雌二醇-ERβ信号轴通过调控M2型巨噬细胞分泌IGF-1促进雌鼠急性骨骼肌炎肌纤维修复

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[摘 要] 目的：雌激素的主要成分为雌二醇，在组织损伤修复中的作用因组织的再生能力而异。在具有强大再生潜能的骨骼肌中，其作用机制尚不明确。本研究旨在探究雌二醇信号对心脏毒素(cardiotoxin，CTX)诱导的小鼠急性骨骼肌炎中肌纤维再生修复的影响。**方法：**在体实验：选取野生C57BL/6雄鼠80只、雌鼠205只，对75只雌鼠进行卵巢去势(ovariectomized，OVX)。CTX诱导小鼠急性骨骼肌炎后，给予β-雌二醇(β-estradiol)、4-[2-苯基-5,7-双(三氟甲基)吡唑并[1,5-a]嘧啶-3-基]苯酚{4-[2-phenyl-5,7-bis(trifluoromethyl)pyrazolo[1,5-a]-pyrimidin-3-yl]phenol，PHTPP}等试剂肌肉注射，将小鼠分为control、β-estradiol、PHTPP、OVX、OVX+β-estradiol组。酶联免疫吸附分析(enzyme-linked immunosorbent assay，ELISA)法比较各组小鼠血清中雌二醇、肌酸激酶(creatine kinase，CK)表达；实时反转录聚合酶链反应(real-time reverse transcription PCR，real-time RT-PCR)检测雌鼠损伤肌内单核细胞趋化蛋白-1(monocyte chemoattractant protein-1，MCP-1)、白细胞介素(interleukin，IL)-13的信使RNA(messenger RNA，mRNA)表达；real-time RT-PCR、蛋白质印迹法(Western blotting，WB)检测损伤肌内雌激素受体(estrogen receptor，ER)α、ERβ、G蛋白偶联受体(G-protein coupled receptor，GPR)30的表达；流式细胞术检测雌鼠损伤肌内巨噬细胞(macrophage，Mac)表型与表达胰岛素样生长因子-1(insulin-like growth factor-1，IGF-1)、肝细胞生长因子(hepatocyte growth factor，HGF)的M2型Mac比例；免疫荧光检测雌鼠单核/Mac的浸润、磷酸化IGF-1受体(phosphorylated insulin-like growth factor 1 receptor，p-IGF-1R)、胚胎型肌球蛋白重链(embryonic myosin heavy chain，eMHC)的表达。细胞实验：用2%马血清(horse serum，HS)分化培养C2C12小鼠成肌细胞72 h，C2C12小鼠成肌细胞为成熟肌管后，给予γ干扰素(interferon gamma，IFN-γ)、β-estradiol、PHTPP等处理24 h。分为单独培养体系与共培养体系。单独培养体系，主要分为control(C2C12+HS)、IFN-γ、IFN-γ+β-estradiol、IFN-γ+PHTPP、IFN-γ+β-estradiol+PHTPP组。WB检测单独培养体系中C2C12细胞的细胞间充质-上皮转化因子(cellular mesenchymal-epithelial transition factor，c-Met)、p-IGF-1R、配对盒蛋白7(paired box protein 7，Pax7)等蛋白质的表达水平。共培养体系，经IFN-γ、β-estradiol、PHTPP等处理24 h后，将C2C12细胞与小鼠腹腔Mac共培养4 h，主要分为control(C2C12+HS+Mac)、IFN-γ、IFN-γ+β-estradiol、IFN-γ+PHTPP、IFN-γ+β-estradiol+PHTPP组。流式细胞术检测Mac表型、表达HGF或IGF-1的M2型Mac比例；WB检测共培养体系中C2C12细胞c-Met、p-IGF-1R、Pax7的蛋白质表达水平。**结果：**在体实验显示，雌鼠血清雌二醇与损伤肌内ERβ水平均显著高于雄鼠(均P<0.05)；与control组比较，OVX组损伤肌内表达IGF-1的M2型Mac比例、eMHC与p-IGF-1R的表达、*IL-13*的mRNA表达均显著下调(均P<0.05)。细胞实验显示，在单独培养体系中，各组C2C12细胞c-Met、p-IGF-1R、Pax7蛋白表达的差异均无统计学意义(均P>0.05)；在共培养体系中，与IFN-γ组比较，IFN-γ+β-estradiol组表达IGF-1的M2型Mac比例与C2C12细胞的p-IGF-1R、Pax7均显著上调(均P<0.05)；IFN-γ+PHTPP组趋势相反(P<0.05)。**结论：**雌二醇-ERβ信号轴通过促进雌鼠损伤肌内M2型Mac浸润及其IGF-1表达，进而缓解炎症并促进肌纤维再生修复。

[关键词] 雌激素；急性骨骼肌炎；雌激素受体β；胰岛素生长因子1；肌纤维再生与修复

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Estradiol-ERβ signaling axis promotes myofiber repair in female mice with acute skeletal muscle inflammation by regulating IGF-1 expression from M2 macrophages

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ABSTRACT

Objective: Estradiol is the principal form of estrogen, and its role in tissue injury and repair varies according to the regenerative capacity of the tissue. However, its mechanism of action in skeletal muscle, which has a strong regenerative capacity, remains unclear. This study aims to investigate the effects of estradiol signaling on myofiber regeneration and repair in a mouse model of cardiotoxin (CTX)-induced acute skeletal muscle inflammation.

Methods: In vivo experiments: A total of 80 wild-type male and 205 wild-type female C57BL/6 mice were included, of which 75 female mice underwent ovariectomy (OVX). Following the induction of acute skeletal muscle inflammation with CTX, the mice received intramuscular injections of β-estradiol, 4-[2-phenyl-5,7-bis(trifluoromethyl)pyrazolo[1,5-a]pyrimidin-3-yl]phenol (PHTPP), or other agents. The mice were divided into a control group, a β-estradiol group, a PHTPP group, a OVX group, and an OVX+β-estradiol group. Serum estradiol and creatine kinase (CK) levels were measured using enzyme-linked immunosorbent assays (ELISA). Real-time reverse transcription polymerase chain reaction (real-time RT-PCR) was used to determine the messenger RNA (mRNA) expression of monocyte chemoattractant protein-1 (MCP-1) and interleukin (IL)-13 in injured muscle from female mice. The expression of estrogen receptor (ER)α, ERβ, G protein-coupled estrogen receptor (GPR) 30, in injured muscle was assessed using real-time RT-PCR and Western blotting (WB). Flow cytometry was used to determine macrophage phenotypes and the proportions of M2 macrophages expressing insulin-like growth factor-1 (IGF-1) or hepatocyte growth factor (HGF) in injured muscle from female mice. Immunofluorescence staining was performed to evaluate monocyte/macrophage infiltration and the expression of phosphorylated IGF-1 receptor (p-IGF-1R) and embryonic myosin heavy chain (eMHC). In vitro experiments: C2C12 mouse myoblasts were differentiated into mature myotubes in medium containing 2% horse serum (HS) for 72 hours and then treated with interferon gamma (IFN-γ), β-estradiol, PHTPP, or combinations of these agents for 24 hours. Both monoculture and coculture systems were established. In the monoculture system, the main groups were control (C2C12+HS), IFN-γ, IFN-γ+β-estradiol, IFN-γ+PHTPP, and IFN-γ+β-estradiol+PHTPP. WB was used to assess the expression of mesenchymal-epithelial transition factor (c-Met), p-IGF-1R, paired box protein 7 (Pax7), and other proteins in C2C12 cells. In the coculture system, after 24 hours of treatment with IFN-γ, β-estradiol, PHTPP, or their combinations, C2C12 cells were cocultured with mouse peritoneal macrophages for 4 hours. The main groups were control (C2C12+HS+macrophages), IFN-γ,

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IFN-γ+β-estradiol, IFN-γ+PHTPP, and IFN-γ+β- estradiol+PHTPP. Flow cytometry was used to determine macrophage phenotypes and the proportions of M2 macrophages expressing HGF or IGF-1. WB was used to assess c-Met, p-IGF-1R, and Pax7 protein expression in C2C12 cells in the coculture system.

