Alveolar Macrophages

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Alveolar macrophages (AMΦ) are the most abundant immune cells residing in terminal airways, where they play important functions in lung development, integrity, surfactant metabolism, and host defense responses, rendering them prominent targets for therapeutic intervention. The traditional view that AMΦ belong to the mononuclear phagocyte system with bone marrow-derived monocytes as developmental intermediates has been

recently challenged. Accumulating evidence has recently indicated that many tissue resident macrophages, including AMΦ, are derived from embryonic precursors, and are self-maintained with minimal contribution from circulating bone marrow-derived precursors in steady states. Fetal monocytes, as AMΦ precursors, seed into the lung prior to birth, expand massively, and then develop into mature AMΦ during the first week after birth. These differentiated AMΦ persist through adulthood via proliferative self-renewal independent of circulating monocytes. However, under certain conditions, such as bone marrow transplantation after lethal irradiation, AMΦ can be replenished from bone marrow-derived monocytes, which serves as an emergency pathway of AMΦ ontogeny. During AMΦ development, they undergo profound changes of the surface profile, which are characterized by increased expression of CD11c, Siglec-F, F4/80, and CD64, and concomitantly down-regulation of CD11b. Local environmental signals, such as granulocyte-macrophage colony-stimulating factor (GM-CSF), instruct AMΦ via PPAR-γ to acquire such signature phenotypes and functions. Moreover, GM-CSF is also required for AMΦ maintenance in promoting proliferation. Although emerging evidence highlights proliferative self-renewal as the main mechanism for AMΦ maintenance in both steady state and under stress conditions, mechanisms that link mitogenic stimuli, such as GM-CSF, to proliferative renewal programming, remain largely unknown.

Proliferating animal cells must tightly coordinate cell-cycle progression with cell growth and proliferation associated bioenergetic demand. Mechanistic target of rapamycin complex (mTOR), a highly conserved serine-threonine kinase, serves a key sensor for metabolic cues to regulate cell growth and proliferation. mTOR forms at least two known distinct complexes, mTOR complex 1 (mTORC1) and mTORC2. mTORC1 contains mTOR, Deptor, mLST8, PRAS40 and the adapter protein Raptor, and is sensitive to the immunosuppressant rapamycin. mTORC1 acts downstream of the PI(3)K-Akt-Tsc1/2 pathway to phosphorylate translational regulators, the S6 ribosomal kinase (S6K), and the translation initiation factor 4E binding protein 1 (4E-BP1). Subsequently, S6K phosphorylates the ribosomal protein S6 to promote ribosomogenesis. Furthermore, activation of mTORC1 promotes the downstream anabolic processes, such as glycolysis, by activating the transcriptional factors *Hif1α* and *c-Myc*, as well as *de novo* lipid biosynthesis via up-regulating SREBPs, while suppressing catabolic processes such as autophagy. As such, essential roles of mTORC1 and its tight regulation by TSC1 have been demonstrated to regulate both innate and adaptive immunity. While inhibition of mTORC1 can reduce pro-inflammatory cytokine production and M1 polarization by macrophages, constitutive mTORC1 activation due to Tsc1 deletion leads to enhanced pro-inflammatory responses and macrophage M1 polarization, but resistance to IL-4-induced M2 polarization and endotoxin-tolerance. Despite extensive progress in our understanding of the

role of mTORC1 in macrophage function, the importance of mTORC1 signaling in the development or maintenance of macrophages is rarely reported.

We have made the following findings:

1. Myeloid specific Raptor deficiency leads to selective disruption of AMΦ homeostasis: Loss of mTORC1 did not impair initial establishment of the AMΦ pool; Rather, it caused impaired maintenance of this population of cells; Raptor/mTORC1 was selectively required for AMΦ homeostasis and had minimal role in the maintenance of other macrophages.
2. RptorΔmyel AMΦ Exhibit Abnormal Surface Phenotype and Phagocytosis Function: Deletion of Raptor broadly affected phenotypic characteristics of AMΦ; Raptor deficiency may not affect AMΦ development, but may result in diminished AMΦ with altered surface profile over time; There was impaired capacity of RptorΔmyel AMΦ to engulf apoptotic cells.
3. mTORC1 Dependent Metabolic Checkpoint Mediates AMΦ Proliferation: mTORC1 may be dispensable for AMΦ survival; Defective in cell-cycle entry of RptorΔMyel AMΦ may potentially contribute to impaired self-renewal and diminished AMΦ population in these mice; An essential role of Raptor was indicated for AMΦ growth, nutrient uptake, and mitochondria biosynthesis, which may contribute to cell-cycle entry and self-renewal of AMΦ.
4. Raptor Regulates Repopulation of AMΦ Post Irradiation Induced Replenishment: Raptor/mTORC1 intrinsically regulates the phenotypic characteristics and metabolism in AMΦ. Thus, mTORC1 is important for self-renewal of AMΦ in vivo.
5. mTORC1 Signaling Confers AMΦ Optimal Proliferating Capacity in Response to GM-CSF: GM-CSF has been demonstrated to be a potent upstream mTORC1 activator in AMΦ; mTORC1 signaling is necessary for GM-CSF induced AMΦ expansion *in vitro* and *in viv*o; mTORC1 is required for AMΦ to respond optimally to GM-CSF to trigger proliferation and control metabolic reprogramming, such as glycolysis and lipid biosynthesis, to meet the energy demand for proliferation.

   Therefore, our results demonstrate an essential role of mTORC1 for AMΦ homeostasis by regulating proliferative renewal.

Key words: Alveolar Macrophage; mTOR; GM-CSF; Proliferation; Phagocytosis

NIR 远程响应型纳米载体用于膀胱癌的靶向递药和热化疗研究

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通过扩大 EPR 效应（增加的血管渗透和滞留效应)，基于体内外刺激响应型纳米载药体系的治疗策略已广泛应用于获得有效的肿瘤靶向性。我们合成了热/pH 双敏感性的壳聚糖/聚 N-异丙基丙烯酰胺@单臂碳纳米管复合物（CS/PNIPAAm@SWCNTs），并将其应用于肿瘤的化疗/光热结合治疗。阿霉素（DOX）是一种治疗膀胱癌的有效药物，包载于纳米复合中，载药率可高达 45%；近红外光（NIR）照射后，经系统注射的纳米复合物在鼠 MB49 膀胱癌模型中肿瘤部位的积累能取得显著提高。同时，包载 DOX 的纳米复合物结合激光治疗将展现较好的协同治疗效应。

目的：

1. 通过建立小鼠 MB49 膀胱癌皮下肿瘤模型，探索近红外光介导下，NanoCOM-DOX 纳米颗粒在肿瘤部位的靶向积累情况；
2. 利用小动物活体成像技术，观察 NanoCOM-Cy7.5 纳米颗粒在动物体内的分布情况；
3. 通过建立 MB49 膀胱癌皮下肿瘤模型，探讨 NanoCOM-DOX 在动物水平的抗肿瘤效果；
4. 通过观察小鼠活动状态、体重变化、血生化分析以及对重要脏器进行 HE 染色，评价纳米颗粒在动物体内的毒副反应。

方法：

1. 建立小鼠 MB49 膀胱癌皮下肿瘤模型：将 100ul 2×106/ml MB49 细胞混悬液注射于 C57BL/6 小鼠右后肢皮下。
2. 纳米颗粒在近红外介导下的肿瘤靶向性评价：当肿瘤体积达到 1000mm3 时，通过眼眶静脉丛分别注射 DOX（阿霉素）和 NanoCOM-DOX 溶液，肿瘤部位暴露于 0.35 W/cm2 NIR 激光 15 min，未照射组作为阴性对照。1h 后，取小鼠脾脏和肿瘤组织进行离体成像，观察比较组织中的 DOX 荧光密度值；利用近红外染料 Cy7.5 替代 DOX，观察 NanoCOM-Cy7.5 纳米颗粒在活体小鼠体内的动态分布。
3. NanoCOM-DOX 纳米颗粒的体内抗肿瘤疗效评价：当小鼠肿瘤体积达到 60mm3 时，将小鼠随机分为 5 组（n=5），包括 PBS+NIR 组、DOX+NIR 组、NanoCOM-DOX 组、NanoCOM+NIR 组、NanoCOM-DOX+NIR 组。荷瘤小鼠眼眶静脉丛注射后 30min 进行 NIR 激光（808 nm, 1 W/cm2, 5 mm）照射 5 min，照射过程中红外热成像仪监测肿瘤部位温度。治疗后每两天测量肿瘤大小，14 天后将小鼠处死、取肿瘤组织并称重，并进行石蜡包埋、切片及 HE 染色。

4. NanoCOM-DOX 纳米颗粒在动物体内的毒副作用评价：治疗期间，观察小鼠活动状态、称体重。治疗后第 14 天，取小鼠全血并收集血清，检测血清中 ALT、AST、 ALP、 BUN、TBIL 和 LDH 水平；收集小鼠心脏、肝脏、脾脏、肺脏、肾脏固定过夜，石蜡包埋、切片及 HE 染色进行组织学检查。

结果：

1. 纳米颗粒在近红外介导下的肿瘤靶向性评价：对肿瘤组织进行离体成像结果显示，NanoCOM-DOX 组靶向传递更多的 DOX 分子至肿瘤部位，为 DOX 组的 5-8 倍，且 NIR 照射后显著增强；NanoCOM-Cy7.5 纳米颗粒的活体成像结果显示，NIR 照射下更多纳米颗粒传递至肿瘤部位并能维持 24h 以上。
2. NanoCOM-DOX 纳米颗粒的体内抗肿瘤疗效评价：治疗后第 14 天，NanoCOM-DOX+NIR 组平均肿瘤重量明显小于其他治疗组，抑瘤率达 90.0%；DOX+NIR 组和非照射的 NanoCOM-DOX 组显示一定的抗肿瘤效果，抑瘤率分别为 10.9%和 49.5%。肿瘤组织的 HE 染色结果显示，NanoCOM-DOX+NIR 治疗组肿瘤细胞破坏较严重，NanoCOM+NIR 治疗组肿瘤细胞破坏程度较轻。
3. NanoCOM-DOX 纳米颗粒在动物体内的毒副作用评价：不同治疗组小鼠的体重未见明显变化，组间无统计学差异；相比 PBS 组，其他治疗组血生化水平均在正常范围内；组织学检查结果显示，两组内脏器均未见明显异常。

结论：本研究在动物水平验证了 CS/PNIPAAm@SWCNTs-DOX 纳米颗粒在 NIR 介导下具有更有效的肿瘤靶向性，包载 DOX 的纳米颗粒结合激光治疗具有协同抗肿瘤作用，且纳米材料无毒副作用、生物相容性好，具有较好的应用前景。

关键词：纳米颗粒；刺激响应性；膀胱癌；靶向；DOX

GM-CSF/TNFα 细胞膜表面双重修饰的 LNCaP 前列腺癌治疗性疫苗的制备及其生物学效应鉴定

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鉴于常见治疗方法的各种局限性，有必要研究出更为安全有效的前列腺癌治疗方法。肿瘤细胞疫苗是从机体的肿瘤组织中提取出导致肿瘤细胞，经灭活处理后使肿瘤细胞失去原有的致瘤性，但仍然保持本来的免疫原性，然后诱导机体的主动抗肿瘤免疫反应。

粒细胞-巨噬细胞集落刺激因子 GM-CSF 是一种调节造血细胞增殖和分化的细胞因子。在疫苗接种的部位，局部大量表达的粒细胞-巨噬细胞集落刺激因子 GM-CSF 能够提高抗原提呈细胞 APC 数量，从而有

效地捕获、加工和提呈抗原给 T 细胞。TNFα 在体内、体外均能高效率的杀死某些肿瘤细胞，或抑制肿瘤细胞的增殖，是非常重要的免疫刺激因子，与 GM-CSF 联合应用有免疫协同效应。

我们利用自行研制的 Streptavidin (SA)-hGM-CSF 和 SA-hTNFα 双功能融合蛋白，通过细胞膜表面锚定修饰技术获得了 GM-CSF/TNFα 双重膜表面修饰的 LNCaP 前列腺癌治疗性疫苗；并利用 hPBMC-NOD/SCID 嵌合小鼠前列腺癌模型，验证该疫苗的疗效。

方法

1. 分别制备有活性的 SA-hGM-CSF 和 SA-hTNFα 双功能融合蛋白。
2. 分别制备有活性的肿瘤疫苗：收集 LNCaP 细胞悬液、生物素修饰细胞膜表面；SA-hGM-CSF 或/和 SA-hTNFα 蛋白通过与生物素结合、继而锚定在细胞膜表面。
3. 建立 hPBMC-NOD/SCID 嵌合小鼠前列腺癌模型：在小鼠背部的皮下注射 LNCaP 肿瘤细胞（5×10^5 个/mouse），将小鼠分为 GM-CSF/TNFα 双锚定组、GM-CSF 单锚定组、TNFα 单锚定组、固定细胞组、PBS 组。根据分组，在皮下注射肿瘤细胞后的第 0、7、14 天后分别给小鼠腹腔注射疫苗（2×10^6 个/mouse）。
4. 疗效评价：检测 hPBMC-NOD/SCID 嵌合小鼠外周血中是否有 $hCD4^+$T 细胞、$hCD8^+$T 细胞浸润，观察小鼠的生存状况、肿瘤的增长状况、小鼠的生存率，检测肿瘤、脾脏、肝脏、淋巴结组织中 $hCD4^+$T 细胞、$hCD8^+$T 细胞的浸润情况，检测小鼠肝脏损伤状况，判断小鼠模型是否出现移植物抗宿主病 GVHD。

结果

1. SDS-PAGE 鉴定发现，纯化、复性后的 SA-hGM-CSF 和 SA-hTNFα 双功能融合蛋白能形成多聚体结构，并具有相应 hGM-CSF 或 hTNFα 的生物活性。
2. 流式细胞术分析表明，SA-hGM-CSF 和 SA-hTNFα 双功能融合蛋白能有效地锚定在已灭活的 LNCaP 细胞表面，且锚定率达到 95% 以上，并验证了相应疫苗具有 hGM-CSF 或/和 SA-hTNFα 的生物活性。
3. 流式细胞术分析表明，人外周血单个核细胞移植入 NOD/SCID 小鼠腹腔 4 周后，人 $CD4^+$T 细胞、$CD8^+$T 细胞可出现在小鼠外周血中。免疫组化分析鉴定显示，在不同治疗组的小鼠的肿瘤肿瘤、脾脏、肝脏、淋巴结组织中有不同程度的 $CD4^+$T、$CD8^+$T 细胞浸润，其中 GM-CSF/TNFα 双锚定组浸润 T 细胞数量最多，且无明显的移植物抗宿主病。
4. 三次疫苗连续接种一周后，GM-CSF/TNFα 双锚定组有 2 只小鼠存活（2/5），GM-CSF 单锚定组有 1 只存活（1/5），TNFα 单锚定组有 1 只小鼠存活（1/5），固定细胞组有 1 只小鼠存活（1/3），PBS 组全部死亡（0/3）。此外，GM-CSF/TNFα 双锚定组小鼠肿瘤的横截面积显著小于比其他治疗组。

