·重要食源性寄生虫病:广州管圆线虫病防控专题·

Acan-Gal-1 通过膜 Annexin A2 调控巨噬细胞免疫表型

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摘　要:【目的】探讨广州管圆线虫来源的*Acan*-Gal-1 调控巨噬细胞免疫表型和 Annexin A2/S100A10 膜表面易位的分子机制。【方法】采用 IFA 方法检测巨噬细胞中 Annexin A2 和 S100A10 的定位;采用流式细胞术和 ELISA 检测巨噬细胞功能变化;通过 siRNA 干扰 Annexin A2,检测 *Acan*-Gal-1 对各指标的影响。通过 Western blot、qRT-PCR 等方法检测相关基因和蛋白的表达。通过生物信息学方法分析 *Acan*-Gal-1 蛋白结构特征,利用软件进行序列比对,预测其与 Annexin A2 的相互作用模式。【结果】本研究发现 *Acan*-Gal-1 能够显著上调巨噬细胞 M2 型标志物(Arg-1、IL-10、TGF-β),同时抑制 LPS 诱导的 M1 型标志物(iNOS、IL-6、IL-1β)表达。*Acan*-Gal-1 能减少免疫激活状态下巨噬细胞中 Annexin A2 的蛋白水平;干扰 Annexin A2 表达后,*Acan*-Gal-1 不仅无法诱导巨噬细胞转化为免疫抑制表型,也无法抑制 LPS 诱导巨噬细胞转化为免疫激活表型。*Acan*-Gal-1 不影响巨噬细胞中 Annexin A2 及其伴侣蛋白 S100A10 的表达,但 *Acan*-Gal-1 能促进二者向细胞膜表面易位。【结论】*Acan*-Gal-1 可促使 Annexin A2/S100A10 复合物在膜表面富集发挥其在巨噬细胞免疫调控中的作用。

关键词:*Acan*-Gal-1;巨噬细胞极化;膜联蛋白 A2;钙结合蛋白 A10;经典活化型巨噬细胞;替代性活化型巨噬细胞;广州管圆线虫

中图分类号:R383.1+9　　**文献标志码:**A　　**文章编号:**1672-3554(2026)01-0017-12

DOI:10.11714/jsysu.med.YX20250132

Acan-Gal-1 Modulates Macrophage Immune Phenotypes through Membrane Annexin A2

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Abstract:【Objective】To investigate the molecular mechanisms by which Acan-Gal-1 regulates macrophage immune phenotypes and Annexin A2/S100A10 membrane translocation in Angiostrongylus cantonensis. 【Methods】Immunofluorescence assay (IFA) was performed to determine the subcellular localization of Annexin A2 and S100A10 in macrophages. Flow cytometry and ELISA were used to assess functional changes in macrophage. siRNA interference of Annexin A2 was used to detect the effect of Acan-Gal-1 on various indicators. The expression levels of related genes and proteins were analyzed by Western blot, qRT-PCR, and ELISA methods. Bioinformatics analyses were conducted to characterize the structural features of Acan-Gal-1, and sequence alignment and molecular interaction prediction were performed to explore its potential interaction with Annexin A2.【Results】This study found that Acan-Gal-1 can reverse LPS-induced promotion of macrophage migration and phagocytic function; it significantly upregulated M2-type markers in macrophages (Arg-1, IL-10, TGF-β) while inhibiting the expression of M1-type markers (iNOS, IL-6, IL-1β) induced by LPS. Acan-Gal-1 reduced the protein levels of Annexin A2 in macrophages under an immunologically activated state; after interfering with Annexin A2 expression, Acan-Gal-1 not only fails to induce macrophages to transition to an immunologically inhibitory phenotype but also fails to inhibit LPS-induced transition of macrophages to an

收稿日期:2025-09-19　　**录用日期:**2026-01-13

基金项目:浙江省自然科学基金(LY22C070001)

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immunologically activated phenotype. Although Acan-Gal-1 did not affect the expression of Annexin A2 or its chaperone protein S100A10 in macrophages, it markedly promoted their translocation to the plasma membrane.【Conclusions】Acan-Gal-1 facilitates the enrichment of the Annexin A2/S100A10 complex at the macrophage membrane, thereby exerting immunomodulatory effects on macrophage polarization.

Key words: *Acan*-Gal-1; macrophage polarization; annexin A2; S100 calcium binding protein A10; classically activated macrophage; alternatively activated macrophage; *Angiostrongylus cantonensis*

[J SUN Yat-sen Univ(Med Sci), 2026, 47(1): 17-28]

广州管圆线虫(*Angiostrongylus cantonensis*)第五期幼虫可移行至中枢神经系统及眼部,导致嗜酸性脑膜脑炎、脑膜炎或眼部病变[1-3]。半乳糖凝集素(galectin)是一类对β-半乳糖苷具有特殊亲和力的糖结合蛋白,一些虫源性半乳糖凝集素能在虫体入侵宿主的过程中发挥抑制宿主免疫应答的作用[4]。广州管圆线虫的五期幼虫及成虫高表达了一种半乳糖凝集素galectin 1(*Acan*-Gal-1),该蛋白与秀丽隐杆线虫(*Caenorhabditis elegans*)Lectin 1及人类galectin 9具有高度同源性[5]。巨噬细胞作为机体固有免疫系统的重要组成细胞,其在抗病原体入侵、调控免疫反应等方面发挥着作用。巨噬细胞具有极强的可塑性,可因组织微环境的变化而表现出不同的激活状态,并表现出不同的特性和作用,从而在机体的生理和病理活动中发挥不同的调节功能[6-7]。膜联蛋白A2(Annexin A2)是一种钙依赖性磷脂结合蛋白,与伴侣蛋白S100A10形成异源四聚体(A2t),参与细胞膜动力学调控[8]。其功能与其在细胞内的定位也密切相关,细胞膜上的Annexin A2可通过与Toll-like Recepter2、病原体表面蛋白等相互作用介导免疫反应。前期研究发现*Acan*-Gal-1能与巨噬细胞表面膜蛋白Annexin A2互作,*Acan*-Gal-1如何影响巨噬细胞中Annexin A2的具体机制有待进一步的研究[9]。故本研究通过基因干扰及膜蛋白定位技术,观察体外实验中*Acan*-Gal-1对巨噬细胞功能的影响过程中Annexin A2/S100A10复合物的定位与变化,旨在揭示Annexin A2在*Acan*-Gal-1调控巨噬细胞功能表型中的作用。

1 材料与方法

1.1 仪器、试剂及材料

PCR扩增仪、垂直电泳仪、凝胶成像系统(美国Bio-rad公司);Nanodrop分光光度计、CO_2培养箱、超低温冰箱(美国Thermo Fisher公司);核酸电泳仪(上海君意电泳设备有限公司);恒温水浴锅(江苏省金坛市医疗仪器厂);低温超高速离心机、台式离心机(德国Eppendorf公司);全温培养摇床(苏州众业有限公司);超声波细胞粉碎机(宁波新芝科器研究所);高压蒸汽灭菌器(上海博讯实业有限公司);多功能酶标仪(美国Thermo公司);蛋白印迹电泳系统(上海天能Tanon科技有限公司);湿转转膜仪(美国Bio-rad公司);荧光化学发光成像系统(上海勤翔生物公司);流式细胞仪(美国贝克曼库尔特公司);激光共聚焦显微镜(德国徕卡公司);超净工作台(美国Thermo Forma公司);微生物培养箱(上海一恒仪器有限公司)。