Results: In vivo experiments: The serum estradiol levels and ERβ expression in injured muscle were significantly higher in female mice than in male mice (both P<0.05). Compared with the control group, the OVX group showed significant decreases in the proportion of IGF-1-expressing M2 macrophages in injured muscle, the expression of eMHC and p-IGF-1R, and the mRNA expression of IL-13 (all P<0.05). In vitro experiments: No significant between-group differences were observed in c-Met, p-IGF-1R, or Pax7 protein expression in C2C12 cells in the monoculture system (all P>0.05). In the coculture system, compared with the IFN-γ group, the proportion of M2-type Mac expressing IGF-1 in the IFN-γ+β-estradiol group was significantly upregulated in both p-IGF-1R and Pax7 of C2C12 cells (all P<0.05), while in the IFN-γ+PHTPP group, the trend was opposite (all P<0.05).

Conclusion: Estradiol-ERβ signaling axis promotes the infiltration of M2 macrophages into injured skeletal muscle and enhances their expression of IGF-1 in female mice, thereby alleviating inflammation and promoting myofiber regeneration and repair.

KEY WORDS estrogen; acute skeletal myositis; estrogen receptor β; insulin-like growth factor 1; myofiber regeneration and repair

雌激素在组织损伤修复中的作用机制因组织再生能力而异。在再生能力有限的器官中，其保护作用主要体现在减轻即刻损伤[1-3]；而在具有强大再生潜能的骨骼肌中，其作用机制尚不明确。急性骨骼肌损伤修复的核心在于炎症的及时消退与肌纤维的有效再生，二者紧密衔接。研究[4]发现，损伤后的再生肌纤维特异性高表达雌激素受体(estrogen receptor，ER)β，这提示雌二醇-ERβ信号轴可能在修复过程中扮演关键角色。巨噬细胞(macrophage，Mac)表型极化是调控炎症-再生转换的核心环节：M1型Mac驱动炎症反应，而M2型Mac则促进炎症消退并分泌再生相关因子[如胰岛素样生长因子1(insulin-like growth factor 1，IGF-1)和肝细胞生长因子(hepatocyte growth factor，HGF)][5-8]。

基于此，本研究旨在通过阐明雌二醇-ERβ信号轴在雌鼠急性骨骼肌炎肌纤维修复中的具体机制，重点探讨该信号轴是否通过调控Mac向M2型Mac极化来促进炎症缓解，并进一步解析M2型Mac来源的IGF-1或HGF是否作为关键介质，驱动下游的肌卫星细胞活化与肌纤维再生，揭示雌激素在雌鼠骨骼肌再生修复中的独特作用路径，为相关治疗策略提供新靶点。

1 材料与方法

1.1 伦理声明

本研究已获得佛山大学实验动物伦理委员会批准(审批号：20250905)。

1.2 动物与细胞

从南方医科大学实验动物中心购买8~12周龄的C57BL/6小鼠，其中雄鼠80只、雌鼠205只，体重为18~22 g，生产许可证号为SCXK(粤)2021-0041。

C2C12小鼠成肌细胞购自美国模式培养物集存库(American Type Culture Collection，ATCC)，Mac为小鼠腹腔Mac。

1.3 主要试剂与仪器

心脏毒素(cardiotoxin，CTX；100 μg/mL)、β-雌二醇(β-estradiol)均购自德国Sigma公司；4-[2-苯基-5,7-双(三氟甲基)吡唑并[1,5-a]嘧啶-3-基]苯酚{4-[2-phenyl-5, 7-bis(trifluoromethyl)pyrazolo[1, 5-a] -pyrimidin-

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3-yl]phenol，PHTPP)购自美国MCE(MedChemExpress)公司；兔抗ERα多克隆抗体、小鼠抗ERβ单克隆抗体、兔抗G蛋白偶联受体(G-protein coupled receptor，GPR)30多克隆抗体、驴抗小鼠抗肌萎缩蛋白(dystrophin)、兔抗小鼠F4/80、兔抗小鼠CD11b、兔抗小鼠配对框(paired box，Pax)7、大鼠抗小鼠胚胎型肌球蛋白重链(embryonic myosin heavy chain，eMHC)、大鼠抗小鼠胰岛素样生长因子1受体(insulin-like growth factor 1 receptor，IGF-1R)、大鼠抗小鼠磷酸化胰岛素样生长因子1受体(phosphorylated insulin-like growth factor 1 receptor，p-IGF-1R)、山羊抗小鼠细胞间充质-上皮转化因子(cellular mesenchymal-epithelial transition factor，c-Met)均购自美国Abcam公司；藻红蛋白(phycoerythrin，PE；标记F4/80)、Alexa fluor 700[标记巨噬细胞甘露糖受体(macrophage mannose receptor，MMR)，CD206]、eFluor 405[标记主要组织相容性复合体(major histocompatibility complex，MHC)-II]、别藻蓝蛋白(allophycocyanin，APC；标记IGF-1)、太平洋蓝[标记肝细胞生长因子(hepatocyte growth factor，HGF)]、驴抗羊二抗、羊抗兔二抗、羊抗大鼠二抗、大鼠抗兔异硫氰酸荧光素(fluorescein isothiocyanate，FITC)、酶标仪均购自美国ThermoFisher公司；小鼠抗3-磷酸甘油醛脱氢酶(glyceraldehyde-3-phosphate dehydrogenase，GAPDH)单克隆抗体、大鼠抗兔免疫球蛋白G-辣根过氧化物酶(immunoglobulin G-horseradish peroxidase，IgG-HRP)、山羊抗大鼠IgG-HRP、兔抗山羊IgG-HRP均购自杭州弗德生物科技有限公司；γ干扰素(interferon gamma，IFN-γ)购自美国Research and Diagnostic Systems公司；小鼠肌酸激酶(creatine kinase，CK)酶联免疫吸附分析(enzyme-linked immunosorbent assay，ELISA)试剂盒、小鼠雌二醇的ELISA试剂盒均购自上海瑞番生物科技有限公司。多维高清流式细胞仪、FACS Aria II细胞分选仪均为美国BD公司(Becton, Dickinson and Company)产品；荧光显微镜为奥林巴斯中国有限公司产品；实时反转录聚合酶链反应(real-time reverse transcription PCR，real-time RT-PCR)仪器为美国ABI(Applied Biosystems)公司产品。