结论

在 hPBMC-NOD/SCID 小鼠前列腺癌模型中，GM-CSF/TNFα 双重膜表面修饰的 LNcaP 前列腺癌治疗性疫苗能更有效地促进人 $CD4^+$T、$CD8^+$T 细胞浸润到癌组织内，以致能显著提高荷瘤小鼠的生存率。

关键词：LNcaP 肿瘤细胞；肿瘤疫苗；GM-CSF；TNFα；NOD/SCID 小鼠

前列腺癌细胞 C-P3 细胞的三维培养及大规模生产工艺的优化

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目的

本课题主要是对前列腺癌 PC-3 细胞体外二维到三维培养条件的逐步优化，并在 Wave20/50 生物反应器中利用微载体 cytodex3 对 PC-3 细胞培养过程中的摆动方式和营养条件进行优化，以便建立一个适合 PC-3 细胞大规模培养的中试工艺，为前列腺癌治疗性疫苗的产业化奠定基础。

方法

1. PC-3 细胞的二维静止培养的条件优化：在二维培养皿中对 PC-3 细胞的基本生长特性，种子细胞培养基进行优化，从文献查阅常用的四种培养基中选出 PC-3 细胞生长形态最好，达到对数期时间最短，生长密度最高的培养基，用倒置显微镜观察细胞的生长形态，用血球计数板绘制细胞的生长曲线。
2. PC-3 细胞的三维静态培养的条件优化：在 10cm 细菌培养皿中对微载体的初始接种密度，细胞的初始接种密度，微载体的类型 cytodex1 和 cytodex3，血清浓度，培养基，扩大培养方式，新旧球的加入比例等条件的优化，使用细胞动力学分析细胞生长活力，通过倒置显微镜观察细胞在微载体上的贴壁和生长状态，用 0.1%结晶紫柠檬酸染液对细胞核染色并绘制生长曲线，用葡萄糖和乳酸试剂盒，对细胞的营养代谢情况在酶标仪进行监测。
3. PC-3 细胞的三维动态培养的条件优化：在 Wave20/50 生物反应器中，设定 5%$CO_2$37℃，0.01 通气流速的情况下，对 PC-3 细胞在小规模三维静止的条件基础上分别采取全摆动，不摆动，间隙摆动的培养方式培养；不同起始血清浓度对细胞贴壁和生长的影响；营养物质（葡萄糖，生长因子）的补充对细胞生长的影响；通过绘制生长曲线和观察细胞生长状态，监测葡萄糖和乳酸代谢情况，最终收获细胞数等参数进行优化。
4. 利用流式细胞术对微载体培养的 PC-3 细胞膜表面生物素化作用、以及锚定修饰 hGM-CSF 的生物学功能进行鉴定。

结果

1. 在二维静止培养方式中，使用了四种含 10%FBS 的培养基分别培养 PC-3 细胞，其中选取含 10%FBS 的 RPIM1640 培养基作为种子细胞培养基，其中细胞的分泌颗粒物最少，细胞形态呈饱满梭形，细胞内空泡较少，细胞老化速度慢，生长密度最高，达到对数期的时间最短，固选为种子培养基对其进行扩大培养。
2. 在 3D 静止培养体系，我们选择 3g/l cytodex3 和 $2x10^5$cell/ml 的基本接种比例条件，摸索不同起始血清浓度对 PC-3 细胞的生长状况的影响，建立一种双相血清浓度培养方法，起始血清浓度为 10%待细胞贴壁后将血清浓度降至 5%。
3. PC-3 细胞在 cytodex3 静止的培养条件下，在细菌培养皿中对其贴满载体的细胞进行不消化直接接种新旧球比例为 1：1-2：1 时，细胞可以通过“侨联”的作用贴满新球，易于传代扩增，在模拟细胞循环培养体系中进行每天半量换液的操作。
4. 在三维动态培养方式中，使用微载体 3g/l cytodex3 和 $2x10^5$cell/ml 细胞密度的接种条件，结合 Wave20/50 生物反应器中，气体和温度易于控制等优点，在波浪式生物反应器中采取静止--间隔--连续摆动的培养方式，待细胞在微载体贴壁后逐步提高转动时间，摆动角度控制在 5°，6-9rpm 范围内，利于营养物质和氧气传递率的扩散，使细胞贴壁和生长效果最好，培养末期细胞收获数最高。
5. 在 Wave20/50 生物反应器微载体培养体系中，在每天 50%换液的培养基中添加 1g/l 的葡萄糖，血清浓度降到 2%的条件下，相对于不添加葡萄糖的培养基，细胞在微载体上的贴壁时间更久，细胞伸展的更好，达到细胞的最高密度相对较高。
6. 流式细胞分析仪检测了二维培养和三维培养的 PC-3 细胞的膜表面 hGM-CSF 修饰效率达 95%以上。

结论

我们利用微载体培养技术，建立了一种适合 PC-3 细胞在 Wave20/50 生物反应器中大规模培养的中试工艺，并对 PC-3 细胞三维培养条件进行逐步优化，建立了双相血清培养浓度和间隔摆动方式的培养方式，从而对 PC-3 前列腺癌细胞实现了大规模培养。

关键词：前列腺癌；肿瘤疫苗；PC-3 细胞；微载体培养；大规模培养

重组大肠杆菌产 SA-hGM-CSF 高密度发酵工艺的研究

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针对重组大肠杆菌 *E. coli* BL21 (DE3) -pET24a-SA-hGM-CSF，我们通过单因素优化实验在摇瓶中对培养基成分和培养条件进行一系列优化，以便获得最佳发酵条件，并通过正交试验进行了 10L 发酵罐(BioFlo® 415)的发酵工艺摸索，从而确定 SA-hGM-CSF 高密度、高表达的发酵中试工艺，为该双功能融合蛋白的大规模生产奠定基础。

方法

1. SA-hGM-CSF 融合蛋白的发酵工艺

1）摇瓶发酵的研究：通过单因素优化实验，在摇瓶中对培养基的成分以及诱导表达条件进行优化，包括：碳源、有机氮源、无机盐、诱导剂浓度、诱导时间、pH 和温度。

2）10L 发酵罐发酵的研究：在摇瓶发酵的基础上，采用四因素三水平的正交试验（L_9（4^3））对 10L 发酵罐的发酵过程中的 pH、补料开始时间、诱导温度、诱导时机进行优化。

2. SA-hGM-CSF 融合蛋白的纯化与复性

收集工程菌，菌体经高压破碎仪破菌，将获得的包涵体经包涵体洗涤液洗涤后再用包涵体溶解液溶解，随后，对融合蛋白进行 DEAE FF 离子交换层析纯化条件的摸索，采用 DEAE FF 柱上复性法将纯化后的目的蛋白进行复性。RP-HPLC 检测 SA-hGM-CSF 融合蛋白的纯度。

3. SA-hGM-CSF 融合蛋白的生物学活性测定

CCK-8 法检测 SA-hGM-CSF 融合蛋白和 hGM-CSF 标准品对 TF-1 细胞增殖的活性，比较两者活性的大小；流式细胞术检测 SA-hGM-CSF 融合蛋白对已生物素化的 MB49 膀胱癌细胞膜表面的锚定修饰效率。

结果

1. SA-hGM-CSF 融合蛋白的发酵工艺

1) 摇瓶发酵：通过摇瓶单因素的优化实验，初步摸索出最佳的培养基（即发酵培养基）：蛋白胨 20g/L，酵母粉 12g/L，NaCl 10g/L，葡萄糖 10g/L，K_2HPO_4 $3H_2O$ 1.5g/L，$KH_2PO_4$2.3g/L，$(NH_4)_2SO_4$2.4g/L，$MgS0_4$ $7H_2O$0.25g/L；摇瓶的表达参数是： IPTG 诱导浓度为 0.5mM，诱导时间为 5h， pH=7.0，温度 37℃，整个摇瓶发酵持续时间为 9 小时。

2) 10L 发酵罐发酵：正交试验结果分析，最后确定出适合 SA-hGM-CSF 融合蛋白高密度发酵的工艺路线。即：将发酵培养基在 10L 发酵罐（BioFlo® 415）中原位灭菌后，种子液按 5%接种量接种到 10L 发酵罐中。罐中的起始工作体积为 10L，发酵参数为：转速 250-650 rpm，温度 37℃，氧容量控制在 50±5%，通气量、通氧气、搅拌速度与 DO 连动，pH=7.0。当发酵至 3h 时，采用连续补料的方式给与添加补料，待菌密度 OD_{600}=3.0 时，一次性加入 IPTG 进行诱导，使其终浓度为 0.5mM，并设定诱导温度为

32.5℃。控制搅拌转速及通氧量使发酵液保持一定的溶氧量，以 pH 调节物稳定发酵液 pH，发酵持续时间约 10 小时，每升培养液可收获湿菌体达 30g 左右，表达水平达 50%以上。

2. SA-hGM-CSF 融合蛋白的纯化与复性

经包涵体洗涤液洗涤后，目的蛋白的纯度已达到 60%左右，经过 DEAE FF 离子交换层析纯化和柱上复性后，SDS-PAGE 和 RP-HPLC 分析，融合蛋白的纯度达到 95%、回收率达 70%以上。复性后，SA-hGM-CSF 融合蛋白主要以单体和多聚体形式同时存在。

3. SA-hGM-CSF 融合蛋白的生物学活性鉴定

SA-hGM-CSF 双功能融合蛋白具有显著的增殖 TF-1 细胞的活性，且呈剂量依赖性，和 hGM-CSF 标准品相比，两者无明显的差别。其 EC50 分别为 1.460 和 1.455，活性分别是 0.685×10^6 和 0.687×10^6；流式细胞术及检测了 SA-hGM-CSF 双功能融合蛋白对已生物素化的 MB49 膀胱癌癌细胞的锚定率，其锚定率可高达 99%左右。

结论

本研究获得了重组大肠杆菌 *E. coli* BL21 (DE3) -pET24a-SA-hGM-CSF 高密度、高表达的发酵中试工艺。

关键词：SA-hGM-CSF 双功能融合蛋白；条件优化；正交试验；高密度发酵；工艺

生物素-亲和素-时间分辨荧光免疫分析检测

肌酸激酶同工酶 MB 方法的建立及其临床初步验证

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诊断急性心肌梗死（AMI）有许多生化指标,如:心肌肌钙蛋白 T(cTnT)、心肌肌钙蛋白 I(cTnI)、肌酸激酶（CK）、肌酸激酶同工酶 MB(CK-MB)、肌红蛋白(Mb)等。AMI 时心肌细胞受损，膜的完整性和通透性改变，这些大分子从细胞内逸出，可在外周血中检测到。胞浆中酶蛋白 CK 主要由两个亚基聚合而成，不同亚基的组合形成了三种同工酶：CK-MM、CK-MB、CK-BB，其中 CK-MB 主要存在于心肌组织中，是急性心肌梗死后升高较早、升高幅度较大的一种标志物。CK-MB 的升高程度可以准确地反映心肌梗死的范围，其高峰出现的时间是否提前，对于判断溶栓治疗是否成功具有重要临床价值。因此，CK-MB 被视为诊断 AMI 较敏感的指标之一。

本研究旨在通过原核表达可溶性人 CK-MB，获得稳定性高、互通性好的人 CK-MB 蛋白校准品；同时，建立生物素-亲和素-时间分辨荧光免疫分析（BA-TRFIA）检测 CK-MB 的方法，并进行临床初步验证。

方法

1. pET28a-CK-MB 原核表达载体的构建、可溶性 CK-MB 重组蛋白的诱导表达、纯化及其抗原性鉴定。
2. CK-MB 重组蛋白稳定性评价：测定不同保存体系条件下重组蛋白 CK-MB 的活性。
3. BA-TRFIA 检测 CK-MB 方法的建立：将抗 CK-MB 捕获抗体 X 共价偶联到聚苯乙烯材料的固相板上，将检测抗体 Y 进行生物素标记，用 Eu^{3+}对链霉亲和素（SA）进行标记。采用双抗体夹心法，初步建立 BA-TRFIA 检测 CK-MB 方法。然后从捕获抗体包被量、生物素标记检测抗体量、Eu^{3+}-SA 用量等方面进行优化，确定最佳反应条件，建立了 BA-TRFIA 检测 CK-MB 方法。
4. 方法学评价及临床初步验证：对建立的 BA-TRFIA 检测 CK-MB 进行方法学评价，评价指标包括特异性、标准曲线、最低检测限、批内/批间精密度和干扰实验，以及对临床标本进行检测，并与化学发光免疫分析方法（CLIA）进行比较分析等。

结果

1. CK-MB,重组蛋白标准品的成功制备：将构建好的 pET28a-CK-MB 重组质粒转化入大肠杆菌 BL21（DE3）感受态细胞，30℃，250rpm，0.5mmol/L IPTG 诱导表达 5h，CK-MB 蛋白表达量占菌体总蛋白的 55%以上，经 SDS-PAGE 鉴定目的蛋白在上清及包涵体中均有表达，分子量约为 92KD。对收集破菌上清进行镍柱亲和层析，重组蛋白 CK-MB 多在 100mmol/L 咪唑梯度洗脱，纯化后融合蛋白的纯度在 90%以上，浓度高于 300μg/L。
2. CK-MB 重组蛋白抗原性已完整保存：制备的 CK-MB 重组蛋白能与羊抗人 CK-MB 单克隆抗体特异性结合。
3. CK-MB 重组蛋白的稳定性好：在含 10% BSA、2mmol/L EDTA 的蛋白保护剂中， 4℃保存 16d 重组蛋白活性丧失平均低于 5%；在-20℃条件下，重组蛋白在观察半年活性基本不变
4. BA-TRFIA 检测 CK-MB 最佳优化条件为：捕获抗体 X 包被固相板最佳使用量 3μg/ml；生物素标记抗体及 Eu^{3+}-SA 稀释度 1:100；孵育温度为 37℃，反应时间均为 30min。
5. 方法学评价优异：该方法检测出口出口目标的标准曲线工作范围为 0.64-400ug/L，剂量-反应线性相关系数为 0.9423，最低检测限为 0.1ug/L。批内、批间精密度分别为 3.6%~9.2%、8.0%~10.3%，与肌钙蛋白 I、肌钙蛋白 T、肌红蛋白及牛血清白蛋白均无交叉反应。
6. 临床健康体检人员血清标本的检测结果符合要求：对 150 例临床健康体检人员血清标本进行检测，结果显示检测的健康体检人员血清标本 CK-MB 蛋白含量为 0~0.81ug/L，检测的阴性预测值为 96%。
7. 方法学比对结果优：对 100 例 CK-MB 阴性标本和 150 例 CK-MB 阳性标本同时使用 BA-TRFIA 和 CLIA 进行检测,且与 CLIA 检测结果具有很强的相关性（R=0.9249），BA-TRFIA 检测 150 例 CK-MB 阳性