细胞株鼠巨噬细胞系Raw264.7细胞、pCold Ⅲ质粒由温州医科大学寄生虫实验室保存;Trizol® Reagent购自上海雅酶生物有限公司;三氯甲烷、氯化钠、异丙醇、甲醇等试剂购自上海麦克林生化科技有限公司;HiScript® Ⅱ 1st Strand cDNA Synthesis Kit等试剂购自南京诺唯赞生物科技股份有限公司;PCR相关试剂购自宝日医生物技术(北京)有限公司;50× TAE缓冲液、4S Green Plus核酸染料购自生工生物工程(上海)股份有限公司;高纯度低电渗琼脂糖购自擎科生物;DL2000 Plus和DL10000 Plus DNA Marker购自新胜科技有限公司;Plasmid Mini Kit、Gel Extraction Kit购自OMEGA公司;限制性内切酶Xba Ⅰ、BamH Ⅰ及T4 DNA连接酶购自New England Biolabs公司;IPTG、Ampicillin、琼脂粉、蛋白胨等购自上海麦克林生化科技有限公司及OXOID茂捷公司;His标签蛋白纯化试剂盒购自碧云天生物技术公司;DH5α和BL21(DE3)感受态细胞购自北京索莱宝科技有限公司;超滤管(3 kDa)购自美国默克Millipore公司;MagpoinsTM His-Tag Immunoprecipitation Kit购自

Sino Biological公司；细胞膜蛋白与细胞浆蛋白抽提试剂盒购自碧云天生物技术公司；Annexin A2（D11G2）、iNOS、Arg-1、GAPDH等抗体购自美国CST公司或武汉三鹰生物技术有限公司；HRP标记的二抗购自biosharp公司；ECL发光液购自上海雅酶生物有限公司；IL-6、TGF-β ELISA检测试剂盒购自上海雅酶生物有限公司；Lipofectamine 2000/3000转染试剂购自美国Thermo Fisher公司；siRNA由北京梓熙生物科技有限公司合成；OPTI-MEM培养基购自美国Gibco公司。

1.2　细胞培养、重组蛋白的诱导表达与纯化

培养RAW264.7细胞并获得去除内毒素的*Acan*-Gal-1，采用BCA法测定蛋白浓度，通过制作标准曲线计算样品蛋白含量。

1.3　流式细胞术检测细胞的吞噬功能活性

检测巨噬细胞吞噬功能：首先将荧光微球与质量分数5%牛血清白蛋白（BSA）按1∶100比例混合并于37 ℃避光孵育30 min进行调理，随后将调理后的荧光微球加入细胞培养孔中，37 ℃避光共孵育1 h使巨噬细胞充分吞噬；完成孵育程序后，弃培养上清，使用PBS，反复漂洗三次，彻底清除未被吞噬的荧光标记微球。随后加入适量PBS缓冲液，采用无菌细胞刮刀刮取贴壁细胞，经吹打混匀和滤网过滤后制备单细胞悬液。立即使用流式细胞仪进行检测，分析荧光阳性细胞群的比例，以此定量评估实验组细胞的吞噬功能活性。

1.4　免疫荧光法检测巨噬细胞吞噬功能

将RAW264.7细胞以1×10^{5}个/孔的密度接种于24孔培养板中，待细胞完全贴壁后给予*Acan*-Gal-1处理。荧光标记微球与质量分数5%BSA按体积比1∶100混合，37 ℃避光预孵育30 min。经*Acan*-Gal-1刺激12 h后，每孔加入预处理的荧光微球悬液，继续避光培养3 h。随后用PBS充分洗涤3次以去除未被吞噬的微球，40 g/L多聚甲醛溶液室温固定15 min。采用抗F4/80一抗4 ℃孵育过夜进行巨噬细胞特异性标记，TBST缓冲液洗涤3次后，加入Alexa Fluor 488标记的二抗室温避光孵育1 h，最后用DAPI溶液染色15 min标记细胞核；最后封片并在荧光显微镜下观察，计数每个巨噬细胞内吞噬的荧光微球数量以评估其吞噬功能。

1.5　流式细胞术检测细胞CD206

检测RAW264.7细胞表面CD206分子表达，方法同上。

1.6　qRT-PCR

按常规方法，针对不同的目的基因，采用华大基因合成的特异性引物，建立SYBR Green荧光定量PCR反应体系，设置95 ℃预变性1 min，95 ℃变性15 s、60 ℃退火/延伸30 s，共40个循环的反应程序，最后通过熔解曲线分析和Ct值计算，定量检测各基因的表达变化。

1.7　ELISA

收集细胞培养上清液后，以500×*g*离心力离心5 min，取上清分装保存于-80 ℃备用。根据说明书依次进行37 ℃孵育、生物素标记抗体结合、酶复合物反应等步骤，期间严格进行5次洗涤以去除非特异性结合，最后加入TMB显色液反应后测定吸光度值，通过标准曲线计算样品中细胞因子的具体浓度。

1.8　Western blot

按常规方法电泳、转膜、封闭、洗涤，与一抗（1∶1 000稀释，4 ℃过夜）和二抗（1∶5 000稀释，室温1 h）孵育，使用增强型化学发光检测试剂进行免疫反应显色，通过仪器采集化学发光信号，并对特异性条带进行灰度值定量，计算目的蛋白的相对表达量。

1.9　siRNA转染

取对数生长期的RAW 264.7细胞，调整密度，接种于12孔细胞培养板，采用脂质体-siRNA混合液形成稳定的转染复合物。通过蛋白质免疫印迹或实时定量聚合酶链反应技术检测靶基因在蛋白水平或mRNA水平的表达变化，评估RNA干扰效率。

1.10　免疫荧光实验检测巨噬细胞中Annexin A2和S100A10蛋白表达

RAW 264.7细胞，调整密度，采用常规清洗、通透、封闭；加入BSA稀释的一抗4 ℃过夜孵育，PBS清洗三次；避光条件下加入荧光标记的二抗37 ℃孵育1 h，PBS洗涤后DAPI染核15 min；样品封片后采用抗荧光衰减封片剂处理，于荧光显微镜下观察并采集荧光图像。

1.11　生物素标记法分离提取巨噬细胞表面蛋白

细胞经PBS洗涤后，使用1 mg/mL Sulfo-NHS-SS-Biotin溶液室温标记10 min，冰预冷TBS清洗去除游离生物素。刮取细胞后加入含蛋白酶抑制剂的RIPA裂解液冰浴裂解2 h，12 000×*g*离心15 min收集上清液。上清与平衡后的NeutrAvidin琼脂糖

珠4 ℃孵育2 h,经严格洗涤后采用50 mmol/L DTT洗脱缓冲液解离生物素标记蛋白。最终洗脱产物与4×loading buffer混合,100 ℃变性5 min后进行Western blot分析。

1.12 MTT法

将细胞悬液以每孔100 μL接种于96孔板(细胞密度1 000~10 000个/孔),边缘孔用PBS填充,37 ℃、体积分数5%CO_2培养至细胞贴壁后加入梯度浓度药物(5~7个浓度,每孔100 μL,3~5个复孔);药物处理12 h后每孔加入20 μL MTT溶液(5 mg/mL)继续培养4~6 h形成紫色甲臜结晶;去除培养液后每孔加入150 μL二甲基亚砜(DMSO)溶解甲臜结晶,低速振荡10 min促进充分溶解。采用酶标仪在双波长(490 nm,570 nm)条件下测定吸光度值。实验设置空白对照组(仅含培养基和试剂)和阴性对照组(含细胞和溶剂),通过比较各组吸光度差值定量分析药物对细胞增殖的抑制作用。