1.4 方法

1.4.1 造模

卵巢去势(ovariectomized，OVX)模型：小鼠采用腹腔注射氯胺酮(90 mg/kg)和甲苯噻嗪(4.5 mg/kg)麻醉，开腹摘除双侧卵巢，缝合伤口后注射青霉素(22 000 U/kg)[9]制备。

急性骨骼肌损伤模型：在小鼠胫骨前肌(tibialis anterior，TA)注射50 μL CTX(50 μg/mL)以诱导急性骨骼肌损伤[10]。OVX鼠于术后28 d注射CTX诱导损伤。该模型稳定、闭合性好、无菌，可排除外源性干扰[10]。

β-estradiol或PHTPP干预雌鼠：采用CTX注射后1 d，动物分别接受雌二醇激动剂β-estradiol(50 μL/d，50 mg/L)或ERβ拮抗剂PHTPP(50 μL/d，2 g/L)皮下注射[11-12]制备。

1.4.2 血清雌二醇检测与雌鼠急性骨骼肌损伤模型的鉴定

1.4.2.1 血清雌二醇检测

创伤后机体常出现以雌激素升高为特征的性激素水平变化[13]。为了验证CTX致急性骨骼肌损伤后小鼠血清中雌二醇的表达水平，取雄鼠70只、雌鼠70只进行血清雌二醇水平动态检测。雄鼠、雌鼠随机分为对照(PBS-Male、PBS-Female)组和损伤(CTX-Male、CTX-Female)组；损伤组小鼠注射CTX诱导急性骨骼肌损伤、对照组小鼠注射磷酸盐缓冲盐水(phosphate buffered saline，PBS)，分别于当天(D0)、第1天(D1)、第3天(D3)、第7天(D7)、第10天(D10)、第15天(D15)、第20天(D20)取材(n=5)；低温离心分离血清，按照小鼠雌二醇的ELISA试剂盒说明书检测血清雌二醇浓度。

OVX鼠不同时间段的血清雌二醇验证：取OVX鼠15只，于OVX后的D0、第14天(D14)、第28天(D28)检测血清雌二醇(n=5)。低温离心分离血清，按照小鼠雌二醇的ELISA试剂盒说明书检测血清雌二醇浓度。

1.4.2.2 雌鼠急性骨骼肌损伤模型的鉴定

取1.4.2.1中的CTX-Female组的D0、D3、D7、D10、D15、D20的骨骼肌，制备厚度为6 μm的冰冻切片。对切片进行苏木精-伊红(hematoxylin and eosin，HE)染色，即将冰冻切片经过固定、苏木精染色、分化与返蓝、伊红染色、脱水、透明、封片等步骤，检测雌鼠骨骼肌的炎性细胞浸润、溃变的肌纤维及中央核肌纤维情况以鉴定雌鼠急性骨骼肌损伤模型。

1.4.3 损伤肌ER信使RNA和蛋白质的检测

组织损伤后，雌二醇信号主要由ER(ERα、ERβ或GPR30)接收[14]。为明确急性骨骼肌损伤后雌二醇信号主要经由哪类受体介导，本研究通过real-time RT-PCR和蛋白质印迹法(Western blotting，WB)检测损伤局部ER的表达。

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提取D0、D3、D7、D10雄鼠与雌鼠的损伤肌组织总RNA(*n*=5)，real-time RT-PCR检测*ERα*、*ERβ*、*GPR30*的信使RNA(messenger RNA，mRNA)表达，引物序列见附表1(https://doi.org/10.57760/sciencedb.xbyxb.00180)。

取雄鼠、雌鼠各10只，分别于损伤后D3、D7取材(*n*=5)，提取各组损伤肌内总蛋白质，WB检测损伤肌内ERα、ERβ、GPR30等蛋白质的表达。

1.4.4 HE染色及免疫荧光染色

为了进一步探究炎症不同处理对雌鼠损伤肌炎症的影响，对急性骨骼肌损伤模型雌鼠采用OVX、β-estradiol、PHTPP等处理。取雌鼠50只，分为control、β-estradiol、PHTPP、OVX、OVX+β-estradiol组，分别于损伤后D3、D7取材，制备厚度为6 μm冰冻切片(*n*=5)。后续分别进行HE染色、免疫荧光染色。免疫荧光染色一抗(1∶200)，二抗(1∶600)，染色后于荧光显微镜下观察。

1.4.5 血清CK检测

骨骼肌损伤后，CK可从损伤肌细胞释放至细胞外[15]。为了验证不同组别的小鼠急性骨骼肌损伤后血清中CK表达情况，取急性骨骼肌损伤模型雌鼠30只，分为control、OVX、OVX+β-estradiol组(*n*=5)。按照小鼠CK的ELISA试剂盒说明书检测血清CK浓度。

1.4.6 炎症因子mRNA检测

骨骼肌损伤后，炎症因子可从损伤肌细胞释放至细胞外[16]。为了验证不同组别的小鼠急性骨骼肌损伤后损伤肌内炎症因子表达情况，提取1.4.5中control、OVX、OVX+β-estradiol组雌鼠D3、D7(*n*=5)损伤肌组织总RNA，real-time RT-PCR检测*MCP-1*、*IL-13*的mRNA表达，引物序列见附表2(https://doi.org/10.57760/sciencedb.xbyxb.00180)。

1.4.7 流式细胞术分析

取急性骨骼肌损伤雌鼠30只，分为control、OVX、OVX+β-Estradiol组，分别于损伤后D3、D7取材(*n*=5)。取材后将损伤肌剪碎，于37 ℃下使用0.2%胶原酶II消化骨骼肌，对肌组织进一步匀浆。过滤，离心收集细胞，重悬于PBS中，封闭Fc段，添加荧光抗体(1∶100)，多维高清流式细胞分析仪分析$F4/80^+MHC\text{-}II^+$、$F4/80^+CD206^+$、$F4/80^+CD206^+IGF\text{-}1^+$、$F4/80^+CD206^+HGF^+$细胞的比例。

1.4.8 体外验证

1.4.8.1 C2C12单独培养

为排除体内复杂因素的干扰，进一步对C2C12小鼠成肌细胞单独培养，并进行相关验证。C2C12小鼠成肌细胞培养于含10%胎牛血清(fetal bovine serum，FBS)和青霉素-链霉素的杜尔贝科改良伊格尔培养基/营养混合物F-12(Dulbecco's modified Eagle medium/nutrient mixture F-12，DMEM/F12)中，待融合70%~80%后，换用含2%马血清(horse serum，HS)的分化培养基培养72 h，随后以IFN-γ(3 ng/mL)诱导炎症24 h，并同时给予β-estradiol(1.0 nmol/L)和/或PHTPP(0.1 μmol/L)处理。实验分为5组：control(C2C12+HS)、IFN-γ、IFN-γ+β-estradiol、IFN-γ+PHTPP、IFN-γ+β-estradiol+PHTPP。IGF-1和HGF可分别通过结合p-IGF-1R和c-Met促进肌卫星细胞增殖[8, 17]。为明确雌二醇-ERβ信号轴是否直接调控肌管中c-Met、p-IGF-1R及Pax7的表达，提取C2C12小鼠成肌细胞总蛋白质，WB检测c-Met、p-IGF-1R及Pax7表达。