标本结果与患者心电图、cTnI、BNP 的检测结果相符。

结论

BA-TRFIA检测CK-MB的方法基本符合临床诊断的相关要求，具有检验诊断急性心肌梗死的潜力。

关键词：时间分辨荧光免疫分析；生物素；亲和素；心肌梗死；肌酸激酶同工酶MB

sTNFRⅡ-adiponectin 融合蛋白的制备及其生物学功能的初步鉴定

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本课题拟对我们的新型TNFα拮抗剂（发明专利号ZL200510100468.9）人可溶性肿瘤坏死因子受体II胞外区与人脂联素球部的双功能融合蛋白（sTNFRII-gAD）进行改造，旨在构建一种新型的sTNFRⅡ-adiponectin双功能融合蛋白。该双功能融合蛋白借助脂联素能自发形成同源三聚体、甚至寡聚体的特性，形成三聚体化或多寡聚体化的sTNFRⅡ，以增强其与TNFα的亲和力、从而提高其拮抗TNFα的活性。此外，利用富含“GC”的载体及其CHO细胞高效表达系统，探索无血清悬浮流加培养方法，为高效、快速获取该双功能融合蛋白的中试制备工艺奠定基础。

方法

1. 获取sTNFRⅡ-adiponectin-WT和sTNFRⅡ-adiponectin-MT基因：通过逆转录聚合酶链反应（RT-PCR），从人脂肪组织中获取脂联素cDNA。以该基因为模版，分别得到野生型和突变型adiponectin。再以实验室之前得到的pMH3-sTNFRII-gAD-Fc为模版，扩增sTNFRII，将两者融合，得到sTNFRⅡ-adiponectin-WT和sTNFRⅡ-adiponectin-MT基因。

2. 构建PMH3-sTNFRⅡ-adiponectin-WT、PMH3-sTNFRⅡ-adiponectin-MT、PCA-sTNFRⅡ-adiponectin-WT和PCA-sTNFRⅡ-adiponectin-MT表达质粒：PCR扩增目的基因sTNFRⅡ-adiponectin-WT和sTNFRⅡ-adiponectin-MT，构建PMD18-T克隆载体，转化入大肠杆菌进行扩增，提质粒得到大量sTNFRⅡ-adiponectin-WT和sTNFRⅡ-adiponectin-MT目的基因，将其酶切后分别连入PMH3、PCA表达载体，得到PMH3-sTNFRⅡ-adiponectin-WT、PMH3-sTNFRⅡ-adiponectin-MT、PCA-sTNFR -adiponectin-WT和PCA-sTNFRⅡ-adiponectin-MT表达质粒。

3. 建立表达 sTNFRⅡ-adiponectin-WT 和 sTNFRⅡ-adiponectin-MT 融合蛋白的高表达 CHO 细胞株：通过电转化的方法，将成功构建的表达质粒转入 CHO-S 细胞，经过筛选得到高表达 sTNFRⅡ-adiponectin-WT 和 sTNFRⅡ-adiponectin-MT 融合蛋白的细胞株。

4. sTNFRⅡ-adiponectin-WT 和 sTNFRⅡ-adiponectin-MT 融合蛋白的制备：

1) 贴壁培养：筛选出的高表达株于 T75 中贴壁培养，收集细胞上清，利用镍柱纯化获取目的蛋白 sTNFRⅡ-adiponectin-WT 和 sTNFRⅡ-adiponectin-MT，透析过夜后超滤管浓缩蛋白，并通过 Western-blot、

考马斯亮蓝染色法鉴定目的蛋白大小。

2) 悬浮培养初探：将 T75 中贴壁培养的高表达株进行 B001 悬浮驯化，得到适合悬浮生长的稳定高表达工程细胞株。

5. sTNFRⅡ-adiponectin-WT 和 sTNFRⅡ-adiponectin-MT 融合蛋白活性的测定：通过 MTT 法测定 sTNFRⅡ-adiponectin-WT 和 sTNFRⅡ-adiponectin-MT 中和 TNF-α 杀伤 L929 细胞的活性，比较目的蛋白 sTNFRⅡ-adiponectin-WT 和 sTNFRⅡ-adiponectin-MT、标准蛋白 sTNFRⅡ-Fc 以及实验室原先构建的蛋白 sTNFRII-gAD-Fc、sTNFRII-gAD 拮抗 TNFα 的能力的差异。

结果

1. 成功得到sTNFRⅡ-adiponectin-WT和sTNFRⅡ-adiponectin-MT基因：RT-PCR后用1.2%的琼脂糖检测结果，成功得到与预期条带大小一致的片段。

2. 成功构建PMH3-sTNFRⅡ-adiponectin-WT、PMH3-sTNFRⅡ-adiponectin-MT、PCA-sTNFRⅡ-adiponectin-WT和PCA-sTNFRⅡ-adiponectin-MT表达质粒：提质粒酶切后，用1.2%的琼脂糖鉴定酶切结果，得到条带与预期位置相符，将该质粒送测序鉴定目的基因，与NCBI上获取的基因序列比对后完全一致，提示成功构建PMH3-sTNFRⅡ-adiponectin-WT、PMH3-sTNFRⅡ-adiponectin-MT、PCA-sTNFRⅡ-adiponectin-WT和PCA-sTNFRⅡ-adiponectin-MT表达质粒。

3. sTNFRⅡ-adiponectin-WT和sTNFRⅡ-adiponectin-MT融合蛋白在CHO细胞中高表达：电转染后的细胞经过单克隆化后用G418加压筛选，通过Dot-Blot筛选高表达株，经过2轮筛选后，挑选表达量高的细胞株扩增培养，能得到高表达目的蛋白的细胞株。

4. sTNFRⅡ-adiponectin-WT和sTNFRⅡ-adiponectin-MT融合蛋白的纯化：镍柱纯化时，目的蛋白约在120mM左右浓度的咪唑洗脱下来，纯化后成功得到目的蛋白。经过WB鉴定，目的蛋白单体大小为70KD左右，并且以多聚体的形式存在。

5. sTNFRⅡ-adiponectin-WT和sTNFRⅡ-adiponectin-MT融合蛋白活性的测定：sTNFRⅡ-adiponectin-WT和sTNFRⅡ-adiponectin-MT融合蛋白具有显著抑制TNFα杀伤L929细胞的活性，且随蛋白浓度的升高，抑制TNFα杀伤L929细胞越明显，即呈剂量依赖性。相对于标品sTNFRⅡ-Fc来说，目的蛋白活性明显较强，但活性还是低于之前实验室构建的融合蛋白sTNFRⅡ-gAD。

结论

本研究成功构建了 sTNFRⅡ-adiponectin-WT、sTNFRⅡ-adiponectin-MT 真核表达载体，并在 CHO 细胞中实现了较高水平的表达，并初步建立了无血清悬浮流加培养工艺。

关键词：可溶性肿瘤坏死因子受体 II-脂联素；CHO 细胞；“富含 GC”表达载体；无血清悬浮培养

SIRT1 通过 TNF-α/β-catenin 信号通路促进 B[a]P 诱导肺癌发生的分子机制

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80%~90%的肺癌与吸烟相关。香烟烟雾中多环芳烃类化合物苯并芘（B[a]P）及其代谢产物 B[a]PDE 是最为明确的致肺癌化合物，但具体作用机制尚不明确。沉默信息调节因子 1（SIRT1）是烟酰胺腺嘌呤二核苷酸（NAD^+）依赖性的组蛋白脱乙酰化酶，参与细胞迁移/侵袭、恶性转化等的调控。然而，SIRT1 在肺癌发生发展中的作用及其机制尚不清楚。

我们研究发现:

1. SIRT1 在人肺癌组织中高表达：免疫组化分析表明，SIRT1 蛋白在非细胞肺癌，包括腺癌和鳞癌中的表达水平显著高于肺正常组织。

2. B[a]P 诱导人支气管上皮细胞 BEAS-2B 和小鼠肺组织中 SIRT1 的表达：B[a]P 暴露下，SIRT1 mRNA 水平逐步升高，且具有时间依赖性；Western Blotting 表明，B[a]P 对 SIRT1 蛋白的诱导具有时间依赖性，且 SIRT1 蛋白表达量在 48 h 达到峰值；免疫组化分析发现，支气管灌注 B[a]P 小鼠的肺组织中 SIRT1 的表达量明显高于对照组。此外，TNF-α、NF-κB 和 COX-2 在人肺腺癌和鳞癌中的表达水平显著高于正常组织。

3. B[a]P 暴露下，SIRT1 促进 BEAS-2B 细胞的迁移和侵袭：利用细胞伤口愈合实验和 Transwell 实验证明，8 μM B[a]P 暴露环境中，过表达 SIRT1 能够促进 BEAS-2B 细胞迁移和侵袭，反之亦然。

4. B[a]P 暴露下，SIRT1 促进 TNF-α 的表达：Western Blotting 表明，过表达 SIRT1 能够促进 BEAS-2B 细胞中 NF-κB 和 COX-2 蛋白的表达；而抑制 SIRT1 则降低 NF-κB 和 COX-2 的表达。流式细胞术分析表明，过表达 SIRT1 能够促进 BEAS-2B 细胞中 TNF-α 蛋白的表达；而抑制 SIRT1 则降低 TNF-α 的表达。同时，过表达 SIRT1 能够促进 BEAS-2B 细胞中 TNF-α mRNA 的表达，而抑制 SIRT1 表达则下调 TNF-α mRNA 水平。双荧光素酶报告基因分析表明，B[a]P 暴露下，SIRT1 过表达能够提高 TNF-α 启动子的转录活性，而 SIRT1 表达受到抑制后 TNF-α 启动子转录活性则受到抑制。

5. TNF-α 作为 SIRT1 下游信号分子，参与 SIRT1 调控细胞迁移和侵袭的过程：利用细胞伤口愈合实验和 Transwell 实验表明，8 μM B[a]P 暴露环境中，过表达 TNF-α 能够促进 BEAS-2B 细胞迁移和侵袭，反之亦然。

6. TNF-α 和 Wnt/β-catenin 信号通路之间相互调控：SIRT1 表达量与 β-catenin 呈正相关；且 SIRT1 能增加 β-catenin 在细胞核内的积聚。分别经 Wnt/β-catenin 特异性抑制剂 XAV939 和 TNF-α 敲除质粒作用后发现，B[a]P 暴露下，BEAS-2B 细胞中 TNF-α 和 Wnt/β-catenin 通路之间能相互促进，而 BEAS-2B 细胞膜中的 E-cadherin 蛋白水平则明显下调。

7. 长期 B[a]P 暴露下，SIRT1 能够诱导 BEAS-2B 细胞转化：裸鼠成瘤实验研究表明，相对于对照组细胞，长期暴露于 B[a]P 的 SIRT1 过表达细胞成瘤体积显著增大；并且， SIRT1 过表达细胞形成的肿瘤组织中，TNF-α 和 β-catenin 表达水平显著高于对照组。

因此，SIRT1 通过 TNF-α/β-catenin 信号通路参与了 B[a]P 诱导的支气管上皮细胞转化，故是治疗肺癌的潜在靶点。

关键词：苯并芘；沉默信息调节因子 1；肺癌；肿瘤坏死因子 α；连环蛋白 β

胸腺基质淋巴细胞生成素在过敏监测中的研究

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摘要：胸腺基质淋巴细胞生成素(TSLP)对活化树突状细胞(DCs)和诱导淋巴细胞及其亚群的分化、成熟有着重要的调控作用。TSLP 能通过对髓样和淋巴样细胞进行作用，从而平衡体内天然免疫和适应性免疫，在启动以及促进由 Th2 细胞介导的过敏性炎症中扮演着重要的角色。运动性疲劳可直接影响运动员的竞技运动能力。运动员在长期的高强度的训练后经常会有 Th1/Th2 的免疫失衡，导致运动员抗感染能力下降，易患感冒、上呼吸道感染和皮肤病等。本研究通过持续追踪多名职业自行车运动员，检测他们血清中细胞因子的含量，探索 TSLP 在自行车运动员过敏性疾病监测中作为一种标志物的可能性。发现了在患有过敏性疾病的运动员血清中，TSLP 与 IL-9 具有正相关性。同时通过计算 Th1、Th2 和 Th9 的标志性细胞因子 IFNγ、TSLP 和 IL-9 比值，得到的 Th1/Th2、Th1/Th9 的比值，发现 TSLP、IL-9 可以作为一种运动性免疫失衡的标记物，继而可以为运动疲劳诊断提供依据。

关键词：胸腺基质淋巴细胞生成素，过敏监测，自行车运动员过敏

FUNCTIONS OF THYMIC STROMAL LYMPHOPOIETIN IN ALLERGIC DISEASES SURVEILLANCE

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Abstract：Thymic stromal lymphopoietin (TSLP) palys an important role in activating dendrtic cells and inducing the differentiation and mature of lymphocytes. At the same time, TSLP is essential for the initiation and

development of Th2-mediated allergic inflammation by acting on myeloid and lymphoid populations to coordinate innate and adaptive immunity. Sports fatigue can directly affect the competitive athletic ability of athletes. After a long-term intensive training with fatigue, athletes always gain Th1/Th2 imbalance, which results in decline of anti-infection ability and in susceptibility to colds, upper respiratory tract infection and skin diseases. In this study, we collected several cyclists' serum by one year continuous tracking and detected the expression of cytokines in serum to investigated the role for TSLP in cyclist allergic inflammation monitoring. We found the correlation of serum interleukin 9 and TSLP concentration in atopic cyclist. By calculating the value of IFNγ/TSLP as well as IFNγ/IL-9 representing Th1/Th2 and Th1/Th9 respectively, we demonstrated TSLP and IL-9 were an effective marker for measure the sports immune imbalance.