1.13 统计学方法

数据采用($\bar{x} \pm s$)表示。多组均数比较时,若数据服从正态分布且方差齐性,采用单因素方差分析(One-way ANOVA),组间两两比较采用Bonferroni法。两组间比较时,若数据独立、正态且方差齐性,则采用成组t检验,检验水准α=0.05。所有统计分析均通过GraphPad Prism 8.0实现。

2 结 果

2.1 *Acan*-Gal-1促进巨噬细胞向免疫抑制表型转化

qRT-PCR检测结果显示*Acan*-Gal-1能显著促进巨噬细胞向免疫抑制表型转化。qRT-PCR检测结果显示,*Acan*-Gal-1处理显著上调RAW264.7细胞中M2型巨噬细胞标志炎症因子基因(Fizz-1、IL-10和TGF-β)的转录水平。ELISA分析进一步证实,该处理组细胞培养上清中TGF-β的分泌量较对照组明显增加。并且,qRT-PCR和Western blot结果也显示*Acan*-Gal-1处理组Arg-1在mRNA和蛋白水平的表达量均较空白组有上调,采用GAPDH归一化处理后,相对表达量为0.78±0.05(对照组为0.47±0.05),较对照组显著上调了66.0%(图1A-G)。

2.2 *Acan*-Gal-1抑制巨噬细胞向免疫激活表型转化

为探究*Acan*-Gal-1对巨噬细胞免疫表型的调控作用,采用LPS诱导建立M1型巨噬细胞活化模型。采用qRT-PCR、ELISA和Western blot结果显示,*Acan*-Gal-1能显著抑制LPS诱导的免疫激活反应:相较于LPS处理组,*Acan*-Gal-1处理组的免疫激活型标志因子IL-6和IL-1β mRNA表达量明显下降,并且IL-6的分泌水平也明显下降;*Acan*-Gal-1处理组iNOS在mRNA和蛋白水平的表达量相较于LPS处理组减少,采用GAPDH归一化处理后,*Acan*-Gal-1处理组相对表达量为0.79±0.05(LPS处理组为1.26±0.05),较LPS处理组显著下调了37.0%)(图1H-M)。这些结果表明*Acan*-Gal-1不仅能促进巨噬细胞向免疫抑制表型转化,还能有效抑制其向免疫激活表型的转化。

2.3 *Acan*-Gal-1能减少免疫激活状态下巨噬细胞中Annexin A2的蛋白水平

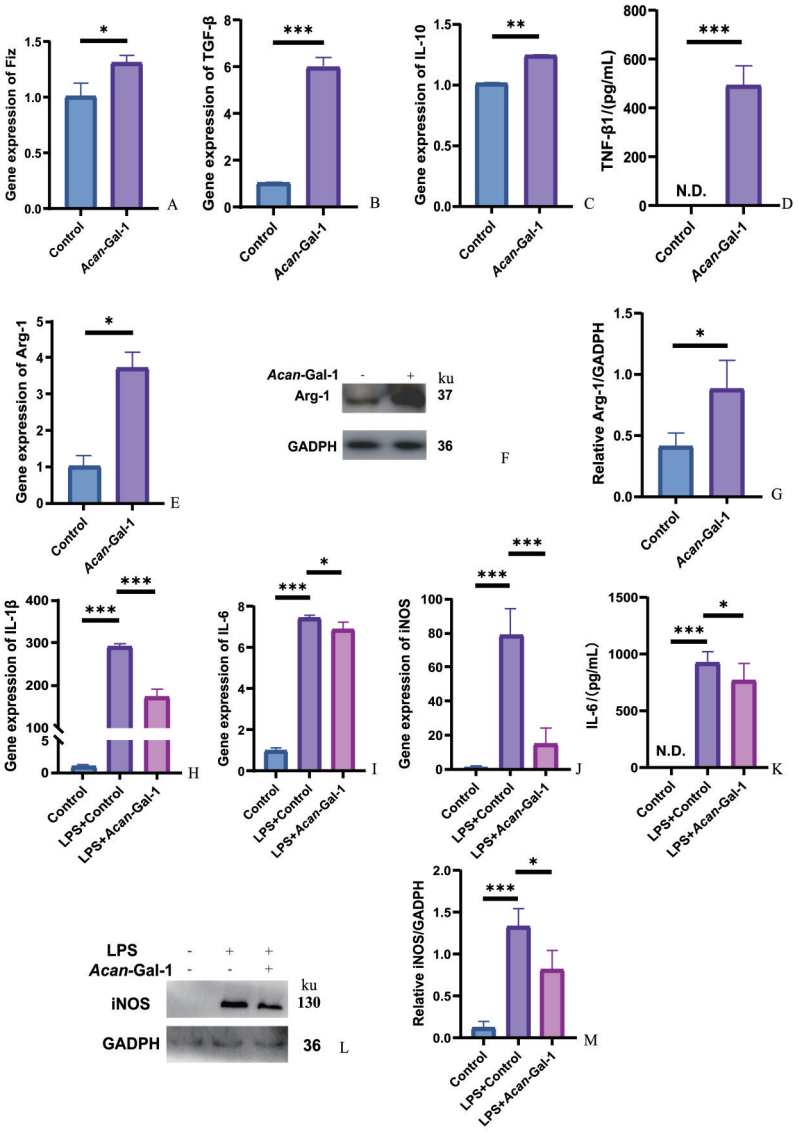
为阐明Annexin A2在*Acan*-Gal-1介导的巨噬细胞免疫表型调控中的分子机制。结果显示,脂多糖刺激显著上调巨噬细胞Annexin A2蛋白表达,采用GAPDH归一化处理后,*Acan*-Gal-1处理组相对表达量为0.58±0.05(LPS处理组为1.19±0.05),较LPS处理组显著下调了51.4%,而*Acan*-Gal-1处理可明显逆转这一效应(图2A-B)。

2.4 干扰Annexin A2表达后,*Acan*-Gal-1无法诱导巨噬细胞转化为免疫抑制表型

采用RNA干扰技术特异性沉默Annexin A2基因表达后(图2C-D),*Acan*-Gal-1促进巨噬细胞向免疫抑制表型转化的效应被显著抑制。具体表现为:与单纯*Acan*-Gal-1处理组相比,Annexin A2敲低组的免疫抑制表型巨噬细胞相关标志基因TGF-β、IL-10 mRNA表达量下降,上清液中细胞因子TGF-β的分泌量减少,标志酶Arg-1蛋白表达水平也减少,采用GAPDH归一化处理后,*Acan*-Gal-1处理组相对表达量为0.62±0.05(LPS处理组为1.01±0.05),较LPS处理组显著下调了38.6%(图2E-H,图3AB)。