1.4.8.2 C2C12与Mac共培养

将腹腔Mac(1×10^6个)置于Transwell内层，与C2C12共培养4 h。实验分为5组：control(C2C12+HS+Mac)、IFN-γ、IFN-γ+β-estradiol、IFN-γ+PHTPP、IFN-γ+β-estradiol+PHTPP。

M2型Mac可分泌IGF-1和/或HGF，而IGF-1、HGF均可促进肌卫星细胞增殖[5-8]。为验证在上述共培养体系中雌二醇信号是否会调控表达IGF-1或HGF蛋白的M2型Mac比例。采用流式细胞术检测Mac表型($F4/80^+MHC\text{-}II^+$、$F4/80^+CD206^+$)及M2型Mac中表达HGF或IGF-1的比例($F4/80^+CD206^+HGF^+$、$F4/80^+CD206^+IGF\text{-}1^+$)；同时提取C2C12蛋白，WB检测c-Met、p-IGF-1R、Pax7的表达水平。

上述所有实验流程数据实验动物样本量汇总见附表3(https://doi.org/10.57760/sciencedb.xbyxb.00180)。

1.5 统计学处理

图片均采用ImageJ处理。采用IBM SPSS 22.0统计学软件进行统计分析。数据采用均数±标准差表示。多组间比较采用单因素方差分析(one-way ANOVA)。若存在显著差异，进一步的两两比较采用Tukey HSD法。2组间比较采用独立样本*t*检验。$P<0.05$为差异具有统计学意义。

2 结 果

2.1 雌鼠急性骨骼肌损伤模型的鉴定

HE染色结果(图1)显示，CTX诱导损伤后，雌鼠肌纤维间炎性细胞(绿色箭头标记)浸润于D3达峰，

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D20恢复至损伤前水平。损伤组肌纤维出现溃变(蓝色箭头标记)，中央核肌纤维增多(黄色箭头标记)；随修复进程，再生肌纤维直径增大、核逐渐移向周边，至D20肌纤维直径基本恢复至D0水平。

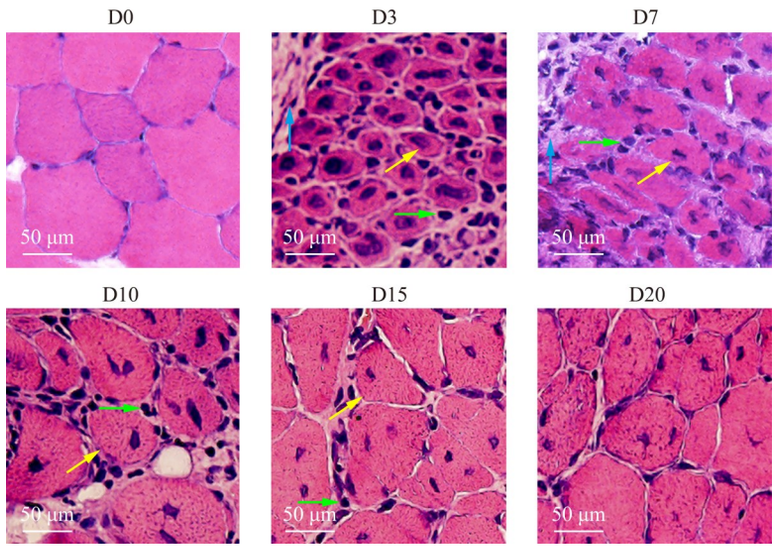


图1　雌鼠急性骨骼肌损伤模型鉴定(*n*=5)

Figure 1　Identification of the acute skeletal muscle injury model in female mice (*n*=5)

HE staining of acute skeletal muscle injury in female mice. Green arrows indicate inflammatory infiltrating cells, blue arrows indicate degenerated muscle fibers, and yellow arrows indicate regenerating muscle fibers. D0: Day 0; D3: Day 3; D7: Day 7; D10: Day 10; D15: Day 15; D20: Day 20; HE: Hematoxylin and eosin.

2.2 肌损伤后血清雌二醇升高及再生肌纤维ERβ表达上调

ELISA检测结果(图2)显示，CTX致急性骨骼肌损伤后，雌、雄小鼠血清中的雌二醇水平均显著升高(均P<0.05)，且雌鼠血清中的雌二醇水平显著高于雄鼠(P<0.05)。

体内实验结果显示，雌鼠损伤肌内*ERβ*的mRNA(图3A)和蛋白质(图3B)水平均显著高于*ERα*和*GPR30*(均P<0.01)，且雌鼠的ERβ蛋白水平显著高于雄鼠(P<0.05)。此外，ELISA结果(图3C)显示，OVX可有效降低雌鼠体内雌二醇水平，且术后28 d尤为显著(P<0.01)。

为排除体内复杂因素的干扰，对C2C12细胞单独培养进行研究，结果(图3D)显示，炎性刺激仅显著上调ERβ蛋白的表达(P<0.01)，而对ERα和GPR30的表达均无显著影响(均P>0.05)。

2.3 雌激素信号通过ERβ调控损伤肌内炎症

HE染色结果(图4A)显示，CTX致急性骨骼肌损伤后，与control组雌鼠比较，β-estradiol组雌鼠损伤肌内炎性细胞浸润显著减少，再生肌纤维直径显著增大；PHTPP组、OVX组雌鼠的趋势相反；OVX组雌鼠予以β-estradiol处理后，趋势得以矫正。

免疫荧光结果(图4B)显示，CTX致急性骨骼肌损伤后，损伤处浸润的炎症细胞以单核/Mac为主。与control组雌鼠比较，OVX组雌鼠的单核/Mac显著增多(P<0.05)；OVX组雌鼠予以β-estradiol处理后，其浸润数量得以矫正(P<0.05)。

ELISA结果(图5A)显示，CTX致急性骨骼肌损伤后，与control组雌鼠比较，OVX组雌鼠血清CK表达显著上调(P<0.05)；OVX组雌鼠予以β-estradiol处理后，CK的表达得以矫正(P<0.05)。

Real-time RT-PCR结果(图5B)显示，CTX致急性骨骼肌损伤后，与control组雌鼠比较，OVX组雌鼠损伤肌内促炎因子*MCP-1*显著上调(P<0.05)，抗炎因子*IL-13*显著下调(P<0.05)；OVX组雌鼠予以β-estradiol处理后，*MCP-1*、*IL-13*的表达均得以矫正(均P<0.05)。

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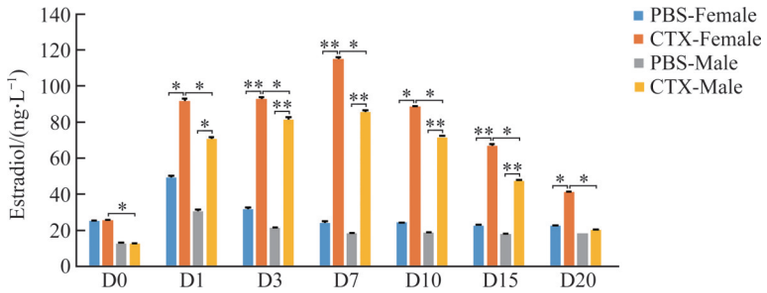


图2 CTX致急性骨骼肌损伤后小鼠血清中雌二醇的表达水平(*n*=5)

Figure 2 Expression levels of estradiol in mouse serum after CTX-induced acute skeletal muscle injury (*n*=5)

ELISA detection of estradiol levels in mouse serum after muscle injury. Data are represented with mean±standard deviation. **P*<0.05, ***P*<0.01. Independent samples *t*-test was used for statistical analysis. PBS: Phosphate buffered saline; CTX: Cardiotoxin; ELISA: Enzyme-linked immunosorbent assay; D1: Day 1.