Key words: Thymic stromal lymphopoietin, monitoring of allergic, allergy in cyclists

金枪鱼眼制备透明质酸的研究

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摘要：透明质酸(hyaluronic acid, HA)又称玻璃酸，由 N-乙酰氨基葡萄糖(Glc-Nac)以 β-1，3 糖苷键与葡萄糖醛酸(GIcA)交替连接而成。由于独特的分子结构和理化性质，透明质酸已在医药、化妆品、眼科、骨科手术等方面得到了广泛的应用。本文采用海洋生物资源—金枪鱼眼为原料，将鱼眼解冻后剥得玻璃体，经丙酮脱脂处理，粉碎、减压干燥，提取、除蛋白、表面活性剂沉淀，沉淀解离后经过乙醇沉淀、脱水，干燥等步骤，获得精制 HA 产品，终产品中 HA 纯度 78.36%，蛋白含量为 1.48%。最终得到一条工艺简单的透明质酸制备路线，获得了良好的透明质酸产品，为产业化应用打下了基础。

关键词：透明质酸，金枪鱼眼，提取，纯化

PRODUCTION OF HYALURONIC ACID USINING ENZYME MEMBRANE BIOREACTOR

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Abstract: Hyaluronic acid (HA) is a mucopolysaccharide with unique molecular structure and the physical and chemical properties.It has been successfully used in cosmetics, arthritis therapy and medicine.The research was mainly to study the extraction and purification process of hyaluronic acid from marine resources—tuna to get

optimum technological conditions. Thawed out tuna eye and get the vitreous，Crude HA is obtained via acetone degrease, crushing, decompression drying, extraction, protein precipitation process。and then by ethanol precipitation, dehydration, drying and other steps, purified HA is obtained. Hyaluronic acid Purity in the final product was 78.36%, protein content 1.48%. This study obtained a simple process route and access to high quality HA products, which lay a foundation for industrial application.

Key words: Hyaluronic acid, tuna eye, purification.

核酸适体分离细菌内毒素的研究

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摘要：本论文筛选了一种针对细菌内毒素的核酸适体，并开展了将其应用于内毒素分离的研究。基于 SELEX 筛选技术，经过 15 轮正筛和 5 轮反筛，共得到 13 个克隆子，测序，获得 1 条高频序列，命名为 EAQ3。通过 DNAMAN 6.0.3.99 软件对 13 条序列进行一级结构和二级结构分析，结果表明，一级结构分析表明这 13 条序列的一致性为 75.15 %，其中：随机序列区域中大多数富含 G、C 碱基；有 25 条序列的随机序列区域以 T，G 结尾。所以很有可能随机序列区域中的 G、C 碱基和 T、G 尾巴与靶标的结合有关。二级结构分析表明所有 13 条序列都形成茎环结构，其中 EAQ3 有可能形成 G-四聚体结构。对三条序列 EAQ1，EAQ2，EAQ3 与内毒素结合的亲和性和特异性测定。检测结果表明，EAQ3 与内毒素结合的亲和性最高，特异性最好。以 EAQ3 制备核酸适体-磁珠分离细菌内毒素。经过吸附动力学曲线拟合，该核酸适体-磁珠在 10 分钟内对内毒素的吸附量最高达到 1199 EU/g，吸附过程快速，可应用于生物制品中内毒素的去除。

关键词：核酸适体，内毒素，分离，SELEX

SELECTION OF DNA APTAMERS FOR SEPARATION OF ENDOTOXIN

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Abstract： We selected DNA aptamers against endotoxin, and investigated their applications in separating endotoxin. Based on the technology of SELEX (Systematic Evolution of Ligands by Exponential Enrichment), after 15 rounds of SELEX and five rounds of reverse screening, 13 sequences were acquired, among them three sequences appeared frequently called high frequency sequence, EAQ3. The 13 sequences were analyzed by DNAMAN 6.0.3.99 and their primary and secondary structures were acquired. The primary structure analysis showed that the consistency of the 13 sequence was 78.12%, among them: most of the random sequences area were rich in G, C bases. What was more, there were 25 sequences ending with T or G in their random region. It seemed that the C-rich, G-rich parts and T,G tail in random region may have impact on targets combing. The secondary structure analysis showed that all the 13 sequences form the stem loop structure and the EAQ3 may

form a G-quartet structure.Binding affinity and specificity of aptamers to endotoxin were detected. The results showed that the EAQ3 had the highest affinity to endotoxin, and its specificity to endotoxin was also the best. So the EAQ3 can be used to separate endotoxin specifically. Aptamer-magnetic beads for endotoxin separation were designed. The adsorption quantity reached extreme in first 10 min and the extreme was 1199 EU/g. And the process of adsorption was relatively fast. It is expected that these aptamer-magnetic beads will have a feasible and usefulapplication in separating endotoxin from biological products.

Key Words: Aptamer，Endotoxin，SELEX，Separation

大肠癌中 GRP78 对 Wnt 信号通路的调控及黄连素的干预作用

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目的：本研究旨在探讨大肠癌中 GRP78 蛋白对 Wnt 信号调节作用及黄连素抗肠癌的机制。**方法：**培养人肠癌细胞 SW480 和 HT-29，分别分组：正常组（SW480 或 HT-29 正常培养 24h）、低浓度黄连素组（20μmol/L 黄连素处理 24h）、高浓度黄连素组（100μmol/L 黄连素处理 24h）、 LiCl 组（用 20mmol/L LiCl 处理 25h）、LiCl+黄连素组（先用 20mmol/L LiCl 处理 1h 后用 20mmol/L LiCl 和 100μmol/L 黄连素处理 24h）、BiX 组（用 1μmol/L BiX 处理 1h）、BiX+黄连素组（用 1μmol/L BiX 处理 1h 后用 100μmol/L 黄连素处理 24h），采用免疫印迹法检测 GRP78、β-catenin 以及 Wnt 信号通路下游靶基因的表达，用免疫荧光检测 GRP78 亚细胞定位、β-catenin 细胞核转位变化，用免疫共沉淀法检测 GRP78-β-catenin、β-catenin-E-cadherin 和 β-catenin-APC 蛋白相互作用的变化。**结果：**与正常组相比，LiCl 组激活了 Wnt 信号通路，β-catenin、GRP78 表达上调，BiX 组 GRP78 表达上调，两组均出现 GRP78 在胞质积累，β-catenin 向核内转移增多，GRP78-β-catenin、β-catenin-E-cadherin 和 β-catenin-APC 蛋白相互作用增强，在黄连素存在下（低浓度黄连素组、低浓度黄连素组、LiCl+黄连素组、BiX+黄连素组），核内 β-catenin、GRP78 表达下调，GRP78 向膜上转移，β-catenin 向核内转移减少，GRP78-β-catenin、β-catenin-E-cadherin 和 β-catenin-APC 蛋白相互作用减弱。**结论：**黄连素可以通过干预 GRP78 蛋白的表达及其与 Wnt 信号关键因子相互作用而起到抗肠癌作用。

关键词：GRP78，Wnt 信号，黄连素

蛋白质组学方法研究丹参酮ⅡA 保护 ox-LDL 诱导的内皮细胞损伤的分子机制

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目的 利用蛋白质组学技术研究丹参酮ⅡA 对 ox-LDL 诱导的人脐静脉内皮细胞（Human umbilical vein endothelial cells，HUVEC）细胞损伤的保护作用及其分子机制。**方法** 体外培养 HUVEC，利用 MTT 法确定 ox-LDL 和丹参酮ⅡA 的最佳作用浓度，并用不同浓度的丹参酮ⅡA 和 50 mg/L ox-LDL 处理 HUVEC 细胞 24 h。利用流式细胞仪检测 HUVEC 细胞中活性氧（Reactive oxygen species，ROS）含量。检测 HUVEC 细胞内丙二醛（Malondialdehyde，MDA）含量和超氧化物歧化酶（Superoxide dismutase，SOD）活性的变化。利用双向荧光差异凝胶电泳（Two dimension difference gel electrophoresis，2D-DIGE）结合质谱鉴定技术筛选并鉴定丹参酮ⅡA 作用前后差异表达的蛋白质。利用 BioPUBINFO 网站建立蛋白质相互作用网络。**结果** 与正常组比较， 0-8 μmol/L 丹参酮ⅡA 对 HUVEC 细胞增殖无影响（P>0.05），浓度大于 50 mg/L 的 ox-LDL 对细胞增殖具用显著的抑制作用（P<0.05）。经过流式细胞仪检测，ox-LDL 诱导 HUVEC 细胞中 ROS 含量显著增加（P<0.01），而不同浓度丹参酮ⅡA 作用后，随着其浓度的增加 ROS 含量逐渐降低。丹参酮ⅡA 作用后，与 ox-LDL 组相比，MDA 含量下降，而 SOD 活性提高（P<0.05）。④蛋白质组学实验表明：8μmol/L 丹参酮ⅡA 作用后，ox-LDL 损伤的 HUVEC 细胞中 29 个蛋白质表达发生变化，通过建立的蛋白质相互作用网络图筛选出 11 个关键节点进行功能分析，发现这些节点主要涉及细胞代谢、氧化磷酸化作用、细胞组分及细胞骨架。**结论** 50 mg/L 的 ox-LDL 作用 HUVEC 细胞 24 h，能成功建立内皮细胞损伤模型。丹参酮ⅡA 能够显著降低受损内皮细胞内 ROS 和 MDA 含量，以及提高 SOD 活性，表明丹参酮ⅡA 能显著增强 HUVECs 抵御氧化损伤的能力。结合蛋白质相互作用网络图谱分析丹参酮ⅡA 主要通过调节细胞中氧化磷酸化途径和抗炎抗氧化方面保护内皮细胞。

关键词：丹参酮ⅡA，HUVEC 细胞，Ox-LDL，2D-DIGE，蛋白质相互作用

绞股蓝总皂苷对 HDL_2 诱导的 HepG2 细胞蛋白质组表达的影响

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[摘 要] 目的：利用双向电泳与质谱鉴定技术研究绞股蓝总皂苷（Gypenosides，GPS）对 HepG2 细胞 HDL_2 诱导的 HepG2 细胞蛋白质组的影响，寻找 GPS 作用靶点，分析 GPS 抗动脉粥样硬化的作用机制。

基金项目：国家自然科学基金青年科学基金（63411126）

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方法：体外培养 HepG2 肝癌细胞，利用 MTT 实验确定 GPS 的最佳作用浓度，并用不同浓度的 GPS 和 300 mg/L HDL_2 处理 HepG2 细胞 24 h。提取细胞总蛋白，利用双向电泳结合硝酸银染色技术扫描得到不同实验组间的蛋白质图谱，最后通过质谱鉴定获得绞股蓝总皂苷作用前后差异表达的蛋白质。**结果：**经质谱鉴定得到 40 个差异表达蛋白质，在模型组中表达升高、GPS 组中表达降低的蛋白点有热休克蛋白 60、硫氧还蛋白相关蛋白、calumenin、EF-hand 结构域、钙网蛋白、nucleobindin、丝氨酸/精氨酸丰富的剪接因子、半乳糖凝集素 1、M2 型丙酮酸激酶、valosin-containing protein、3-羟基异丁酸脱氢酶前体、内质网蛋白 29、异质性胞核核糖核蛋白 K、异质性胞核核糖核蛋白 C、DEAD 盒蛋白 Abstrakt、ZNF254 蛋白、烯酰辅酶 A 水合酶、原肌球蛋白结合蛋白 3、stomatin 样蛋白 2、真核翻译起始因子 3、鸟嘌呤核苷酸结合蛋白、蛋白酶体 β3、辅酶 Q 细胞色素 C 还原酶核心蛋白、半乳糖激酶 1、NAD+依赖型异柠檬酸脱氢酶，在模型组中表达降低、GPS 组中表达升高的蛋白点有过氧化物酶 2、超氧化物歧化酶、血红素结合蛋白 1、谷胱甘肽 S 转移酶 P1、钠氢交换调节因子 1、视网膜母细胞瘤结合蛋白 7、热休克蛋白 27、Stathmin1/oncoprotein18、WD-40 重复蛋白、26S 蛋白酶体、Ran 结合蛋白 1、PSMA3、蛋白酶体 Y、cargo selection protein TIP47、焦磷酸酶 1。**结论：**绞股蓝总皂苷能够通过调节细胞中抗炎抗氧化蛋白、钙离子调控蛋白、细胞代谢、能量代谢等相关蛋白的表达，来维持细胞氧化还原自稳状态、胞内钙离子平衡、以及细胞骨架、细胞代谢、能量代谢稳定等，为胆固醇逆运转的进行提供了保障。

[关键词] 绞股蓝总皂苷；动脉粥样硬化；HepG2 细胞；HDL_2；蛋白质组

EGCG 对巨噬细胞源性泡沫细胞蛋白质组影响的研究

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摘要【目的】研究 EGCG 对 Raw264.7 巨噬细胞源性泡沫细胞蛋白质组表达的影响，探讨其抗动脉粥样硬化的机理。【方法】20 μg/ml ox-LDL 作用 24h 诱导巨噬细胞分化为泡沫细胞，加入 EGCG 作用后，应用蛋白质组及质谱技术分析并鉴定 EGCG 作用前后细胞内蛋白质组的差异，找寻 EGCG 细胞内的分子作用靶点。【结果】通过双向图谱分析及质谱鉴定，本实验共得到 18 个差异蛋白质点：相对于巨噬细胞，泡沫细胞中 M2 型丙酮酸激酶，葡萄糖-6-磷酸酶，磷酸丙糖异构酶，ATP 合酶，stathmin 1，蛋白酶体 26S，泛素结合酶 E2N，环指蛋白 130，乙二醛酶类表达下降，加药后表达上升。β-肌动蛋白，原肌球蛋白（Tropomyosin alpha-3 chain），A-X 肌动蛋白（A-X actin），热休克蛋白 70（heat shock protein 70），腺嘌呤磷酸核糖转移酶（Adenine phosphoribosyl- transfera se），巨噬细胞游走抑制因子（macrophage migration inhibitory factor

13kda protein），反义碱性成纤维细胞生长因子（anti-sense basic fibroblast growth factor）泡沫细胞中表达上调，加药后下调。而硫氧还蛋白（thioredoxin domain containing 12 precursor）和谷胱甘肽 S-转移酶（glutathione S-transferase mu 1）在泡沫细胞中表达升高，加药后表达继续上调。【结论】 EGCG 可通过调节细胞骨架，减少 CD36 介导的脂质吞噬；调节细胞能量代谢相关酶类，提高 ATP 水平，促进胆固醇逆转运；影响细胞内泛素-蛋白酶体系统，调节抗氧化酶类的表达，减轻细胞氧化应激；抑制巨噬细胞炎性浸润，减轻炎症反应，发挥抗动脉粥样硬化的作用。