2.5 干扰Annexin A2表达后,*Acan*-Gal-1无法抑制LPS诱导巨噬细胞转化为免疫激活表型

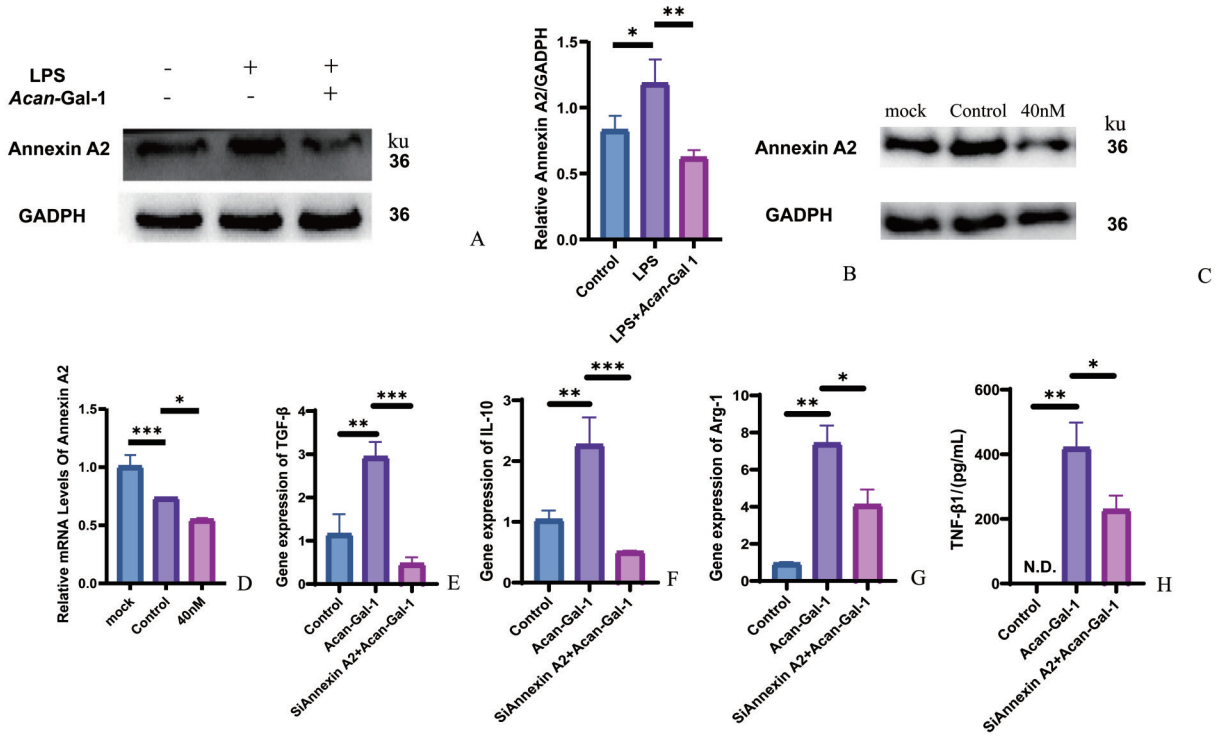
在LPS刺激的巨噬细胞免疫激活模型中,Annexin A2基因沉默显著减弱了*Acan*-Gal-1的抑制作用。Western blot和qPCR分析显示,在Annexin A2正常表达的对照组中,*Acan*-Gal-1处理可显著抑制LPS诱导的促炎因子IL-6的mRNA表达、IL-1β mRNA表达以及iNOS蛋白合成,采用GAPDH归一化处理后,Annexin A2干扰组相对表



A-C: RT-PCR detection of Fizz-1, IL-10, and TGF-β transcription levels revealed statistically significant intergroup differences by independent samples *t*-test (t=3.779, P=0.019 5; t=27.66, P=0.001; t=30.18, P < 0.001); D: ELISA assay of TGF-β secretion in cell supernatants (N.D. denotes not detected). Independent samples *t*-test revealed statistically significant intergroup differences (t=10.15, P < 0.001); E: RT-PCR detection of Arg-1 transcription levels; independent samples *t*-test revealed statistically significant intergroup differences (t=7.403, P=0.02); F: Western Blot analysis of Arg-1 expression; G: Statistical analysis of Western Blot data, with statistically significant differences between groups as determined by independent samples *t*-test (t=3.072, P=0.04); H-J: RT-PCR detection of IL-1β, IL-6, and iNOS transcription levels. Analysis of variance revealed statistically significant differences among the three groups (F=441.0, P < 0.001; F=779.3, P < 0.001; F=45.28, P < 0.001); Using the Bonferroni method for pairwise comparisons, statistically significant differences were observed between the *Acan*-Gal-1-treated group and the LPS-treated group (all P < 0.001), while statistically significant differences were also found between the LPS-treated group and the untreated group (all P < 0.001); K: ELISA assay of IL-6 secretion in cell supernatants (N.D. denotes not detected) revealed statistically significant differences among the three groups following ANOVA (F=44.88, P < 0.001); pairwise comparisons using Bonferroni correction revealed statistically significant differences between the *Acan*-Gal-1-treated and LPS-treated groups (P < 0.001), while the LPS-treated group also differed significantly from the untreated group (P=0.010 8); L: Western Blot detection of iNOS expression; M: Statistical analysis of Western Blot data. Analysis of variance revealed statistically significant differences among the three groups (F=30.08, P < 0.001); Using the Bonferroni method for pairwise comparisons, statistically significant differences were observed between the *Acan*-Gal-1 treatment group and the LPS treatment group (P < 0.001), while the LPS treatment group also showed a statistically significant difference compared to the untreated group (P=0.04). n=3 independent experiments; data expressed as ($\bar{x} \pm s$), * P<0.05, ** P < 0.01, ***P < 0.001, ns P>0.05.

图1　*Acan*-Gal-1调控巨噬细胞免疫表型转换

Fig. 1　*Acan*-Gal-1 modulates the transition of macrophage immune phenotypes



A: Western blot detection of Annexin A2 expression; B: Statistical analysis chart of Western blot experimental data. Analysis of variance revealed statistically significant differences among the three groups (F=15.32, P=0.004); Using the Bonferroni method for pairwise comparisons, statistically significant differences were observed between the *Acan*-Gal-1 treatment group and the LPS treatment group (P=0.004), while the LPS treatment group also showed statistically significant differences compared to the untreated group (P=0.03); C: Western Blot analysis of Annexin A2 expression following SiAnnexin A2 administration; D: Statistical analysis of Western Blot data. Analysis of variance revealed statistically significant differences among the three groups (F=45.26, P<0.001); Using the Bonferroni method for pairwise comparisons, statistically significant differences were observed between the mock group and untreated group (P < 0.001), and between the untreated and SiAnnexin A2-treated groups (P = 0.02); E-G: Following SiAnnexin A2 treatment, RT-PCR detected the transcriptional levels of TGF-β, IL-10, and Arg-1. Analysis of variance revealed statistically significant differences among the three groups (F=44.37, P<0.001) (F=32.34, P<0.001) (F=39.13, P=0.002); Using the Bonferroni method for pairwise comparisons, statistically significant differences were observed between the *Acan*-Gal-1-treated group and the LPS-treated group (P < 0.001) (P < 0.001) (P = 0.02), while differences between the LPS-treated group and the untreated group were also statistically significant (P = 0.002) (P = 0.04) (P = 0.002); H: Following SiAnnexin A2 administration, ELISA assays measured TGF-β secretion in cell supernatants (N.D. denotes undetectable levels). Analysis of variance revealed statistically significant differences among the three groups (F=54.11, P=0.001); Using the Bonferroni method for pairwise comparisons, statistically significant differences were observed between the *Acan*-Gal-1-treated group and the LPS-treated group (P=0.03), while the LPS-treated group also showed statistically significant differences compared to the untreated group (P=0.001); * P<0.05, ** P<0.01, ***P<0.001, ns P>0.05.