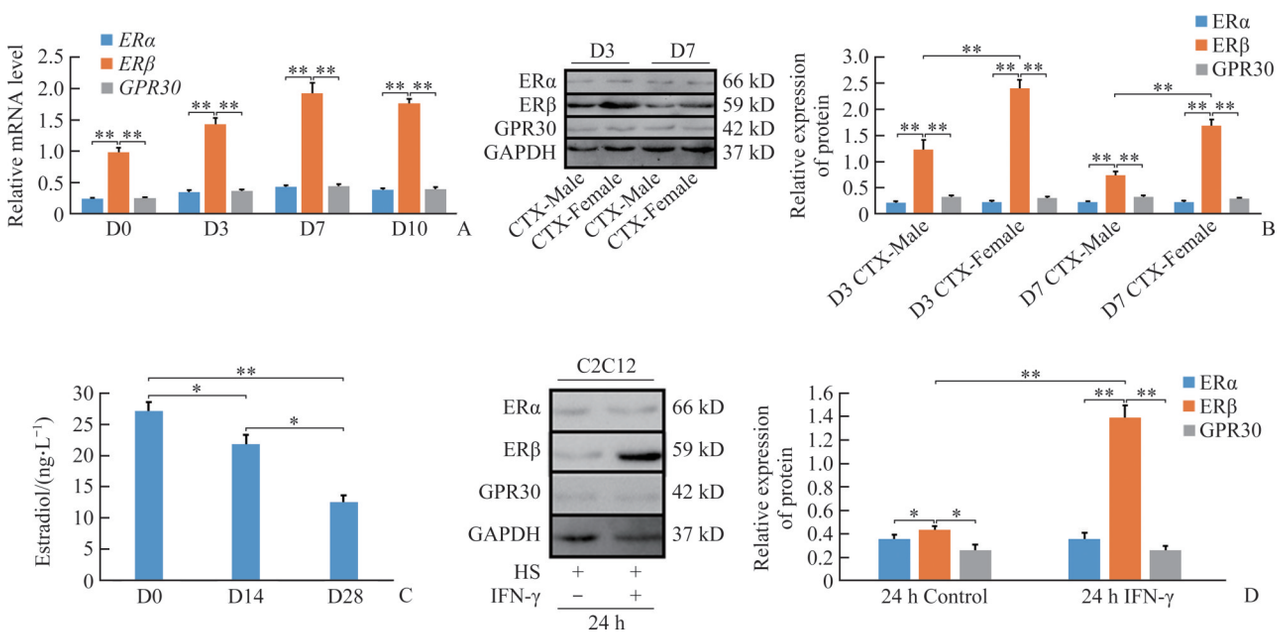


图3 肌损伤诱导再生肌纤维ERβ表达上调及OVX模型对雌二醇的验证(*n*=5)

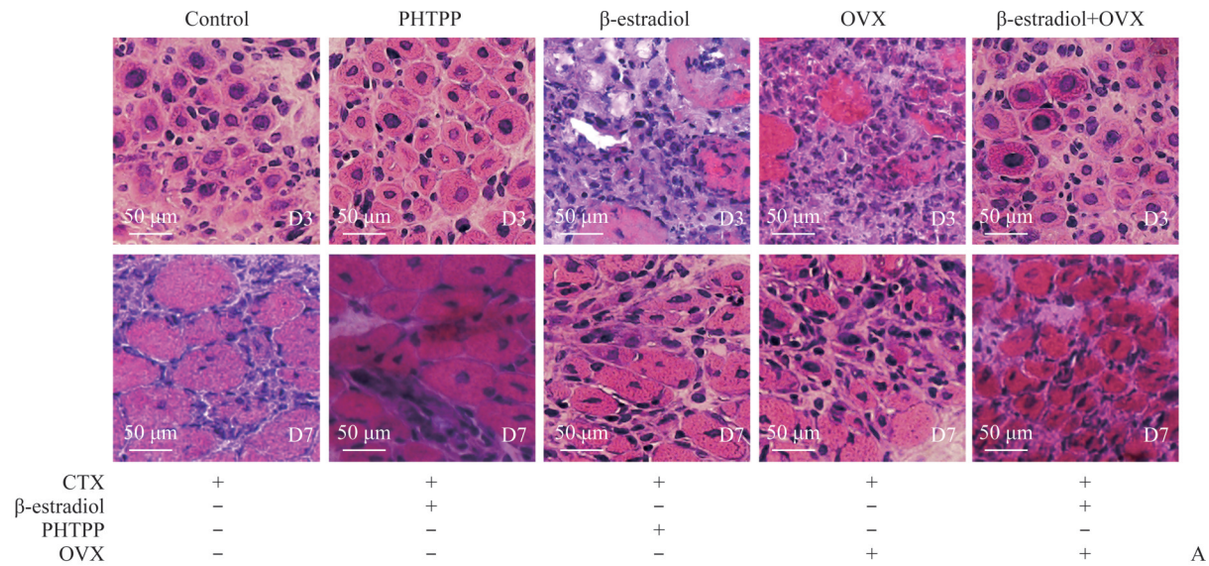
Figure 3 ERβ upregulation in regenerating myofibers post-injury and estradiol validation via OVX model (*n*=5)

A: mRNA levels of *ERα*, *ERβ*, and *GPR30* in injured female mouse muscle. B: Protein expression of ERα, ERβ, and GPR30 in injured muscle detected by WB. C: Serum estradiol levels in female mice at different time points after OVX treatment. D: Protein expression of ERα, ERβ, and GPR30 in inflamed C2C12 myotubes by WB. Data are represented with mean±standard deviation. **P*<0.05, ***P*<0.01. Tukey's HSD test was used for statistical analysis. mRNA: Messenger RNA; *ERα*: Estrogen receptor α; *ERβ*: Estrogen receptor β; *GPR30*: G-protein coupled receptor 30; WB: Western blotting; OVX: Ovariectomized; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; IFN-γ: Interferon gamma; HS: Horse serum.