关键词：EGCG；Raw264.7 巨噬细胞；泡沫细胞；蛋白质组

Galectin-1 在 ox-LDL 诱导的血管内皮细胞氧化损伤中的作用及丹参酮 IIA 的干预

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摘要：目的：通过细胞实验研究 galectin-1 在 ox-LDL 诱导的人脐静脉内皮细胞氧化损伤中的作用及丹参酮 IIA 的调控机制。 **方法：**体外培养 HUVEC， galectin-1siRNA 转染细胞，并用不同浓度的丹参酮ⅡA 和 60mg/L ox-LDL 处理 HUVEC 细胞 24 h。检测 HUVEC 细胞内丙二醛（Malondialdehyde，MDA）含量和活性氧（Reactive oxygen species，ROS）含量的变化；qPCR 法检测不同组细胞 ICAM-1、VCAM-1、Galectin-1 mRNA 含量；Western Blot 法检测不同组细胞 ICAM-1、VCAM-1、Galectin-1 的蛋白含量。**结果：**荧光定量 PCR 和 Western Blot 法检测结果显示，ICAM-1，VCAM-1 及 galectin-1 mRNA 和蛋白质水平在内皮细胞氧化损伤过程中上调，丹参酮ⅡA，galectin-1 si RNA 处理后，ICAM-1，VCAM-1 及 galectin-1 mRNA 和蛋白质表达水平均下调。 **结论：**丹参酮 IIA 可能通达调节 galectin-1 的表达，保护 ox-LDL 诱导的血管内皮细胞氧化损伤。

关键词：Galectin-1；HUVEC；Ox-LDL；丹参酮ⅡA

项目基金：浙江省自然基金项目 (LY13H270005、LQ14H280004)，国家自然科学基金项目（81102841）

Map-based cloning of a spotted-leaf mutant gene *OsSL5* in *Japonica* Rice

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Abstract: A *Japonica* rice mutant, spotted-leaf 5 (*sl5*), was identified from YUN32 by EMS mutagenesis. The number of spots in leaves increased from maturity to late maturity in *sl5*, however, the leaves did not dry and

withered. The *sl5* mutant exhibited significantly lower height, spike length, primary branch number, second branch number, and 1000-grain weight than YUN32. Genetic analysis shows that *sl5* is controlled by a single recessive gene. *SL5* was mapped into a 40-kb interval flanked by markers MX4 and MX5 on chromosome 7 by map-based cloning. Four ORFs, including one *SPL5* gene, were identified in this region. Sequencing reveals that the G base at site 3,647 of the *SL5* coding region was changed to A. The mutant *SL5* site was different from that of the *SPL5* mutant, with the background of *indica* rice. *SL5* is thus a new *SPL5* allele which encodes a putative splicing factor 3b subunit 3. QPCR shows that *SL5* expression in the *sl5* mutant is significantly lower than that in YUN32. The spotted leaf-related genes *RLIN1*, *SPL28*, and *SPL18* expressions were significantly decreased, whereas the *SPL7* and *SL* gene expressions significantly increased. The *SL5* gene may be important for rice cell apoptosis regulation.

基于肿瘤干细胞的抗体药物与肿瘤靶向治疗

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肿瘤干细胞是指肿瘤细胞群体中的未分化细胞，能够自我更新及无限增殖；通常具有正常干细胞样的多潜能性，可以分化产生异质性的肿瘤细胞及组织，对于传统的化疗药物具有耐药性。肿瘤干细胞与正常干细胞有一定的差异，如某些信号通路异常活化、细胞表面表达特异的分子等。针对肿瘤干细胞的这些特性，科学家们提出新的肿瘤治疗策略，即通过设计特异的抗体药物靶向信号通路或者细胞表面分子等，从根源上杀死肿瘤起始细胞。从而达到彻底治愈恶性肿瘤的目的，目前针对不同信号通路如 Notch 和 Wnt，或肿瘤细胞表面标志分子如 EpCAM 和 CD44 等抗体药物处于临床研究阶段。抗体药物包括：（1）单克隆抗体及其片段，如单链抗体（single chain antibody fragment, scFv）、双特异抗体（bispecific antibody）等；（2）抗体与毒素或化学物质形成的偶联物（antibody-drug conjugates, ADCs），（3）免疫毒素（immunotoxins），即抗体和毒素蛋白融合形成的免疫毒素。抗体药物可以通过抗体介导的细胞毒性反应（ADCC）、补体介导的细胞毒性反应（CDC）和阻断细胞生长的信号通路或者诱导细胞凋亡等机理来杀伤肿瘤细胞。

我们实验室已经克隆和表达了胶质瘤等肿瘤干细胞的标志分子 CD133 胞外结构域，利用噬菌体抗体库，筛选单链抗体，进而通过基因工程方法，在 CHO 细胞体系中表达完整抗体，在体外培养的肿瘤细胞中并在移植性小鼠模型中进行抗肿瘤作用研究。

寻找能够在肿瘤细胞中表达的标志分子设计抗体药物，对于恶性肿瘤的临床治疗及新药研发都具有重要的意义。

国家自然科学基金(批准号: 81430081)、浙江省重大科技专项（计划编号：2009C13041）资助

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抗 CD33 单链抗体及免疫毒素的制备及活性检测

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白血病严重威胁人类的健康。据统计，我国白血病的发病率在各种肿瘤中居第六位。目前针对白血病的治疗仍主要采用骨髓移植和化疗等方法，但存在术后复发、毒副反应等问题。抗体免疫疗法是治疗白血病的新方法, 具有毒副作用低、特异性强等优点，引起广泛重视。近年来随着抗体人源化技术的进步，抗体药物在肿瘤临床治疗领域取得了突破性进展, 并逐渐成为全球生物制药领域的热点。

本实验室以构建好的全人源白血病噬菌体抗体库为基础，成功筛选出与白细胞分化抗原 CD33 胞外区蛋白特异性结合的单链抗体（scFv）。表达与 CD33 胞外区蛋白结合性高的 scFv 并检测其相关活性；构建 scFv 与人颗粒酶 B（Gz B）融合的免疫毒素 scFv-Gz B，并对其进行表达及活性检测；将活性高的单链抗体及免疫毒素成功转入酵母表达体系并大量表达。

通过 ELISA 等方法测定单链抗体与 CD33 胞外区蛋白的结合力，选取结合力较强的序列分别设计相应引物，用体外 PCR 方法扩增阳性噬菌体抗体基因，通过引入酶切位点将目的片段重组至表达载体 PET30a(+)中，进而转化至 *E. coli* BL21 中，表达出单链抗体并检测其活性；同时设计相应引物及 Linker 将 scFv 转入实验室已构建并保存的人颗粒酶 B（PET30a-Gz B）重组载体中，表达免疫毒素并测定其生物学活性。结果：成功构建三株单链抗体及对应的单链免疫毒素表达载体，并在大肠杆菌和甲醇酵母体系中表达、纯化得到活性蛋白。经 ELISA 及 Western Blot 验证，所表达三株 scFv 与 CD33 均有较强的结合；经体外细胞毒性测定，这 3 种 scFv-Gz B 均对 CD33 阳性白血病细胞钧具有特异杀伤作用。为后期进一步的功能分析打下了基础，为 CD33 阳性白血病的治疗提供了可能。

国家自然科学基金(批准号: 81430081)、浙江省重大科技专项（计划编号：2009C13041）资助

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Characterization and fine mapping of the rice gene *OsARVL4* regulating leaf morphology and leaf vein development

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Abstract: Leaf morphology and chlorophyll content are closely related to the photosynthetic efficiency, which would potentially contribute to crop yield. In this study, we isolated an EMS-mutagenized rice mutant displaying abaxial rolling and vein-albino leaves, and thus designated it as *Osarvl4*. Compared to the wild type 'Nipponbare', *Osarvl4* mutant had abnormal development of clear cells, parenchyma cells, sclerenchymatous cells, mesophyll cells, bulliform cells and vascular bundles. As a result of the defective leaf development, the chlorophyll content and photosynthetic efficiency were significantly affected in the mutant. Genetic analysis using map-based cloning indicated that the mutation was controlled by a single recessive karyogene localized within a 44 kb region on the long arm of chromosome 4. Sequence analysis and alignment indicated that the three candidate genes in this region showed no difference at the DNA level. However, quantitative real-time PCR analysis showed that the expression of the LOC_Os04g33580 gene in the mutant was significantly lower than that of wild type, while expression of the other two candidate genes (LOC_Os04g33560 and LOC_Os04g33570) exhibited no significant difference. Therefore, we speculate that LOC_Os04g33580 might be the target gene which regulates leaf vein development and leaf morphogenesis in rice and this locus might be subject to epigenetic regulation, such as DNA methylation, Thus, our finding suggests that the *OsARVL4* gene is involved in the regulation of chlorophyll content and photosynthetic efficiency in plants, and provides a genetic basis for the future study of genes related to leaf development in rice.

Fine Mapping and Function Analysis of (*Semi-dominant Dwarf1*) *Si-dd1* Gene in Rice (*Oryza sativa* L.)

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Abstract: A rice mutant characterized by Semi-dominant Dwarf (named as *Si-dd1*) was obtained from *japonica* rice variety Nipponbare by tissue culture mutagenesis. Morphological analysis showed that, *Si-dd1*(AA) and *Si-dd1*(Aa) exhibited shorter plant height, decreased seed setting rate, increased seedling stage, primary branch and secondary branch compared to the wild type (Nipponbare). Moreover, it showed that both the mutant *Si-dd1*(AA) and wild type are increased in height by treating with exogenous hormone GA_3, however no obviously reaction to BR. Western blot showed GID2 expression between *Si-dd1* and wild type is not related to GA. In addition, the cytological analysis showed that *Si-dd1* has smaller stomas in the leaf midrib, increased

mesophyll cells and stem vascular bundles, and dense cells compared to wild type. Genetic analysis and map-based clone showed that the *Semi-Dominant Dwarf* (*Si-dd1*) is controlled by a single semi-dominant gene, which locates in a 244 kb region on chromosome 6.

Key words: Rice, semi-dominant, dwarf, genetic analysis, map-based clone

猪流行性腹泻病毒全长 S1 蛋白在昆虫细胞中的表达及鉴定

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摘要:猪流行性腹泻由猪流行性腹泻病毒(PEDV)引起的一种高度接触性肠道传染病，临床表现为呕吐、腹泻和食欲下降，各阶段猪均易感，日龄越小，发病率和病死率越高。1971 年首发于英国，并相继在欧洲、亚洲等多个国家爆发，给各国养猪业带来严重的经济损失。

PEDV 主要结构蛋白包括：纤突糖蛋白(S)、膜蛋白(M)、核衣壳蛋白(N)和小包膜蛋白(E)。其中，S 蛋白包括 S1 区和 S2 区，S1 暴露于病毒表面，主要识别、结合宿主细胞表面受体，是介导中和抗体产生的主要抗原决定簇。故，S1 蛋白是 PEDV 基因工程疫苗研究中最重要的一个靶蛋白。本研究拟构建 S1 重组杆状病毒在昆虫细胞中高效表达或病毒表面展示 S1 蛋白，建立以 S1 蛋白为靶标的猪流行性腹泻免疫检测方法，研制重组 S1 蛋白亚单位疫苗，或者 PEDV 病毒样颗粒（VLP）作为疫苗。

本研究化学合成 S1 基因，利用 Bac-to-Bac 系统构建 S1 重组杆状病毒，通过报告基因、间接免疫荧光、免疫印迹及胶体金免疫电镜分析全长 S1 蛋白的表达及在细胞和病毒膜上的定位。报告基因及间接免疫荧光分析表明，S1 蛋白可分泌表达于细胞质或者细胞表面；免疫印迹分析重组病毒感染的 sf9 细胞样品表明，S1 兔多抗、报告基因或者融合标签单抗均能检测到相应大小的单体或者多聚体目的蛋白带。胶体金免疫电镜分析表明，重组病毒表面能够附着金颗粒。

全长 S1 蛋白能够通过杆状病毒表达系统实现在 sf9 细胞中的高效表达；S1 蛋白可通过融合分泌表达展示在病毒表面；在 S1 蛋白的羧基端融合报告基因后可显著提高表达量。下一步，我们将制备 S1 蛋白样品，检测猪场送检血清中 PEDV 抗体，验证昆虫细胞表达的 S1 蛋白作为靶标的检测效果；同时扩增并分离表面展示 S1 蛋白的重组病毒（VLP），与 S1 蛋白、CV777 分别免疫接种小鼠，分析抗血清的中和能力。

关键词：PEDV，S1，重组杆状病毒，昆虫细胞

Thymosin from Bombyx mori Upregulated by BmNPV Showing Antivirus Function

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摘要：胸腺素分布于多个物种，在进化过程中高度保守，对动物免疫调节及抗病防病具有重要作用。本实验通过分子和细胞学分析以及药效学研究，试图揭示家蚕胸腺素抗 BmNPV 的作用机制。qRT-PCR 实验证明经过接种 BmNPV 处理的家蚕，其体内各组织的 BmTHY 的转录量都有明显的变化，但是变化的趋势是不一样的。细胞学实验发现给药组细胞在透光性、完整性、形态性等生长状态方面明显比病毒对照组要好。BmTHY 可以提高家蚕的抗病毒能力，该蛋白的开发利用对家蚕资源的保护和新品种的培育具有一定意义。

关键词：家蚕胸腺素，表达，纯化，BmN，BmNPV

Transcriptome Analysis of White Spotted Bamboo Shark Liver and Identificationof Differentially Expressed Transcripts in Response to Liver Regeneration

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Abstract

Background:The white spotted bamboo shark (Chiloscyllium plagiosum) is an economically important cartilaginous fish and marine animal model for liver regeneration.However, the

molecular mechanisms underlying liver regenerationin cartilaginous fish remain unclear because of limited information on the white spotted bamboo sharkgenome.This study aims to characterize the shark normal liver transcriptome and to identify the differentially expressedtranscripts at 3 h after 70% hepatectomy using Illumina–Solexa sequencing.

Results:After removal of low-quality sequences and assembly, a total of 108,855 unigenes was obtained from the normalliver group. Further blast analysis showed that 65,356 unigenes successfully matched the known genes in the database. GOanalysis revealed that 9,989 unigenes took part in 50 categories of biological processes, cellular components, andmolecular functions. Among the 25 clusters of orthologous group categories (KOG), the cluster for ‘‘General function

prediction only’’ represented the largest group, followed by ‘‘Signal transduction mechanisms’’ and ‘‘Posttranslational modification, protein turnover, chaperones’’.KEGG analysis showed that 10,313 unigenes were involved in 36 categories. Through comparison of normal and the liver after partial hepatectomytranscriptome data, we identified 19 significantly differentially expressed unigenes, which were further confirmed by real-time

polymerase chain reaction. Of the 19 unigenes, ten matched the known genes in the database(slc22a16, Slc39a4 ,Slc1a2 ,ALDH1L2, NR5A2, hoga1, ESR2 , MAT1A , DIO1 , Atp2a2 , LRP1). The remaining nine novel unigenes that did not match any known genes in the databasemay provide a basis for the discovery of new transcripts associated with liver regeneration.