图2 Annexin A2介导*Acan*-Gal-1对巨噬细胞免疫表型的调控

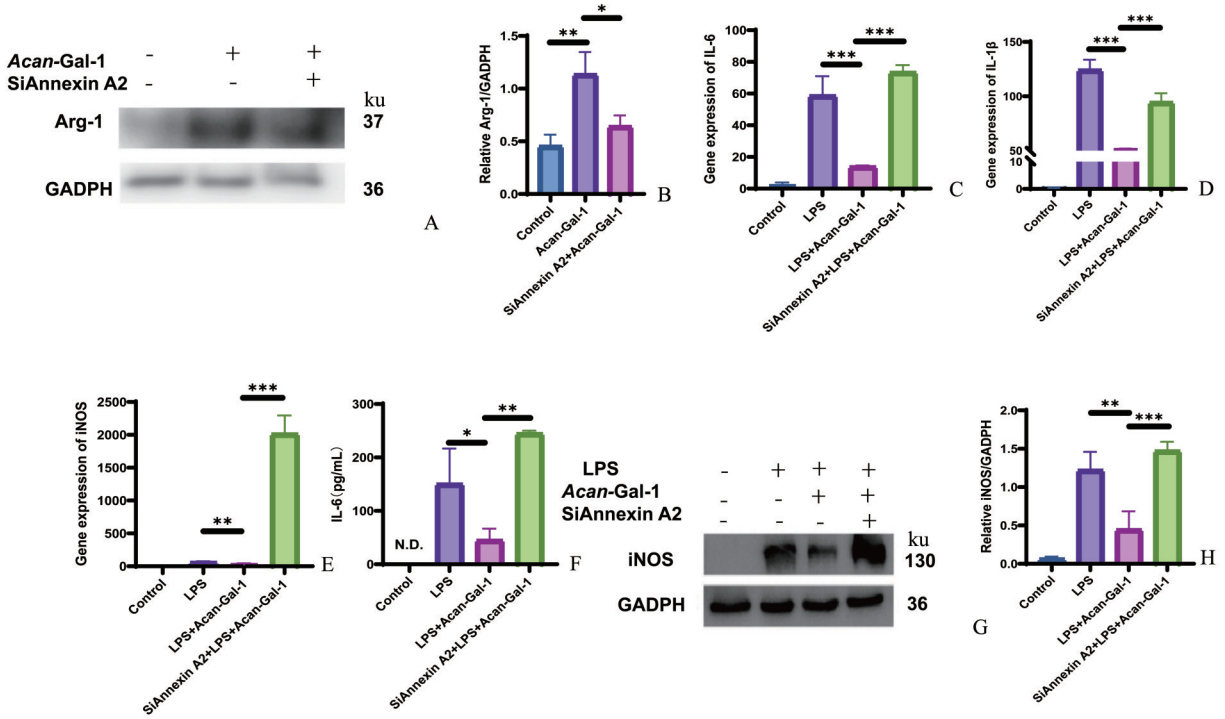
Fig. 2 Annexin A2 mediates the regulation of macrophage immune phenotype by *Acan*-Gal-1

达量为1.26±0.05(*Acan*-Gal-1处理组为0.42±0.05),较*Acan*-Gal-1处理组显著上调200.0%(图3)。特别值得注意的是,Annexin A2敲低后,*Acan*-Gal-1处理组的各项指标与LPS阳性对照组相比均无显著差异,提示Annexin A2是介导*Acan*-Gal-1免疫调控功能的关键分子。

2.6 *Acan*-Gal-1不影响巨噬细胞中Annexin A2和其伴侣蛋白S100A10的表达

为探究*Acan*-Gal-1对巨噬细胞中Annexin A2及其伴侣蛋白S100A10表达的调控作用,本研究首先采用不同浓度梯度的*Acan*-Gal-1处理巨噬细胞。基因和蛋白表达分析表明,不同浓度*Acan*-Gal-1处理对巨噬细胞Annexin A2的转录水平(qRT-PCR)和蛋白表达量(Western blot)与未处理组相比均未产生显著影响(图4A,C-D)。免疫荧光染色结果进一步证实,Annexin A2在细胞内的分布模式也未发生明显改变(图4G-H)。

随后,在检测Annexin A2伴侣蛋白S100A10表



A: Western blot analysis of Arg-1 expression following SiAnnexin A2 administration; B: Statistical analysis of Western blot data following SiAnnexin A2 administration. Analysis of variance revealed statistically significant differences among the three groups (F=16.08, P=0.004); Using the Bonferroni method for pairwise comparisons, statistically significant differences were observed between the *Acan*-Gal-1-treated group and the LPS-treated group (P=0.02), and between the LPS-treated group and the untreated group (P=0.004); Following C-E: SiAnnexin A2 administration, RT-PCR detected transcription levels of IL-6, IL-1β, and iNOS. Analysis of variance revealed statistically significant differences among the four groups (F=117.3, P<0.001) (F=211.9, P<0.001) (F=199.3, P<0.001); Using the Bonferroni method for pairwise comparisons, statistically significant differences were observed between the *Acan*-Gal-1 treatment group and the LPS treatment group (P < 0.001) (P < 0.001) (P < 0.001), and between the *Acan*-Gal-1 treatment group and the SiAnnexin A2 treatment group (P < 0.001) (P < 0.001) (P = 0.002); F: Following SiAnnexin A2 administration, ELISA assays measured IL-6 secretion in cell supernatants (N.D. denotes undetectable levels). Analysis of variance revealed statistically significant differences among the four groups (F = 31.68, P < 0.001); pairwise comparisons using the Bonferroni method revealed statistically significant differences between the *Acan*-Gal-1-treated group and the LPS-treated group (P=0.03), and between the *Acan*-Gal-1-treated group and the SiAnnexin A2-treated group (P=0.001); G: Following SiAnnexin A2 administration, Western Blot analysis detected iNOS expression; H: Statistical analysis of Western Blot data post-SiAnnexin A2 treatment. Analysis of variance revealed statistically significant differences among the four groups (F=40, P< 0.001); Using the Bonferroni method for pairwise comparisons, statistically significant differences were observed between the *Acan*-Gal-1 treatment group and the LPS treatment group (P=0.003), and between the *Acan*-Gal-1 treatment group and the SiAnnexin A2 treatment group (P<0.001). n=3 independent experiments; data expressed as ($\bar{x} \pm s$); * P<0.05, ** P<0.01, ***P<0.001, ns P>0.05.

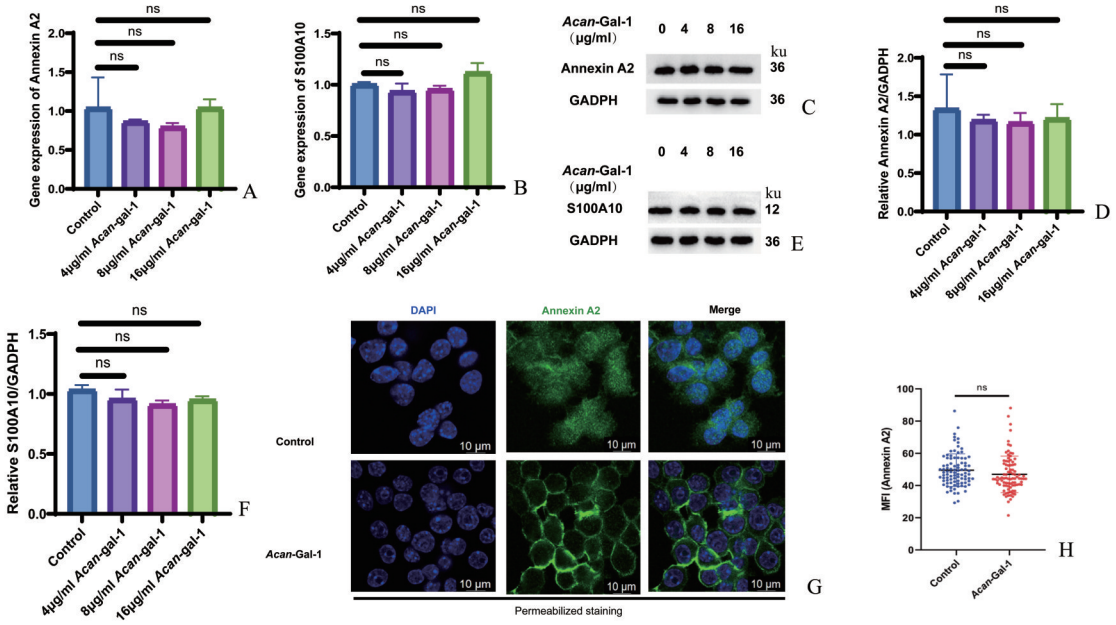
图3　Annexin A2介导*Acan*-Gal-1对炎症巨噬细胞免疫表型的调控