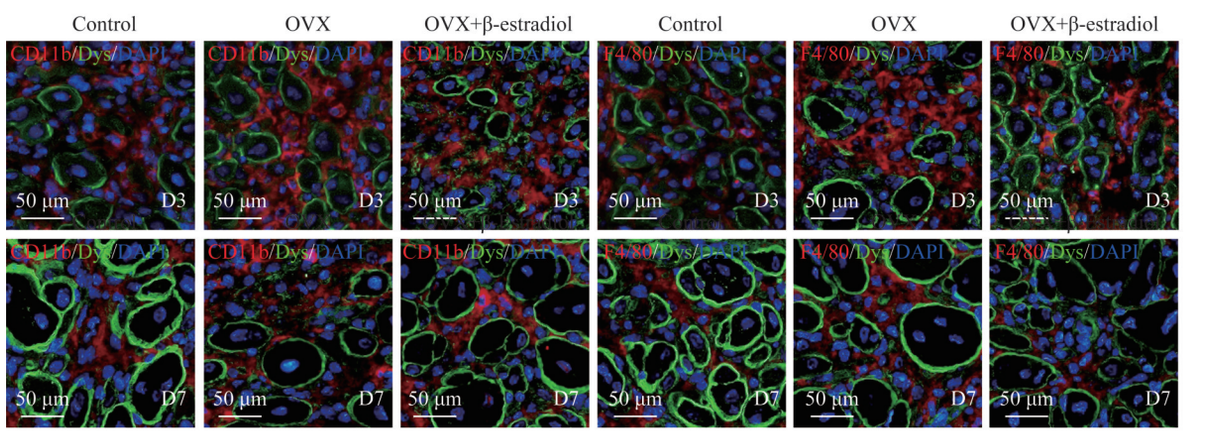
2.4 雌二醇-ERβ通过调控损伤肌内M2型Mac IGF-1的表达影响肌纤维再生与修复

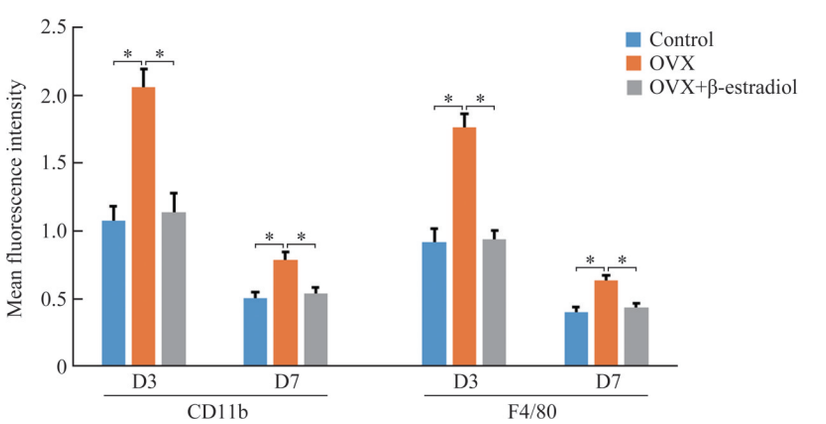
采用流式细胞术分析雌鼠损伤肌内Mac的表型，结果显示，与control组雌鼠比较，OVX组雌鼠的M1型Mac(F4/80$^+$MHC-II$^+$)比例显著上调(P<0.05)，M2型Mac(F4/80$^+$CD206$^+$)比例显著下调(P<0.01)；OVX组雌鼠予以β-estradiol处理后，M1、M2型Mac比例均得以矫正(均P<0.05，图6)。

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A





B

图4　不同处理对雌鼠急性损伤肌内炎症细胞浸润的影响(*n*=5)

Figure 4　Effects of different treatments on inflammatory cell infiltration in acute injured female mouse muscle (*n*=5)

A: HE staining to detect the inflammation condition in injured female mouse muscle after β-estradiol, PHTPP, or OVX treatment. B: Immunofluorescence detection of monocyte/macrophage infiltration in injured female mouse muscle. Data are represented with mean±standard deviation. **P*<0.05. Tukey's HSD test was used for statistical analysis. CTX: Cardiotoxin; PHTPP: 4-[2-Phenyl-5,7-bis (trifluoromethyl)pyrazolo[1,5-a]- pyrimidin-3-yl]phenol; OVX: Ovariectomized.

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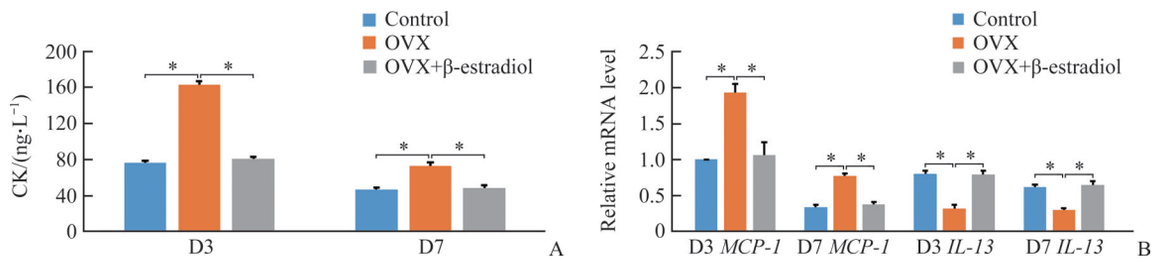


图5 不同处理对雌鼠急性骨骼肌损伤后炎症指标的影响(*n*=5)

Figure 5 Effects of different treatments on inflammatory markers in female mice after acute skeletal muscle injury (*n*=5)

A: ELISA detection of serum CK levels in wild-type, OVX, or β-estradiol-treated female mice. B: Real-time RT-PCR detection of mRNA expression of inflammatory factors in injured female mouse muscle. Data are represented with mean±standard deviation. **P*< 0.05. Tukey's HSD test was used for statistical analysis. CK: Creatine kinase; *MCP-1*: Monocyte chemoattractant protein-1; *IL-13*: Interleukin-13.