Conclusion:This study provided a gene expression pattern for normal shark liver and for the previously unrecognizedchanges in gene transcription that are altered during liver regeneration. Our data revealed useful information for futurestudies on the white spotted bamboo shark genome and provided new insights into the molecular mechanism of liver regeneration.

The Detection of piRNAs in Whitespotted Bamboo Shark's Liver

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Abstract: Piwi-interacting RNAs (piRNAs) are 26-31 nt small non-coding RNAs. piRNAs has been reported mostly in germ-line cells and cancer cells. However, the reports about piRNAs in whitespotted bamboo shark's liver haven not been shown yet. In the previous study of microRNAs in shark's liver, some piRNAs were detected from small RNAs sequenced by Solexa technology. A total of 4857 piRNAs were found and predicted from shark's liver. We further selected 17 piRNAs with the high and significantly differential expression for normal and regenerative liver tissues to verify by Northern blotting. And 10 piRNAs were further identified - six of them were matched to the knownpiRNAs from piRNA Bank. The actual expression of six known and four novel piRNAs were validated by qRT-PCR. At the same time miRanda was used to predict the potential target genes of the10 piRNAs, and subjected them to GO and pathway analysis. The results indicated that piRNAs were involved in many important biological responses including immune inflammation, cells specific differentiation and development, angiogenesis. This study firstly reports piRNAs in the liver of whitespotted bamboo shark by Solexa technology, and provides a useful resource and further elucidation of regulatory role of piRNAs in whitespotted bamboo shark's liver and also may facilitate the development of therapeutic strategies for liver damage.

Keywords： piRNAs**;** whitespotted bamboo shark; Solexa technology;

Preparation and activity analysis of Sparassis crispa glucan

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Abstract: Sparassis crispa glucan (SCG) have anti-humor activity , clinical trials demonstrated that it have inhibition effect on lung cancer, gastric cancer, colon cancer, breast cancer and other cancers . Therefore, it is likely to be candidate medicine in the future . while , the low efficiency method of extract SCG and complex method for SCG activety detection make it difficult . in this research , first , we establish a new method for glucon activitydetection , second , we successfully get a Sparassis crispa specie , thenoptimize method for preparation of low molecular weight Sparassis crispa glucan ,third , we isolate and purify a single Sparassis crispa glucan and detect its activity by the method we established for further research .

Keywords : Sparassis crispa ; glucan ; activity detection

Transposable Element (TE) Bm_1645 ProducesBmAGO2-Associated Small RNAs to Perform the Function

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Abstract： Transposable element (TE) is a DNA sequence which can change position itself and plays an important role in maintaining the stability and diversity of organisms by transposition. Recent studies have shown that there are nearly half of the genes are TEs in *B. mori*. Here, we performed a systematic identification and analysis of the BmAGO2-associated TEs with abundance more than 100 and their mapped small RNAs also associated with BmAGO2. We identified that Bm_1645 was associated with BmAGO2 with the highest abundance, as the same with the situation of small RNAs, and was regarded as a small RNAs pool. We chose a few small RNAs associated with Bm_1645 and performed northern blotting identifying that some of chosen small RNAs had obvious expression differences in normal and ie1-bacmid-pIEx-1-BmAGO2-infected individuals of different development periods of silkworm. With that, we found 4 TE-siRNAs could combine with BmAGO2 according to the EMSA experiment. Using qRT-PCR, we also identified that the up-regulation of the TE-siRNAs of those four in BmN cells could induce the reduced expression of Bm_1645. Except that, the result showed the evidence that

the transcriptional level of Bm_1645 would be up regulated with the down-regulation of BmAGO2 in the BmN cells. Our analysis suggested that Bm_1645 just like a pool of small RNAs, it can produce BmAGO2-associated small RNAs. With the help of the small RNAs, it may be modulated by different mechanisms to generate different products with diverse function. The specific way of regulation is still need further research.

Key words: Tes, Bm_1645, TE-siRNA, BmAGO2-associated

家蚕 N-乙酰转移酶的克隆表达以及功能研究

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摘要：人类的乙酰转移酶参与各种疾病、损伤过程，比如阿尔茨海默病、溃疡性结肠炎、老年痴呆、肌肉损伤等等，家蚕乙酰转移酶的缺失对色素沉积也有一定作用，同时控制着生理节奏，比如光周期的调节控制；

通过原核表达产生大量的目的蛋白 *Bombyx mori N-acetyltransferase* 产物，经过镍柱纯化，在动物体内免疫产生抗体，利用抗体观察它在各组织和家蚕各时期的组织分布情况，提取总 RNA，通过 qPCR 检测目的基因在不同组织和时期的 RNA 水平变化，在细胞中加以过表达和抑制分别观察细胞状态以阐明其功能，通过免疫沉淀和免疫印迹检测自身乙酰化；

家蚕 *Bombyx mori* 作为鳞翅目昆虫的模式生物，又是一个经济昆虫 ,目前对其转录调控机制研究还比较少 ，而且家蚕中有关乙酰转移酶基因方面的报道也很少。我们根据已公布的 N-乙酰转移酶基因进行了生物信息学分析、克隆、测序和原核表达,并研究了其在家蚕不同时期和组织器官的表达谱,为进一步研究家蚕的转录调控及其在家蚕中的功能提供了参考和依据。

关键词:乙酰转移酶，表达谱，乙酰化

灵芝小 RNA 的分离与鉴定

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摘要：灵芝，为多孔菌科真菌赤芝，是一种珍贵的药食两用菌。迄今为止国内外研究发现其内的活性成分有：多糖、生物碱、蛋白质、脂肪、核苷、甾醇、氨基酸、三萜类、微量元素等等。在这些成分的药用价值上的研究已非常成熟，而对于其内小 RNA 的研究几乎没有。如在抗肿瘤、免疫调节、血糖血脂调节、血压调节、抗衰老等药理机制中灵芝多糖和蛋白起主导作用，并且在它们生理机理的研究上都比较成熟，而对于这些机制在小 RNA 方面的研究非常稀少。本研究旨在从小 RNA 这个角度对灵芝进行初步研究，对灵芝进行分离与鉴定，为灵芝在药用上的研究探索新的思路，给将来灵芝医学药学等领域开辟新的道路。本研究主要内容是：采集新鲜的灵芝子实体，进行小 RNA 提取，对小 RNA 进行浓度和纯度测及电泳检测，其后对小 RNA 进行高通量测序及生物信息分析，再对测序结果进行 qPCR 和 Northern blotting 验证。这是首次对灵芝 miRNA 进行完整的报道。

关键词：灵芝；小 RNA；高通量测序；qPCR；Northern blotting

抗 CD3 单链抗体-MAP30 的构建和表达研究

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近年来，抗体药物在临床肿瘤靶向治疗领域取得突破性进展。至今，美国 FDA 已经批准十多种抗体及其偶联药物上市。筛选和构建人源性抗体是抗体药物开发的源头。

本论文首先从本实验室已构建好的全人源白血病噬菌体单链抗体库中筛选出与白细胞分化抗原 CD33 胞外区蛋白特异性结合的单链抗体（scFv），表达并鉴定 scFv 蛋白；构建 scFv 与苦瓜蛋白 MAP30 偶联的用于治疗白血病的免疫毒素，并对其进行鉴定分析。

将靶抗原 CD33 胞外区蛋白结合于固相介质 Ni-NTA 亲和层析柱上，加入噬菌体单链抗体库与之孵育，经过多次洗涤、洗脱、扩增噬菌体单链抗体，进行 4 轮富集筛选；随机挑取第四轮筛选后的噬菌斑制备单克隆噬菌体抗体送测，对测序正确，能够通读的噬菌体抗体进行 ELISA 检测，鉴定其对靶抗原的结合力；选取 ELISA 阳性最高值的单链抗体噬菌体侵染 *E.coli* HB2151，经 IPTG 诱导后进行 scFv 蛋白的可溶性表达，并用 Western blot 对其进行鉴定；设计相应引物和 linker，用 PCR 方法扩增阳性的噬菌体抗体基因，重组至 pET30a(+)-MAP30 表达载体中，转化至 *E. coli* TG1，表达出单链免疫毒素（sc-IT）；通过改变温度和 IPTG 的浓度对 sc-IT 的表达条件进行优化，最终得到目的蛋白的表达。

结果：噬菌体抗体库经过 4 轮的筛选后，结果获得 533 倍的富集。经测序和 ELISA 验证后，共获得 3 株与 CD33 特异性结合较强的噬菌体抗体；经 IPTG 诱导表达，获得表达于细菌周质的可溶性 scFv 蛋白，鉴定后为正确。构建好的 pET30a(+)-sc-IT 表达载体经原核表达，获得表达于上清或包涵体的 sc-IT 蛋白，经鉴定正确。

结论：经筛选获得具有较强特异性的噬菌体单克隆抗体，并表达获得可溶性 scFv 蛋白。成功构建单链免疫毒素，并获得表达，为免疫毒素进一步的功能分析与检测打下了基础，为 CD33 阳性白血病的治疗提供了可能。

国家自然科学基金(批准号: 81430081)、浙江省重大科技专项（计划编号：2009C13041）资助

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耐药性的实验研究

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摘要：目的 明确水飞蓟素(Silymarin，Sily) 对人乳腺癌耐药细胞株 MCF-7/ADM 的逆转耐药作用。**方法** 以 CCK-8 法测定阿霉素（Adm）对人乳腺癌敏感细胞株 MCF-7/S 和耐药细胞株 MCF-7/ADM 的毒性作用，计算出耐药倍数。以无细胞毒性的 Sily（10μg/ml）作为逆转耐药剂，联合 Adm 观察其对耐药细胞株 MCF-7/ADM 的逆转耐药作用，计算得逆转倍数。**结果** ①Adm 对 MCF-7/S 和 MCF-7/ADM 的半数抑制浓度(IC_{50})分别为 1.773 μg/ml 和 45.774 μg/ml，耐药倍数为 25.8 倍。②Sily 能够增强 ADM 对 MCF-7/ADM 的细胞毒作用。以 10μg/ml（抑制率为 2.0%）的 Sily 联合 Adm 作用于 MCF-7/ADM 48h 后，耐药细胞株的 IC_{50} 降至 8.101 μg/ml，逆转倍数为 5.7 倍(P <0.01)。**结论** Sily 能够逆转人乳腺癌耐药细胞株 MCF-7/ADM 的耐药性。

关键词： 乳腺癌；水飞蓟素；逆转耐药；CCK-8

Reversal Effect of Silymarin on Multidrug Resistance Breast Cancer Cell MCF-7/ADM

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Abstract : Objective To explore the effect of silymarin on Adriamycin resistance of breast cancer cell line . **Methods** To measure toxicant effect of Adm to MCF- 7/S(sensitive strain) and MCF-7/ADM (multidrug-

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resistant strain) for resistant index by CCK-8 in vitro ; to observe the reversal effect of silymarin （10μg/ml） which has screened for nontoxicant to MCF- 7/ADM by CCK-8.**Results** ①IC_{50} of Adm to MCF-7/S and MCF-7/ADM were 1.773 μg/ml and 45.774 μg/ml respectively .Drug-Resistant index of MCF-7/ADM was 25.8 .②Silymarin could increase cell toxic effect of Adm. After treated with 10μg/ml silymarin （Inhibition rate was 2.0%） combining with Adm to MCF- 7/ADM for 48 hours , cell's IC_{50} declined to 8.101 μg/ml, and reversal index is 5.7 ($P < 0.01$). **Conclusion** Silymarin could reduce partly revese multidrug- resistance to Adm of MCF-7/ADM in vitro.

Key words : Breast carcinoma ; Silymarin ; Reversing drug-resistance ; CCK-8

人源白血病单链抗体库的构建及筛选

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本课题的目的是构建全人源白血病噬菌体单链抗体库，并从中筛选出与白细胞分化抗原 CD33 胞外区蛋白特异结合的单链抗体，表达单链抗体 scFv 蛋白，并对其进行鉴定分析。

通过分离白血病病人外周血淋巴细胞，提取总 RNA，RT-PCR 合成 cDNA 第一链，使用 32 条引物 PCR 扩增抗体重链可变区（VH）和轻链可变区（VL）基因。使用重叠 PCR（SOE-PCR），将 VH 和 VL 基因通过 linker 连接成单链抗体(scFv)基因，再重组至噬菌粒载体 pCANTAB-5E 中，转化至 E. coli TG1，经辅助噬菌体超感染，构建全人源白血病噬菌体单链抗体库，挑取 20 个单克隆送测序；PCR 扩增急性髓系白血病(acute myeloid leukemia, AML)细胞表面高表达抗原 CD33 的胞外区基因，插入质粒 pET28a(+)，转化至表达菌 *E. coli* Rosetta，表达出 CD33 胞外区蛋白。将 CD33 胞外区蛋白结合于 96 空 ELISA 板，单链抗体库与其孵育后，经多次洗涤、洗脱、扩增噬菌体单链抗体，进行 4 轮富集筛选；选取第四轮筛选后的菌落制备单克隆噬菌体抗体，测序正确的进行 ELISA 特异性鉴定和免疫荧光鉴定；选取阳性值最高的单链抗体噬菌体侵染 *E. coli* HB2151，经 IPTG 诱导表达可溶性 scFv 蛋白，用蛋白印迹的方法鉴定 scFv 蛋白。

结果表明：构建出库容为 1.5×10^9 的噬菌体单链抗体库，测序后序列经分析证明 scFv 基因片段插入率达 99%，抗体库多样性良好。噬菌体抗体库经 4 轮筛选后，结果显示富集了 100 倍。经 ELISA 验证、并测序后，共获得 7 株与 CD33 较强特异性结合的噬菌体抗体并表达获得可溶性 scFv 抗体蛋白，为白血病的诊疗打下基础。

国家自然科学基金(批准号: 81430081)、浙江省重大科技专项（计划编号：2009C13041）资助

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口服霍乱毒素 B 亚基与 Aβ42 融合蛋白治疗阿尔茨海默症研究