Fig. 3　Annexin A2 mediates the regulation of Inflammatory macrophage immune phenotype by *Acan*-Gal-1

达时发现，不同浓度*Acan*-Gal-1处理后的巨噬细胞中，S100A10的mRNA表达水平和蛋白含量与对照组相比均无统计学差异（图4）。免疫荧光分析结果显示，*Acan*-Gal-1处理未显著改变S100A10在细胞内的亚细胞定位分布（图5A，B）。结合前述实验结果证实，*Acan*-Gal-1的生物学功能不依赖于对Annexin A2或S100A10表达水平的调控。

2.7　*Acan*-Gal-1促进巨噬细胞中Annexin A2及其伴侣蛋白S100A10向细胞膜表面易位

基于Annexin A2作为*Acan*-Gal-1膜受体的前期发现，本研究重点探究了*Acan*-Gal-1对Annexin A2和伴侣蛋白S100A10膜定位的动态调控作用。通过生物素标记膜表面蛋白，分离获得细胞膜蛋白，并结合Western blot分析发现，*Acan*-Gal-1处理显著提高了膜表面Annexin A2的水平，采用



A-B: RT-PCR detection of Annexin A2 and S100A10 transcription levels revealed no statistically significant differences among the four groups following analysis of variance (F=1.191, P=0.39) (F=4.395, P=0.05); Using the Bonferroni method for pairwise comparisons, no statistically significant differences were observed between the 4 μg/mL *Acan*-Gal-1 treatment group and the untreated group (P=0.55) (P=0.55), nor between the 8 μg/mL *Acan*-Gal-1 group and the untreated group (P=0.69) (P=0.77). while the 16 μg/mL *Acan*-Gal-1 group also showed no statistically significant difference compared to the untreated group (P>0.99) (P=0.22); C: Western Blot detection of Annexin A2 expression; D: Statistical analysis chart of Western Blot experimental data. Analysis of variance revealed no statistically significant differences among the four groups (F=0.2873, P=0.83); Using the Bonferroni method for pairwise comparisons, no statistically significant difference was observed between the 4 μg/mL *Acan*-Gal-1-treated group and the untreated group (P=0.89), nor between the 8 μg/mL *Acan*-Gal-1-treated group and the untreated group (P=0.82). and the 16 μg/mL *Acan*-Gal-1 group showed no statistically significant difference compared to the untreated group (P=0.93); E: Western Blot analysis of S100A10 expression; F: Statistical analysis of Western Blot data. Analysis of variance revealed no statistically significant differences among the four groups (F=3.044, P=0.09); Using the Bonferroni method for pairwise comparisons, no statistically significant difference was observed between the 4 μg/mL *Acan*-Gal-1-treated group and the untreated group (P=0.33), while the difference between the 8 μg/mL *Acan*-Gal-1-treated group and the untreated group was not statistically significant (P=0.07); and the 16 μg/mL *Acan*-Gal-1 group showed no statistically significant difference compared to the untreated group (P=0.27); G: IFA observation of Annexin A2 fluorescence intensity; H: Statistical analysis graph of IFA experimental data. Independent samples t-test revealed no statistically significant differences between groups (t=1.632, P=0.104 5); * P<0.05, ** P<0.01, ***P<0.001, ns P>0.05.

图4 *Acan*-Gal-1调控巨噬细胞Annexin A2/S100A10表达

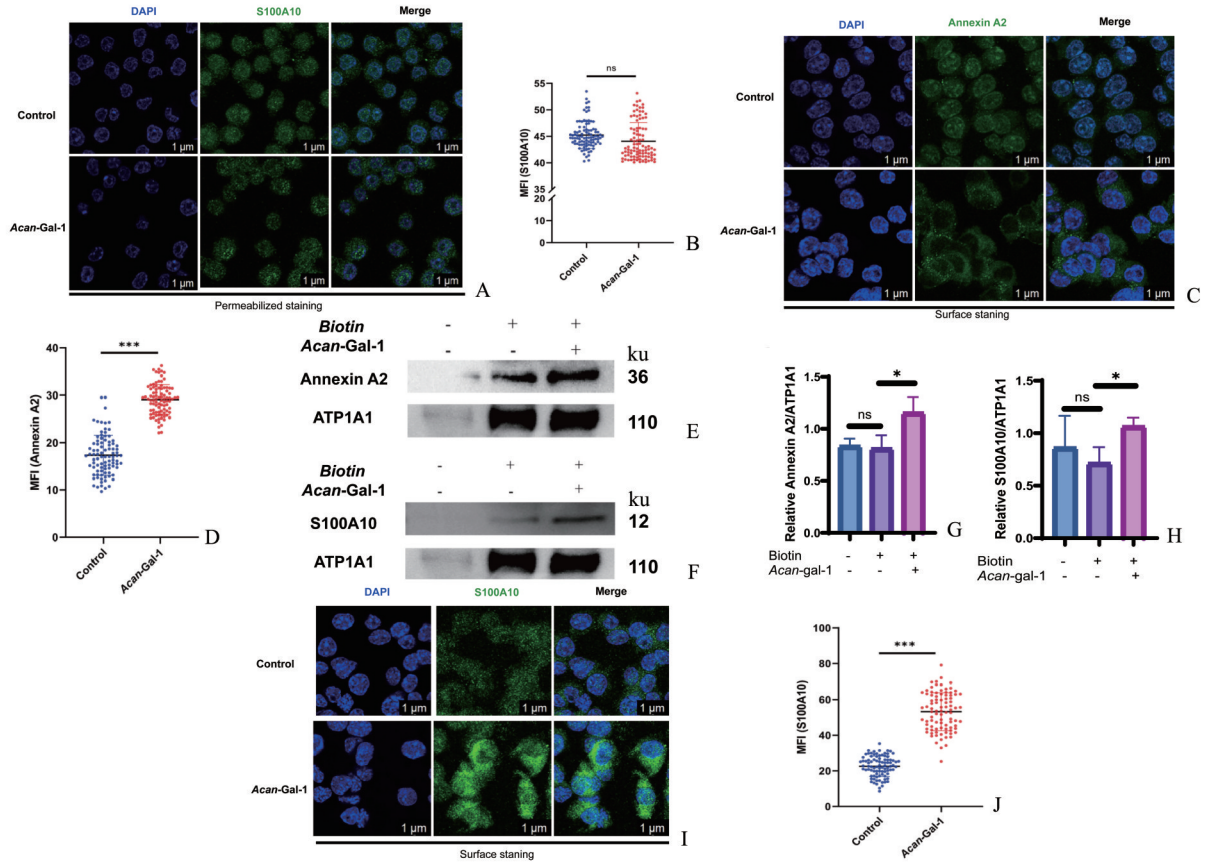
Fig. 4 *Acan*-Gal-1 regulates the expression of Annexin A2/S100A10 in macrophages