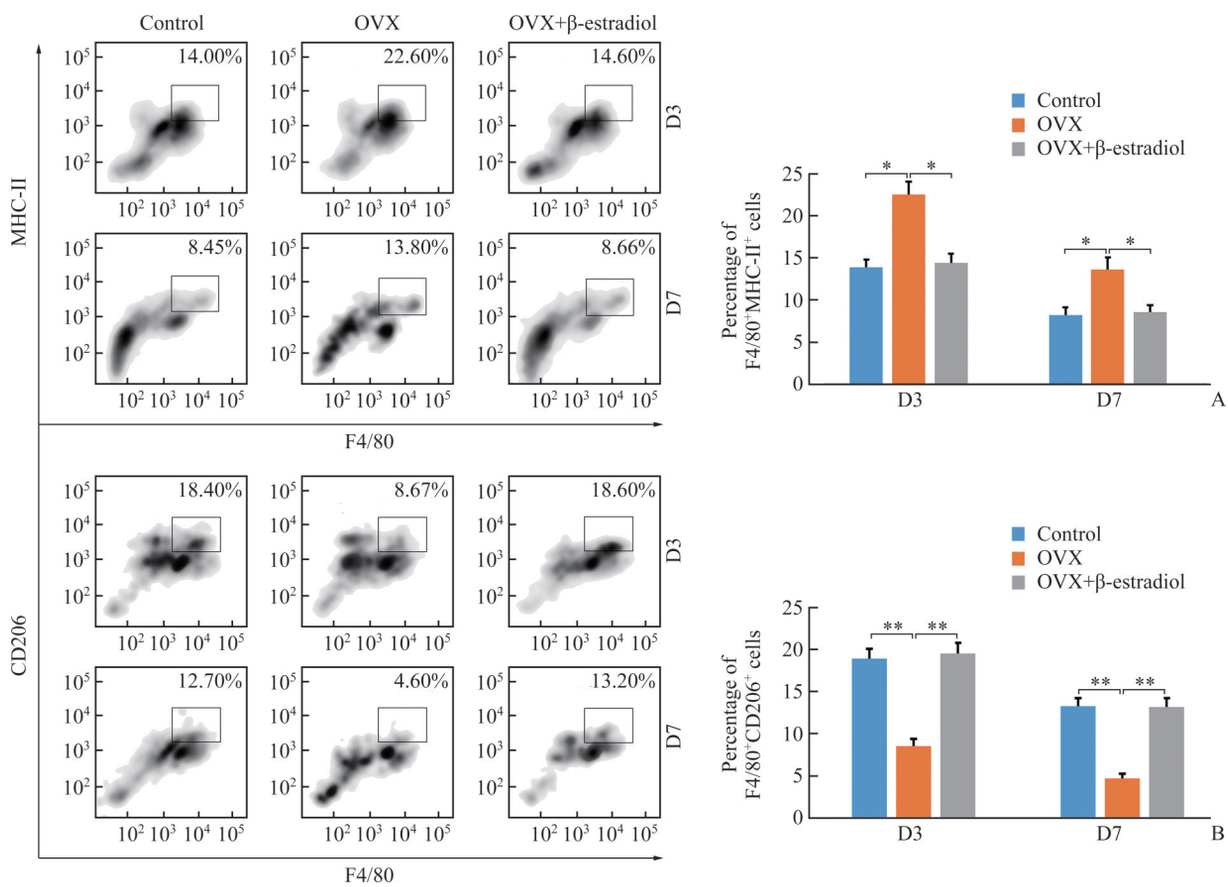


图6 雌激素信号调控雌鼠损伤肌内M1/M2型Mac比例(*n*=5)

Figure 6 Estrogen signaling regulates the proportions of M1/M2 Mac in injured female mouse muscle (*n*=5)

A and B: Flow cytometry detection of the proportions of M1 (A) and M2 (B) macrophages in injured female mouse muscle. Data are represented with mean±standard deviation. **P*<0.05, ***P*<0.01. Tukey's HSD test was used for statistical analysis. MHC: Major histocompatibility complex; Mac: Macrophage.

体外流式细胞术结果(图7A)显示，在C2C12与Mac共培养体系中，与IFN-γ组相比，单独给予β-estradiol可显著下调M1型Mac比例、上调M2型Mac比例(均*P*<0.05)；单独给予PHTPP则效果相反(*P*<0.05)；联合给予β-estradiol可逆转PHTPP引起的改变(*P*<0.05)。进一步分析M2型Mac的功能亚群结

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果(图7B)发现，与IFN-γ组相比，单独给予β-estradiol显著上调表达IGF-1的M2型Mac比例(P<0.05)，而PHTPP则下调该比例(P<0.05)，联合给药可逆转PHTPP的下调作用。相比之下，表达HGF的M2型Mac比例在各组间的差异均无统计学意义(均P>0.05)。

WB结果显示，在C2C12单独培养体系中，与control组相比，其余处理组(含IFN-γ、β-estradiol或PHTPP)上述分子表达差异均无统计学意义(均P>0.05，图8A)。进一步将C2C12与腹腔Mac共培养发现，与control+IFN-γ+Mac组相比，单独给予β-estradiol显著上调p-IGF-1R的表达(P<0.05)，而单独给予PHTPP则显著下调p-IGF-1R的表达(P<0.01)；联合给药可逆转PHTPP引起的p-IGF-1R表达下调(P<0.01)。Pax7的表达趋势与p-IGF-1R一致，而c-Met在各组间的差异均无统计学意义(均P>0.05，图8B)。

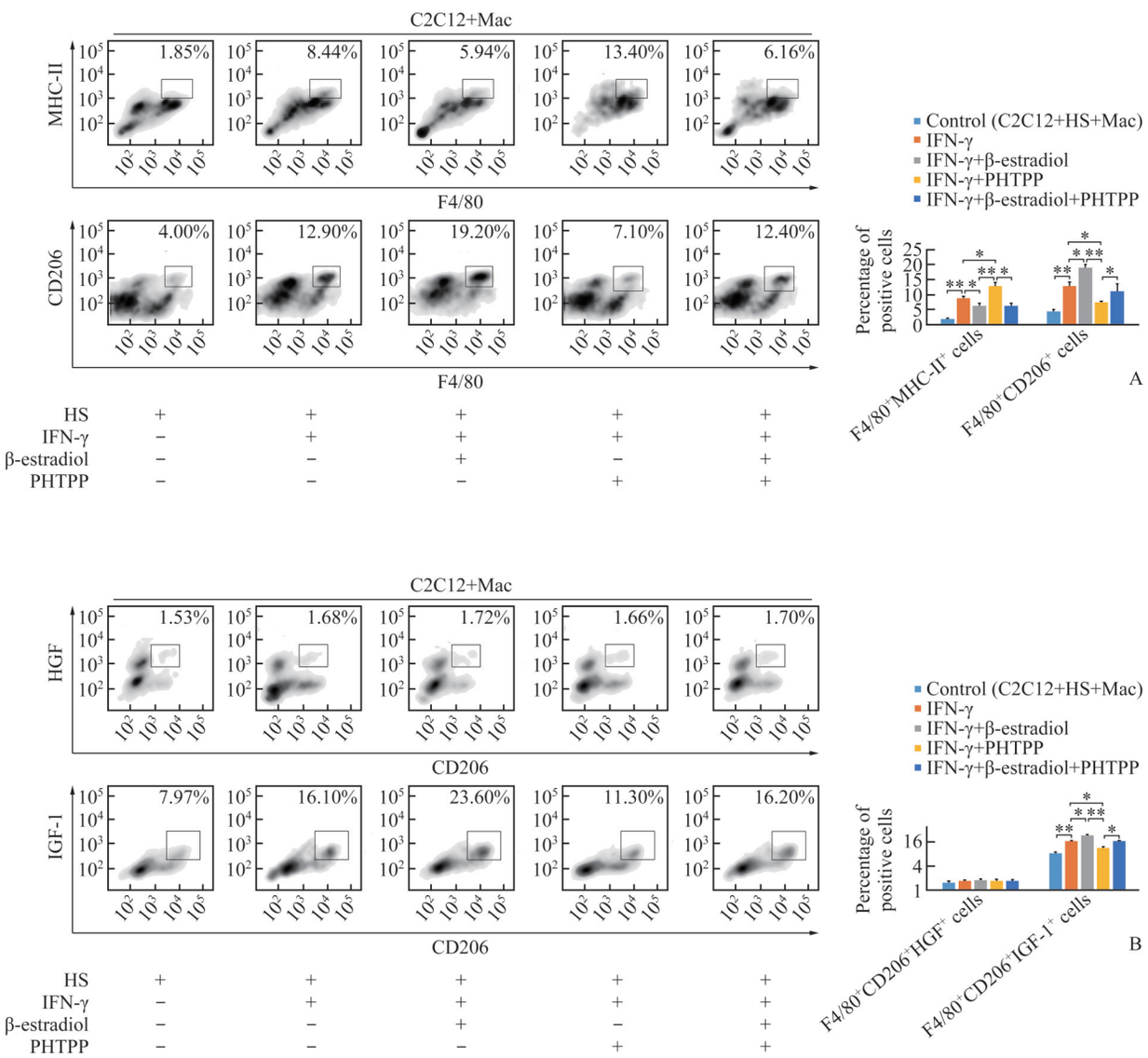


图7　雌二醇-ERβ信号轴调控共培养体系中Mac的表型与功能(n=5)

Figure 7　Estradiol-ERβ signaling axis regulates Mac phenotype and functions in a co-culture system (n=5)

A: Flow cytometry detection of the proportion of M1 and M2 macrophages in different groups of the co-culture system. B: Flow cytometry detection of the proportion of M2 macrophages expressing IGF-1 or HGF in different groups of the co-culture system. Data are represented with mean±standard deviation. *P<0.05, **P<0.01. Tukey's HSD test was used for statistical analysis. HGF: Hepatocyte growth factor; IGF-1: Insulin-like growth factor 1.