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阿尔茨海默症（Alzheimer’s disease，AD)是以记忆力减退、认知功能障碍为特征的中枢神经系统变性疾病。AD 的病理学特征主要包括脑中神经细胞外淀粉样多肽（β-amyloid peptide，Aβ）的沉积导致的老年斑，细胞内过度磷酸化 Tau 蛋白导致的神经纤维缠结和神经细胞元细胞的缺失营养坏死。以 Aβ 为靶点，通过主动免疫或者被动免疫清除脑内 Aβ 对阿尔茨海默症症状有显著的改善，然而注射 Aβ 蛋白不仅对患者带来很大痛苦，而且还会有副作用。在本研究中，我们构建了霍乱毒素 B 亚基（CTB）与人 Aβ42 融合基因，并在家蚕表达系统中高效表达该蛋白，评估了口服该融合蛋白对阿尔茨海默症的预防和治疗效果。我们的研究结果表明口服 CTB-$(Aβ42)_2$ 融合蛋白能显著减少大脑海马区和皮层中 Aβ42 的浓度和老年斑的面积，降低细胞内 Tau 蛋白过度磷酸化程度，改善 AD 模型小鼠的学习记忆能力。另外，口服 CTB-$(Aβ42)_2$ 融合蛋白并没有改变脑内 IL-4，IFN-γ

IL-2，IL-10 等细胞因子的浓度。因此，结合家蚕生物反应器，口服 CTB-$(Aβ42)_2$ 融合蛋白为我们提供了一条经济有效而且低副作用的防治阿尔茨海默症的方法。

关键词：阿尔茨海默症；淀粉样多肽；霍乱毒素 B 亚基；口服

RNA 二级结构对可变剪接调控的作用机制研究

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RNA 可变剪接的调控对生物细胞分化和发育以及疾病发生等至关重要，但对它的作用机制还缺乏了解。大量研究表明，RNA 编辑， RNA 干扰，催化 RNA 等重要作用都依赖着 RNA 分子内和分子间的结构。同样 RNA 二级结构通过不同的机制影响 RNA 分子的剪接。但是，之前关于 RNA 二级结构对剪接影响的研究主要集中在 RNA 二级结构对剪接位点识别的调节上。通过比较基因组分析表明内含子顺式元件可以用来调控 mRNA 前体的互斥剪接，提出了一种较广泛适用外显子互斥剪接调控的机制，并对 RNA 可

变剪接的 RNA 顺式元件与反式作用因子相互作用进行了深入研究。

应用 CRISPR-Cas9 技术研究 Dscam 基因互斥可变剪接的调控机制

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CRISPR/Cas9（Clustered Regularly Interspaced Short Palindromic Repeats）是最新出现的一种由 RNA 指导的 Cas9 核酸酶对靶向基因进行编辑的技术。目前该技术已被应用于多种生物，包括人、小鼠、大鼠、斑马鱼、秀丽隐杆线虫、植物及细菌等。Dscam 基因是唐氏综合症细胞粘附分子(Down Syndrome Cell Adhesion Molecule)的简称,该基因包含着多个可变外显子簇，通过可变剪接可以产生 38016 种不同 mRNA 和蛋白质的异构体。RNA 可变剪接是真核生物基因转录后表达调控的重要环节,可增加蛋白质的多样性与生物体的复杂性,其中 RNA 互斥可变剪接是一种最复杂的类型,其详细机制还有待研究。我们课题组之前的研究中发现 Dscam 的 4 号外显子簇和 9 号外显子簇中具有结构上相似的区域，称为结合位点（IEa），该基因的外显子互斥剪接就是通过这段结构相似且保守的 RNA 竞争互补实现的，结合位点（IEa）序列的突变，会导致外显子簇中所有外显子都无法被剪接，且目前发现的这些外显子中仅存在一个 IEa。我们利用 CRISPR-Cas9 技术敲除，将 Dscam 基因 4 号和 9 号外显子的结合位点（IEa）分别敲除，得到的基因突变的果蝇，并将其与野生型果蝇做比较。利用 RNA-seq（转录组测序技术）检测基因突变果蝇和野生型果蝇的 mRNA, 小 RNA ,和一些非编码 RNA 等序列的差异，从整体的转录水平检测结合位点（IEa）的突变对果蝇 Dscam 基因表达的影响。

关键词：CRISPR-Cas9 技术；RNA 互斥可变剪接；结合位点（IEa）；转录组测序

Nuclear Translocation and Activation of YAP by Hypoxia Contributes to Chemoresistance of SN38 in Hepatocellular Carcinoma Cells

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Abstract: Hypoxia was a prominent feature of hepatocellular carcinoma cells (HCC), contributing to therapeutic resistance towards a variety chemotherapeutic agents including Topoisomerase I inhibitor SN38, with mechanism not yet fully understood, thus remaining a major clinical challenge. Herein, we present evidences that the hypoxia-induced nuclear translocation and accumulation of Yes-associated protein (YAP) acts as a survival input to promote hypoxic-resistance to SN38 in HCC. YAP induction by hypoxia was not mediated by HIF-1α, since the

manipulation of HIF-1α either by CoCl2, exogenous expression nor siRNA of HIF-1α imposed any effect on the phosphorylation or total level of YAP. Instead, mevalonate-HMG-CoA reductase (HMGCR) pathway may modulate the YAP pathway under hypoxia. Combined YAP inhibition by either siRNA or HMGCR inhibitor statins with SN38 achieved improved anti-cancer activities in HCC cells. Moreover, the increased anti-cancer efficacy of statins combined with irinotecan (the prodrug of SN-38) was further validated in a human HCC HepG2 xenograft model in nude mice. Taken together, our findings identify YAP as a novel mechanism of hypoxic-resistance to SN38. These results unveil the combined suppression of YAP (for instance, statins) and SN38 as a potential promising strategy to enhance treatment response of HCC patients, particularly those with advanced stage suffering from hypoxic resistance.

Keywords: Hepatocellular Carcinoma, SN38, Hypoxia, resistance, Yes-associated protein (YAP), statins

Folate Metabolism Regulates Oligodendrocyte Survival and Differentiation by Modulating AMPKα Phosphorylation

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Objectives: Folate, an essential micronutrient, is anessential cofactor in one-carbon metabolism for many cellular pathways including DNA synthesis, metabolism and maintenance. Folate deficiency has been associated with an increased risk of neural tube defects, cancer and motor and cognitive dysfunction. Dihydrofolate reductase (DHFR) is a key enzyme that regulates folate metabolism; however folate/DHFR activity in oligodendrocyte development has not been fully understood. Our preliminary studies have found that folate deficiency caused an obvious delay in oligodendrocytes development in vivo. Based on this, the study aims to explore the regulation of folate/DHFR on oligodendrocytes development and myelination in detail,which not only gives evidence for the function of folate in the CNS development, but also enriches the network of oligodendrocytes regulatory factors, broadening the vision and direction for the clinical treatment of demyelinating disease. **Methods:**1) In situ hybridization, immunostaining, qPCR, western blot analysis were carried out to detect oligodendrocytes development. 2) The effect of folate downstream DHFR on oligodendrocytes and myelin sheath was determined by immunostaining, transmission electron microscope, RT-PCR and western blotting. 3) The proliferation, differentiation and apoptosis of oligodendrocytes caused by folate/DHFR inhibition were detected by immunostaining and western blot. 4) The effects of AMPK signaling pathway on oligodendrocyte regulated by folate metabolism was used to examine by western blot, Immunostaining and qPCR. **Results:** Diet with low-folate during pregnancy blocked oligodendrocytes development, while diet with high-folate facilitated oligodendrocytes development. Consistently,

folate was required for the maturation of primary oligodendroglia progenitor cells. Pharmacological inhibition of DHFR by methotrexate blocked oligodendrocytes differentiation and resulted in severe myelination deficiency in vivo, which could be partially reversed by folate supplement. Folate/DHFR inhibition caused oligodendrocytes apoptosis and differentiation disorders, but had no effect on the proliferation of oligodendrocytes. Folate/DHFR activated AMPKα signaling pathway through upregulating p-AMPKα and AMPKα expression in oligodendrocytes. AMPKα activation or inhibition regulated oligodendrocytes differentiation in vitro. AMPKα activators MET increased the expression of oligodendrocytes markers and myelin sheath compared with DHFR inhibition in vivo and in vitro. **Conclusion:** These findings here identify a previously uncharacterized role of folate/DHFR/AMPKα axis in regulating oligodendrogenesis and myelination during CNS development.

The E3 Ubiquitin Protein Ligase MDM2 Dictates ATRA-induced Osteoblastic Differentiation of Osteosarcoma cells by Modulating the Degradation of RARα

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Abstract: Objective: Retinoic acid receptor alpha (RARα) plays a critical role in the differentiation process of osteosarcoma cells induced by all-trans retinoic acid (ATRA). However, degradation of RARα through ubiquitin proteasome pathway weakens the differentiation efficiency of osteosarcoma cells. Therefore, it is necessary to explore the regulatory mechanisms involved in RARα degradation.

Methods: U2OS cells were mainly used as our research models. (1) Protein levels were detected by Western blotting; (2) The interactions between MDM2 and RARalpha were determined by immunofluorescence and immunoprecipitation; (3) Related proteins and genes were investigated via transfection; (4) The expression levels of MDM2 and OPN in patient biopsies were detected by immunohistochemistry; (5) Alkaline phosphatase activity was assessed by colorimetric assays using the BCIP/NBT Alkaline Phosphatase Color Development kit.

Results: In this report, it demonstrated that interaction with MDM2 leads to strong stimulation of RARα polyubiquitination and degradation by proteasomes. MDM2 appears to function as an ubiquitin E3 ligase in this process, since the MDM2 RING domain mutant inhibits the ubiquitination of RARα. Furthermore, MDM2 is capable of stimulating RARα polyubiquitination under cell-free conditions. Moreover, it also provided evidence that silencing or inhibiting MDM2 promotes the differentiation of U2OS cells as induced by ATRA.

Conclusions: MDM2 serves as an E3 ubiquitin ligase to regulate the degradation of RARα and becomes a novel therapeutic target for ATRA-based differentiation therapeutic approaches in osteosarcoma.

Keywords: Osteosarcoma; RARα; degradation; MDM2; All-trans retinoic acid; differentiation

Gefitinib synergizes with irinotecan to suppress hepatocellular carcinoma by promoting proteasome dependent degradation of Rad51

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Abstract: Chemotherapy is the only choice for most of the hepatocellular carcinoma (HCC) patients, while few agents were available, making it an urgent need to develop new chemotherapy strategies. A phase II clinical trial suggested that the efficacy of irinotecan in HCC was limited due to dose-dependent toxicities. Here, we found that gefitinib exhibited synergistic activity in combination with SN-38, an active metabolite of irinotecan, in HCC cell lines. And the enhanced apoptosis induced by gefitinib plus SN-38 was a result from caspase pathway activation. Mechanistically, gefitinib dramatically promoted the ubiquitin–proteasome-dependent degradation of Rad51 protein, suppressed the DNA repair, gave rise to more DNA-damages, and ultimately resulted in the synergism of these two agents. In addition, the increased antitumor efficacy of gefitinib combined with irinotecan was further validated in a HepG2 xenograft mice model. Taken together, our data demonstrated for the first time that the combination of irinotecan and gefitinib showed potential benefit in HCC, which suggesting that Rad51 is a promising target and providing a rationale for clinical trials investigating the efficacy of the combination of topoisomerase I inhibitors and gefitinib in HCC.

Keywords: hepatocellular carcinoma; irinotecan; gefitinib; apoptosis; Rad51; DNA-damage.

Using lncRNA-ABHD11-AS1 as a novel type of biomarker in the screening of gastric cancer

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Long noncoding RNAs (lncRNAs) play vital roles in tumorigenesis. However, the diagnostic values of most lncRNAs are largely unknown. To investigate whether gastric juice lncRNA-ABHD11-AS1 can be a potential biomarker in the screening of gastric cancer, 173 tissue samples and 130 gastric juice from benign lesion, gastric dysplasia, gastric premalignant lesions, and gastric cancer were collected. ABHD11-AS1 levels were detected by

reverse transcription-polymerase chain reaction. Then the relationships between ABHD11-AS1 levels and clinicopathological factors of patients with gastric cancer were investigated. The results showed that ABHD11-AS1 levels in gastric cancer tissues were significantly higher than those in other tissues. Its levels in gastric juice from gastric cancer patients were not only significantly higher than those from cases of normal mucosa or minimal gastritis, atrophic gastritis and gastric ulcers, but also associated with gender, tumor size, tumor stage, Lauren type and blood carcinoembryonic antigen (CEA) levels. More important, when using gastric juice ABHD11-AS1 as a marker, the positive detection rate of early gastric cancer patients was reached to 71.4%. Thanks to the special origin of gastric juice, these results indicate that gastric juice ABHD11-AS1 may be a potential biomarker in the screening of gastric cancer.

Molecular mechanisms of lncRNAs on gastric cancer

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Long non-coding RNA (lncRNA) is defined as larger than 200 nucleotides without protein coding potential. Recently, deregulated lncRNAs expression has been found associated with gastric cancer. Acting as oncogenes and tumor repressor genes,some lncRNAsare involving the regulation of cell proliferation, migration, and invasion.

By complementary base pairing with mRNA or forming complex with RNA binding protein (RBP), some lncRNAssuch as TINCR,GHET1 and MALAT1 may mediatemRNA stability and splicing. Other lncRNAs, such as HOTAIR, GAPLINC and BC032469are participated in the competing endogenous RNA (ceRNA) network. Under certain circumstances, similar to H19,ANRIL, MEG3, AC130710 and TUSC7 play theirbiological roles by generating to microRNA (miRNA) or associated with miRNAs in gastriccancer cells.

By recruiting histone modifying complex,HOTAIR, ANRIL, FENDRR, PVT1, and MALAT1may regulate transcription in *cis* or *trans*.Through these mechanisms,lncRNAmay form RNA-dsDNA triplex. MEG3, TUSC7, H19, GAS5 and CCAT1play their roles by interacting with tumor suppressor protein P53 or being activated by P53 and onco-protein c-Myc. Finally, lncRNAsmay be used in the diagnosis and treatment of gastric cancer.