GAPDH归一化处理后,*Acan*-Gal-1处理组相对表达量为1.19±0.05(Biotin处理组为0.69±0.05),较Biotin处理组显著上调72.5%(图5E,G)。共聚焦显微镜观察显示,与对照组相比,*Acan*-Gal-1处理组的细胞膜区域Annexin A2荧光信号强度明显增强,表明*Acan*-Gal-1能促进Annexin A2向细胞膜表面的转位(图5C,D)。这一发现为阐明*Acan*-Gal-1通过膜受体Annexin A2调控巨噬细胞功能的分子机制提供了重要实验依据。

实验数据显示,*Acan*-Gal-1处理显著增加细胞膜S100A10的富集程度,采用GAPDH归一化处理后,*Acan*-Gal-1处理组相对表达量为1.07±0.05(Biotin处理组为0.73±0.05),较Biotin处理组显著上调46.6%(图5F,H)。免疫荧光分析进一步证实,与对照组相比,*Acan*-Gal-1处理组细胞膜区域S100A10的荧光强度显著增强(图5I,J)。这些结果共同表明,*Acan*-Gal-1能够特异性促进Annexin A2和S100A10向细胞膜表面的转位。

2.8 抑制剂A2ti-2能抑制巨噬细胞中Annexin A2及其伴侣蛋白S100A10的表面易位,继而调控巨噬细胞免疫表型相关性

为明确Annexin A2/S100A10复合物的结构与表面异位的相关性,并检测免疫表型相关标志物的表达变化。本研究采用特异性四聚体抑制剂A2ti-2



A: IFA observation of S100A10 fluorescence intensity; B: Statistical analysis graph of IFA experimental data. Independent samples t-test revealed no statistically significant differences between groups (t=1.383, P=0.17); C: IFA observation of Annexin A2 fluorescence intensity on the membrane surface; D: Statistical analysis graph of IFA experimental data. Independent samples t-test revealed statistically significant differences between groups (t=21.25, P<0.001); E-F: Western Blot detection of membrane surface Annexin A2 and S100A10 protein levels; G-H: Statistical analysis of Western Blot experimental data. Analysis of variance revealed statistically significant differences among the three groups (F=7.383, P=0.02) (F=10.28, P=0.01); Using the Bonferroni method for pairwise comparisons, statistically significant differences were observed between the *Acan*-Gal-1 treatment group and the Biotin group (P=0.003) (P=0.02); I: IFA observation of S100A10 fluorescence intensity on the membrane surface; J: Statistical analysis of IFA experimental data. Independent samples t-test revealed statistically significant intergroup differences (t=24, P<0.001). n=3 independent experiments; data expressed as ($\bar{x} \pm s$); * P<0.05, ** P<0.01, ***P<0.001, ns P>0.05.

图5　*Acan*-Gal-1调控巨噬细胞Annexin A2/S100A10表达及膜定位

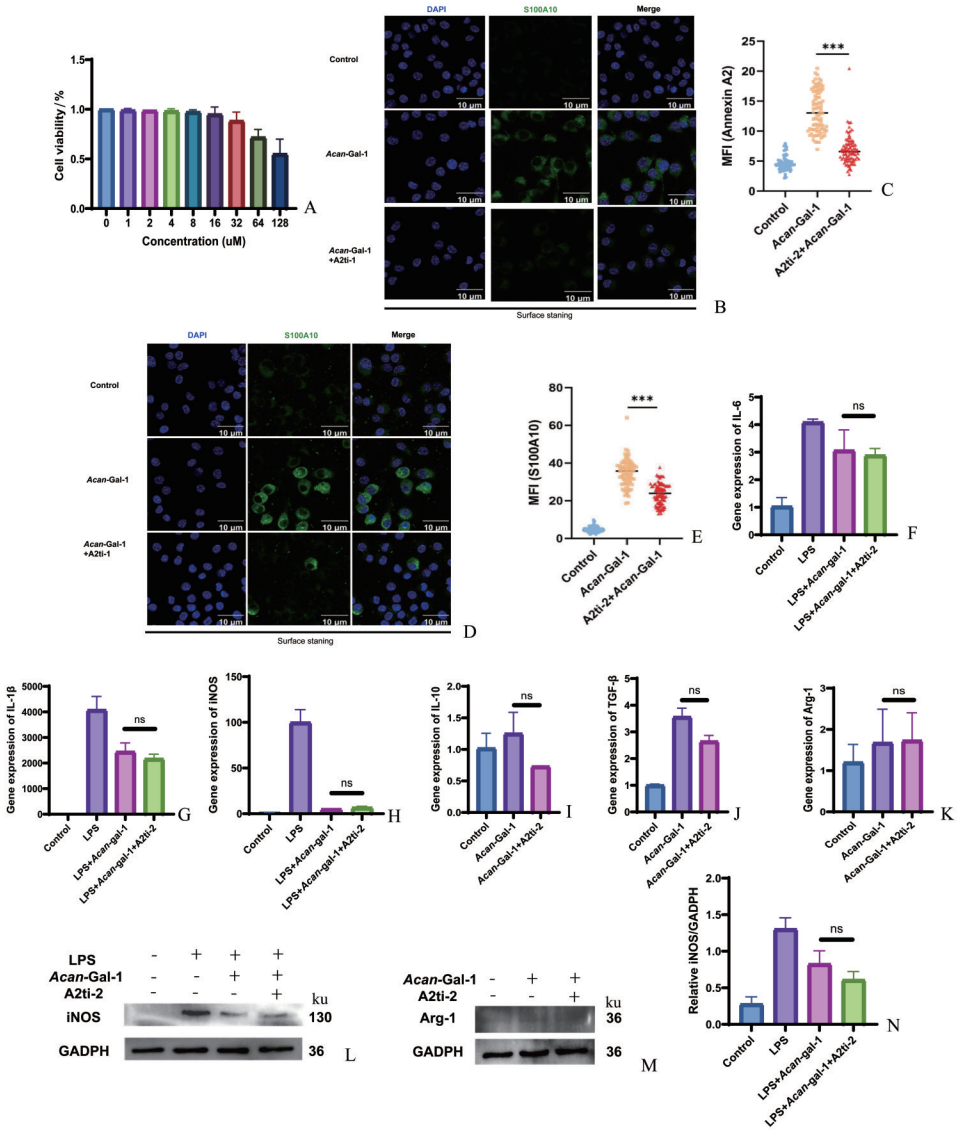
Fig. 5　*Acan*-Gal-1 regulates the expression and membrane localization of Annexin A2/S100A10 in macrophages

干预Annexin A2及其伴侣蛋白S100A10的两者结合。MTT实验确定A2ti-2的最佳作用浓度(图6A)。结果显示，A2ti-2处理能显著抑制*Acan*-Gal-1诱导的Annexin A2和S100A10膜转位(图6B-E)，证实Annexin A2与S100A10的相互作用是其膜定位的必要条件。

qRT-PCR和Western blot分析显示，在A2ti-2处理前后，免疫激活标志物(IL-6、IL-1β、iNOS)和免疫抑制标志物(IL-10、TGF-β、Arg-1)的表达水平均未出现显著差异(图6F-N)。