调节性非编码 RNA 与肿瘤糖代谢

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非编码 RNA(non-coding RNA,ncRNA)是一类不具有蛋白质编码潜能的 RNA，可分为管家 ncRNA 和调节性 ncRNA。管家 ncRNA 包括 rRNA、tRNA、snoRNA、snRNA、转运-信使 RNA(tmRNA)、引导 RNA(gRNA)和端粒酶 RNA 等；而调节性 ncRNA 主要分为短链 ncRNA 和长链 ncRNA。微 RNA(microRNA,miRNA)是研究得比较清楚的一类调节性 ncRNA，不仅可调控细胞分化、增殖和凋亡，还可通过调节糖酵解途径中的限速酶[如己糖激酶(hexokinase, HK)、磷酸果糖激酶(phosphofructokinase, PFK)和丙酮酸激酶(pyruvate kinase, PK)]来调控肿瘤细胞的糖代谢。长链非编码 RNA(long non-coding RNA, lncRNA)是另一类近年来引起重视的调节性 ncRNA，它们可通过调节癌基因 c-*Myc*、葡糖转运蛋白(glucose transporter, GLUT)、HK 和缺氧诱导因子等来调控肿瘤细胞的糖代谢。深入揭示 miRNA 和 lncRNA 等调节性 ncRNA 调控肿瘤细胞糖代谢的分子机制，不仅可以使我们更加深入地了解肿瘤的发生机制，而且可能为肿瘤的预防、诊断和治疗提供新方向。

Evolution of teleost TfR1 under fish-specific genome duplication (FSGD) and accelerated evolution in TfR genes of mammals

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Abstract: Transferrin receptor (TfR) is a homodimeric type II transmembrane protein, and plays a vital role in iron metabolism. Transferrin (TF)-bound iron is taken into cells via binding to TfR and endocytosis of the TfR-TF complex. TfR family can be mainly divided into TfR1 and TfR2, and they are all present in vertebrates except TfR2 which is missing in birds. TfR1 and TfR2 are 45% identical and 66% similar in their extracellular domains, but they are also diverse in many ways according to their difference in protein expression, affinity with diferric transferrin (TF) and functional domains. We speculated that they might possess special evolutionary characteristics. Molecular evolution analysis showed that positive selected sites existed in the ancestral lineage of mammal TfRs rather than in that of poikilotherms. And the positive selection happened in the common ancestor of teleost TfR1a (tel-TfR1a) and tel-TfR1b. The positive selected sites were also detected in the ancestral lineage of tel-TfR1b. The analysis of functional branch length, which represented the level of altered selective constraints of

member genes, showed that all mammal TfRs experienced more functional divergence than those of telesots. The difference in energy demand and aerobic metabolism tendency between endotherms and poikilotherms might induce the evolution of TfRs in endotherms. The fish-specific genome duplication (FSGD) promoted tel-TfR1b to evolve some unique functions.

Keywords: Transferrin receptor (TfR); Endotherms; Poikilotherms; Selection; Functional divergence

A mitogenomic perspective on the phylogenetic position of the *Hapalogenys* genus (Acanthopterygii: Perciformes) and the evolutionary origin of Perciformes

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Abstract: The *Hapalogenys* genus was the most controversial and problematic in its phylogenetic position of Percoidei. In this study, we recheck the taxonomic status of *Hapalogenys* in Percoidei using mitogenomic sequences data. We added a new mitogenomic sequence data from for purposefully chosen species of *Hapalogenys* and conducted phylogenetic analyses using a large mitogenomic data set. The resultant tree topologies were congruent from partitioned Bayesian and Maximum-likelihood methods. The results indicated that *Hapalogenys* was distantly related to Haemulidae and could be removed from Haemulidae. The results supported the *Hapalogeny* was upgraded to a family rank titled Hapalogenyidae, and it should be recognized in a separate family of Hapalogenyidae. A relaxed molecular-clock Bayesian analysis of the divergence times indicated that Perciformes groups had a much older than the old fossil records available for these group. The origin of the common ancestral lineage of Perciformes fish was estimated at the late Jurassic about 149 Myr ago.

Keywords: Mitochondrial genome, Molecular phylogeny, Divergence times, Relaxed molecular clock, Hapalogenys, Perciformes

Mitogenomic perspectives into sciaenid fishes phylogeny and evolution origin in the New world

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Abstract: Sciaenid fishes are widely distributed throughout the coastal waters and estuaries of the world. A total of 23 genera of this family are endemic to the Old World. However, evolutionary relationships among Old World sciaenid fishes and their origin have remained unresolved despite their diversity and importance. Besides, hypotheses that explain the origin and biogeographical distribution of sciaenid fishes are controversial. In this study, the complete mitochondrial genome sequences of seven representative sciaenid species were determined and a well-resolved tree was recovered. This new timescale demonstrated that the sciaenid originated during the late Jurrasic to early Cretaceous Period. The estimated origin time of sciaenid fish is 208 Mya, and the origin of Old World sciaenid is estimated at 126 Mya. Reconstruction of ancestral distributions indicated a plesiomorphic distribution and centre of origin in the New World, with at least one lineage subsequently dispersed to the Old World. Moreover, we conclude that the common ancestors of Old World sciaenid fishes were derived from species of New World.

Keywords: Sciaenidae; mitochondrial genome; phylogeny; divergence time; origin

Advances of IDH studies in Cancers

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Abstract: The point mutations in the active-site arginine residues of isocitrate dehydrogenase (IDH) (IDH1/R132, IDH2/R140, and IDH2/R172) occur frequently in a variety of cancers, including acute myeloid leukemia (AML), brain tumors, and holangiocarcinomas. The IDH mutants catalyze α-ketoglutarate (α-KG) to oncometabolite 2-hydroxyglutarate (2-HG), which dysregulates a set of α-KG-dependent dioxygenases. Various studies have indicated that IDH mutations are associated with DNA hypermethylation at CpG islands which are enriched for genes implicated in stem cell maintenance/differentiation and lineage specification. We herein summarize the advances of IDH1/2 studies in clinical trials: (1) A rapid, sensitive and robust assay approach (pyrosequencing) has been used to detect all types of mutation in either IDH1 or IDH2; (2) IDH1/2 mutation status could be valuable for distinguishing intracranial chondrosarcomas from chordomas; (3) Due to the prognostic information of IDH1/2 mutations, IDH1/2 has been developed as one biomarker for tumor diagnosis; (4) IDH2 mutation plays

a critical oncogenic role in proliferation, apoptosis, invasion, migration, tumorgenesis and senescence, indicating that targeting IDH mutant become a therapeutic strategy in cancers.

Keywords: IDH1, IDH2, mutation, cancers;

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Financial support from the Funding of Zhejiang Provincial Top Key Discipline of Biology, Zhejiang Public Technology Research Program (2014C33234), Science Foundation of Zhejiang Sci-Tech University (14042107-Y), China.

HSP90 Inhibition Is a Novel Therapeutic Strategy in Gastrointestinal Stromal Tumor

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Abstract: Gastrointestinal stromal tumor (GIST) is the most common sarcoma of the gastrointestinal tract. Oncogenic KIT or PDGFRA receptor tyrosine kinase mutations are compelling therapeutic targets in GISTs, and the KIT/PDGFRA kinase inhibitor, imatinib/sunitinib, is standard of care for patients with metastatic GIST. However, most of these patients eventually develop clinical resistance to imatinib and other KIT/PDGFRA kinase inhibitors and there is an urgent need to identify novel therapeutic strategies. Heat shock protein 90 (HSP90) plays a molecular chaperone role to increase the stability and activity of its client proteins including KIT and PDGFRA receptor tyrosine kinases, which also interacts with HSP70, CDC37, mutated p53, AKT, and HIF1-α proteins to regulate cell proliferation and apoptosis through controlling oncogenic protein proper folding, function, and stability. Inhibition of HSP90 and CDC37 inactivates KIT/PDGFRA and downstream intermediates, and suppresses tumor growth in GISTs. Some HSP90 inhibitors including IPI-504, IPI-493, BIIB021, STA9090, and AT13387 are carrying out clinic trials in GISTs. HSP90 inhibition highlights a novel strategy for imatinib-sensitive and -resistant GIST, irrespective of the types of multiple imatinib resistance mutations of KIT/PDGFRA.

Keywords: GIST, HSP90, resistance, imatinib

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Financial support from the Funding of Zhejiang Provincial Top Key Discipline of Biology, Zhejiang Public Technology Research Program (2014C33234), Science Foundation of Zhejiang Sci-Tech University (14042107-Y), China.

The Biological Function of Pyruvate Dehydrogenase in Cancers

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Abstract: Pyruvate dehydrogenase (PDH) is the multi-enzyme complex of mitochondrion. Its catalysis is of great importance in the process of energy metabolism. PDH catalyzes the decarboxylation of pyruvic acid to produce acetyl coenzyme A. And acetyl coenzyme A serves as the primary raw material to bring the glucose into the aerobic oxidation of tricarboxylic acid cycle. Due to insufficiency of PDH, human body suffers from lactic acidosis and atelencephalia. In general, the deficiency of PDH manifests dysfunction caused by the *PDH E_1A* gene mutation. The mutation of *PDH E_1A* changes the structure of PDH and reduces the enzymatic activity. PDH inhibition results in the tumor cells to mainly acquire energy through glycolysis. Activation of glycolysis promotes proliferation and inhibits apoptosis in cancer cells. Besides, due to glycolysis, the microenvironment of tumor can protect cells from attack of the host immune system and reduce the chemotherapy drug efficiency as well as help the invasion and metastasis in cancers. The increasing evidence has shown that PDH is expressed in 256 cases of gastric cancer patients with different level, which is associated with the development and invasion of gastric cancer. Compared with adjacent tissues, PDK-3 in colon cancer tissue is upregulated, which inhibits the activity of PDH. PDK-1 knockdown restores the activity of PDH, and inhibits proliferation and invation in head and neck squamous cell carcinoma. Furthermore, inactivation of PDH promotes the development of melanoma driven by *BRAF V600E* mutation. Herein, we update the crucial oncogenic roles of PDH in the metabolism and signaling transduction of cancer cells.

Keywords: PDH, Mutation, Glycolysis, Metabolism, Cancer

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Financial support from the Funding of Zhejiang Provincial Top Key Discipline of Biology, Zhejiang Public Technology Research Program (2014C33234), science foundation of Zhejiang Sci-Tech University(14042107-Y). China.

家蚕乙酰转移酶的克隆表达及功能研究

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摘要：人类的乙酰转移酶参与各种疾病、损伤过程，比如阿尔茨海默病、溃疡性结肠炎、老年痴呆、肌肉损伤等等，家蚕乙酰转移酶的缺失对色素沉积也有一定作用，同时控制着生理节奏，比如光周期的调节控制；

通过原核表达产生大量的目的蛋白 *Bombyx mori N-acetyltransferase* 产物，经过镍柱纯化，在动物体内免疫产生抗体，利用抗体观察它在各组织和家蚕各时期的组织分布情况，提取总 RNA，通过 qPCR 检测目的基因在不同组织和时期的 RNA 水平变化，在细胞中加以过表达和抑制分别观察细胞状态以阐明其功能，通过免疫沉淀和免疫印迹检测自身乙酰化；

家蚕 *Bombyx mori* 作为鳞翅目昆虫的模式生物，又是一个经济昆虫 ,目前对其转录调控机制研究还比较少 ,而且家蚕中有关乙酰转移酶基因方面的报道也很少。我们根据已公布的 N-乙酰转移酶基因进行了生物信息学分析 、克隆 、测序和原核表达,并研究了其在家蚕不同时期和组织器官的表达谱,为进一步研究家蚕的转录调控及其在家蚕中的功能提供了参考和依据。

关键词:乙酰转移酶、表达谱、乙酰化

灵芝小 RNA 的分离与鉴定

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摘要：灵芝，为多孔菌科真菌赤芝，是一种珍贵的药食两用菌。迄今为止国内外研究发现其内的活性成分有：多糖、生物碱、蛋白质、脂肪、核苷、甾醇、氨基酸、三萜类、微量元素等等。在这些成分的药用价值上的研究已非常成熟，而对于其内小 RNA 的研究几乎没有。如在抗肿瘤、免疫调节、血糖血脂调节、血压调节、抗衰老等药理机制中灵芝多糖和蛋白起主导作用，并且在它们生理机理的研究上都比较成熟，而对于这些机制在小 RNA 方面的研究非常稀少。本研究旨在从小 RNA 这个角度对灵芝进行初步研究，对灵芝进行分离与鉴定，为灵芝在药用上的研究探索新的思路，给将来灵芝医学药学等领域开辟新的道路。本研究主要内容是：采集新鲜的灵芝子实体，进行小 RNA 提取，对小 RNA 进行浓度和纯度测及电泳检测，其后对小 RNA 进行高通量测序及生物信息分析，再对测序结果进行 qPCR 和 Northern blotting 验证。这是首次对灵芝 miRNA 进行完整的报道。

关键词：灵芝；小 RNA；高通量测序；qPCR；Northern blotting

Tetramethylpyrazine blocks TFAM degradation and upregulates mitochondrial DNA copy number by interacting with TFAM

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ATP-dependent mitochondrial Lon protease plays important roles in the quality control of proteins through the degradation of abnormal proteins and protein refolding, as well as in turnover of short-lived regulatory proteins within mitochondrial matrix. 2,3,5,6-tetramethylpyrazine (TMP), a nature small molecular compound and one of the major components of Chinese medicine *Chuanxiong*, is widely applied to clinical therapies for dilating blood vessels and inhibiting platelet aggregation and thrombosis. Recent work demonstrates that TMP may also function as an antitumor agent. However, the mechanism still needs to be further studied. In present study, we investigated the effects of TMP on mitochondrial Lon protease and transcription factor A (TFAM) both *in vivo* and *in vitro.* Here, we demonstrated that TMP specifically blocked Lon-mediated TFAM degradation. Whereas, protease assays indicated that TMP did not inhibit Lon protease activity *in vitro*. We also demonstrated that HeLa cells with tremendously low mitochondrial DNA (mtDNA) copy number treated with TMP results in accumulation of TFAM and subsequently leads to increase of mtDNA content. Furthermore, pull-down assays showed that biotinylated TMP interacted with both TFAM and Lon. These findings suggest a new mechanism for TMP blocking the Lon-mediated TFAM degradation and mtDNA recovery in cells with severe deficits in mtDNA content through direction interaction with TFAM.

分子伴侣系统 Lon/Tid1 在膀胱肿瘤发生发展中的作用机制

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线粒体作为真核细胞的能量工厂，为细胞提供将近 95%的 ATP。线粒体 Lon 蛋白酶作为线粒体基质中关键的蛋白酶，在维持线粒体 DNA 稳定，蛋白质正确折叠，异常蛋白的降解等方面发挥了重要作用。我们发现膀胱癌肿瘤组织中 Lon 蛋白高表达， 并且在膀胱癌肿瘤细胞中下调 Lon 蛋白可降低 ROS 并抑制 JNK/SAPK 的激活以及降低肿瘤细胞的生物能量代谢抑制肿瘤细胞的生长。同时，Tid1 作为协同分子伴侣在这一过程中也发挥了关键的作用。更进一步的，我们发现 Tid1 在协同 p53 进入线粒体，以及可能协同 p53 被 Lon 蛋白降低并增强肿瘤细胞的耐药性方面发挥了十分关键的作用。此外，我们通过对临床病人的组织切片进行分析发现，高表达 Lon 蛋白的膀胱癌病人具有较差的五年生存期，推测 Lon 蛋白可能可以作为膀胱癌病人一个潜在的预后指标和治疗靶点。

致谢：本课题得到了国家自然科学基金（No. 31070710， 31171345）的资助。