3　讨 论

寄生虫与宿主的相互作用涉及多种关键蛋白[10]。广州管圆线虫Ⅲ期幼虫入脑后，其移行及死亡虫体释放的抗原可破坏血脑屏障，并激活天然免疫，招募嗜酸性粒细胞等免疫细胞，加剧中枢神经系统炎症[11]。嗜酸性粒细胞积聚所产生的过氧化物氧化作用加剧组织炎症反应，同时杀死虫体。故虫体需要减弱嗜酸性粒细胞介导的组织炎症反应，以利于L5虫体在脑脊液中寄生。广州管圆线虫分泌的*Acan*-Gal-1是一种具有免疫调节功能的凝集



A: MTT cytotoxicity assay; B: IFA observation of Annexin A2 fluorescence intensity on the membrane surface; C: Statistical analysis chart of IFA experimental data. Analysis of variance revealed statistically significant differences among the three groups (F=63, P < 0.001); Using the Bonferroni method for pairwise comparisons, statistically significant differences were observed between the A2ti-2 treatment group and the *Acan*-Gal-1 treatment group (P < 0.001); D: IFA observation of S100A10 fluorescence intensity on the membrane surface; E: Statistical analysis of IFA experimental data. Analysis of variance revealed statistically significant differences among the three groups (F=75, P < 0.001). Pairwise comparisons using the Bonferroni method indicated a statistically significant difference between the A2ti-2 treatment group and the *Acan*-Gal-1 treatment group (P < 0.001); F-H: RT-PCR detection of IL-6, IL-1β, and iNOS transcription levels revealed statistically significant differences among the four groups (F=78.6, P < 0.001) (F=78.64, P < 0.001) (F=97.13, P < 0.001); Using the Bonferroni method for pairwise comparisons, no statistically significant differences were found between the A2ti-2 and *Acan*-Gal-1 treatment groups (P=0.95)(P=0.73)(P>0.99); I-K: RT-PCR detection of IL-10, TGF-β, and Arg-1 transcription levels revealed statistically significant differences among the three groups (F=3.559, P=0.1) (F=112.5, P < 0.001) (F=54, P < 0.001); Using the Bonferroni method for pairwise comparisons, no statistically significant differences were found between the A2ti-2 treatment group and the *Acan*-Gal-1 treatment group (P=0.08)(P=0.12)(P=0.45); L-M: Western Blot detection of iNOS and Arg-1 expression; N: Statistical analysis graph of Western Blot data, showing statistically significant differences among the three groups (F=28.46, P < 0.001); pairwise comparisons using the Bonferroni method revealed no statistically significant difference between the A2ti-2 treatment group and the *Acan*-Gal-1 treatment group (P=0.3). n=3 independent experiments; data expressed as ($\bar{x} \pm s$); * P<0.05, ** P < 0.01, ***P < 0.001, ns P>0.05; cell viability >95%.

图6 Annexin A2/S100A10表面易位参与*Acan*-Gal-1对巨噬细胞免疫表型的调控

Fig. 6 Surface translocation of Annexin A2/S100A10 is involved in the modulation of macrophage immune phenotype by *Acan*-Gal-1

素，前期研究表明其能抑制宿主免疫反应，改善小鼠类风湿关节炎症状，并促进巨噬细胞凋亡。这些发现提示*Acan*-Gal-1可能在广州管圆线虫感染中通过调控巨噬细胞功能发挥作用。

凝集素是一类具有广泛生物学功能的蛋白质，其可分为C型凝集素、S型凝集素、P型凝集素等半乳糖凝集素（属S型凝集素）发挥着广泛的生物学作用，例如其能参与细胞迁移、增殖、分化、免疫反应等[12]，也能特异性识别β-半乳糖苷，参与免疫调节等多种生理过程[13]。广州管圆线虫分泌的*Acan*-Gal-1属于串联重复型半乳糖凝集素，具有N端和C端两个碳水化合物识别结构域（carbohydrate recognition domains，CRD）[14]，作为人galectin-9的同源物，*Acan*-Gal-1在免疫调节方面展现出潜在应用价值。在感染动物模型中，*Acan*-Gal-1能够有效抑制宿主的炎症反应，表现为减轻脑组织病理损伤并降低血清中促炎细胞因子水平[15]。继而体外实验发现*Acan*-Gal-1可调控巨噬细胞的凋亡进程和炎症因子分泌能力[9]。

巨噬细胞在先天免疫中具有双重作用：既可抑制炎症，又能促进病理性改变[7, 16-17]。前期研究表明，广州管圆线虫幼虫分泌组分可破坏血脑屏障完整性，促进炎性介质向中枢神经系统浸润，从而诱发神经炎症反应。本研究重点探讨了*Acan*-Gal-1能选择性上调M2型表型标志分子（CD206、Fizz-1、IL-10、TGF-β及Arg-1）的表达，并有效抑制脂多糖诱导的M1型特征因子（IL-6、IL-1β和iNOS）的产生。这与本课题组在秀丽隐杆线虫虫体模型中*Acan*-Gal-1增强虫体氧化应激耐受性继而维持存活的结果一致，表明*Acan*-Gal-1通过促进巨噬细胞向M2型极化发挥免疫抑制作用[14]。

巨噬细胞极化受多种膜表面受体调控，这些受体通过识别不同配体激活下游信号通路从而决定极化方向。虽然TLR4/NF-κB通路已被广泛研究[18]，但Annexin A2作为膜受体调控巨噬细胞表型的作用尚不清楚。由于*Acan*-Gal-1能结合Annexin A2，其是否通过该受体调控巨噬细胞极化及代谢重编程值得深入研究。文献研究认为，Annexin A2通过维持内皮细胞连接稳定性调控血管通透性[19]。在免疫细胞中，单核/巨噬细胞表面高表达的Annexin A2可促进其向炎症部位迁移[20]。此外，Annexin A2还参与调控炎症小体活化，影响IL-1β和IL-18等促炎因子的分泌[21]。虽然Annexin A2通常被认为具有抗炎作用，但其功能可能随微环境变化而改变[22]。

本实验发现LPS刺激后，在*Acan*-Gal-1的作用下，Annexin A2的表达会被逆转，这可能涉及多种调控机制。有研究表明，其蛋白水平下降可能与泛素-蛋白酶体途径介导的降解有关，即Annexin A2经泛素化修饰后被蛋白酶体识别并降解[23]；溶酶体途径也可能参与调控，特别是当Annexin A2与配体结合后内化，进而通过自噬-溶酶体途径被清除[24]；在特定炎症环境中，Annexin A2也可能发生亚细胞转位或分泌至胞外，导致其在细胞内检测量降低[25]。A2ti-2（Annexin A2/S100A10异四聚体抑制剂）的加入并不影响*Acan*-Gal-1引起的基因表达水平和总蛋白表达量，但能够显著抑制Annexin A2的表面易位过程。本实验还发现*Acan*-Gal-1可通过调控Annexin A2膜易位过程影响巨噬细胞功能；敲低Annexin A2表达后，*Acan*-Gal-1既不能诱导巨噬细胞向免疫抑制表型转化，也无法拮抗LPS诱导巨噬细胞转化为免疫激活表型。因此，*Acan*-Gal-1引起的Annexin A2及S100A10的膜易位与巨噬细胞表型调控的因果关系还需进一步验证。对上述问题的深入研究，有助于揭示寄生虫免疫逃逸策略，也为开发新型抗炎药物提供了理论依据。

综上所述，本研究证实了*Acan*-Gal-1调控巨噬细胞免疫表型的改变，以及改变AnnexinA2及其伴侣蛋白S100A10的膜转位。结果阐明了*Acan*-Gal-1免疫调节的新机制，也为免疫微环境的重编程提供了潜在干预靶点；后续将继续研究Annexin A2膜易位的下游信号通路，并在实验动物模型中进一步验证。

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（编辑 余 菁）