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流式细胞术结果(图9)显示，与control组相比，OVX组表达IGF-1的M2型Mac比例显著下调(P<0.05)；在OVX的基础上予以β-estradiol处理，其表达得以矫正(P<0.05)；免疫荧光结果(图10A、10B)显示，与control组相比，OVX组肌纤维p-IGF-1R、eMHC的表达均显著下调(均P<0.05)；在OVX的基础上予以β-estradiol处理，p-IGF-1R、eMHC的表达均得以矫正(均P<0.05)。

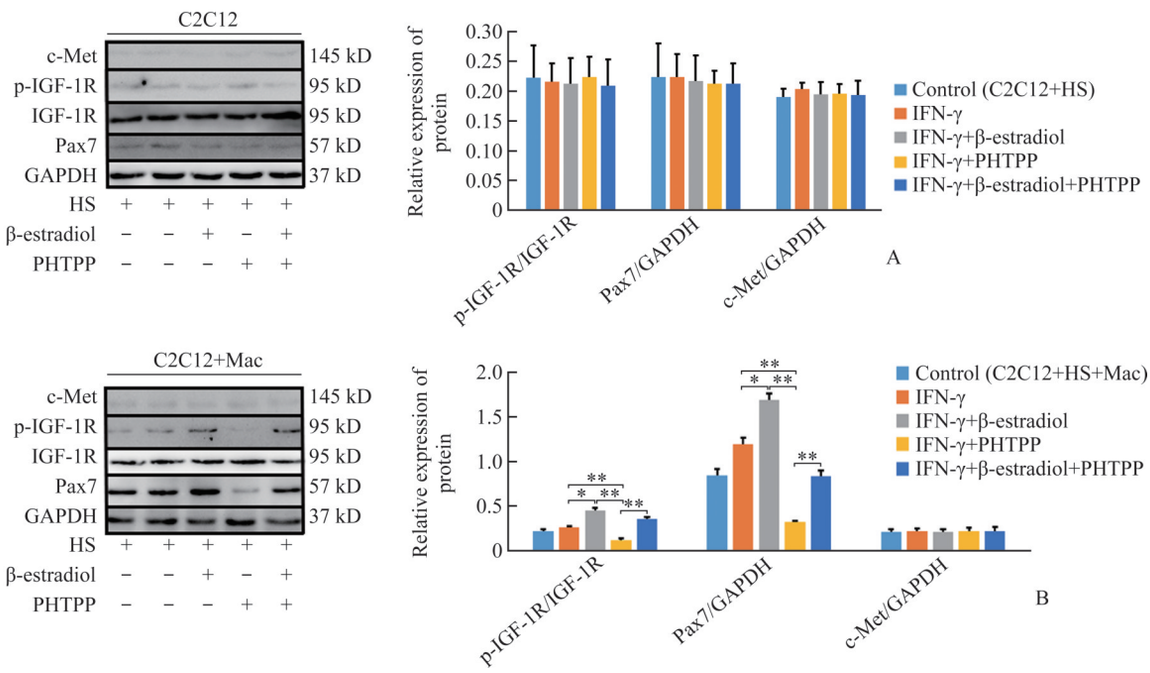


图8 雌二醇-ERβ信号轴调控体外培养体系中C2C12小鼠成肌细胞c-Met、p-IGF-1R、Pax7的表达(n=5)

Figure 8 Estradiol-ERβ signaling axis regulates the expression of c-Met, p-IGF-1R, and Pax7 in C2C12 mouse myoblasts in culture system in vitro (n=5)

A: WB detection of c-Met, p-IGF-1R and Pax7 expression in C2C12 mouse myoblasts from different groups in a monoculture C2C12 system (no Mac). B: WB detection of c-Met, p-IGF-1R and Pax7 expression in C2C12 mouse myoblasts from different groups in the co-culture system. Data are represented with mean±standard deviation. *P<0.05, **P<0.01. Tukey's HSD test was used for statistical analysis. c-Met: Cellular mesenchymal-epithelial transition factor; p-IGF-1R: Phosphorylated insulin-like growth factor 1 receptor; IGF-1R: Insulin-like growth factor 1 receptor; Pax7: Paired box protein 7.

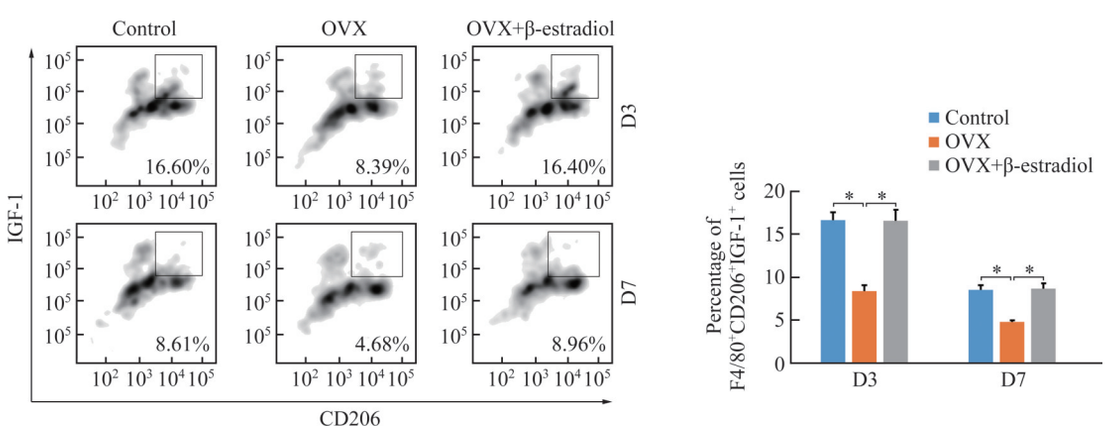


图9 雌激素信号影响雌鼠损伤肌内IGF-1的表达(n=5)

Figure 9 Estrogen signaling affects the expression of IGF-1 in injured female mouse muscle (n=5)

Flow cytometry detection of the proportion of M2 macrophages expressing IGF-1 in injured female mouse muscle. Data are represented with mean±standard deviation. *P<0.05. Tukey's HSD test was used for statistical analysis.

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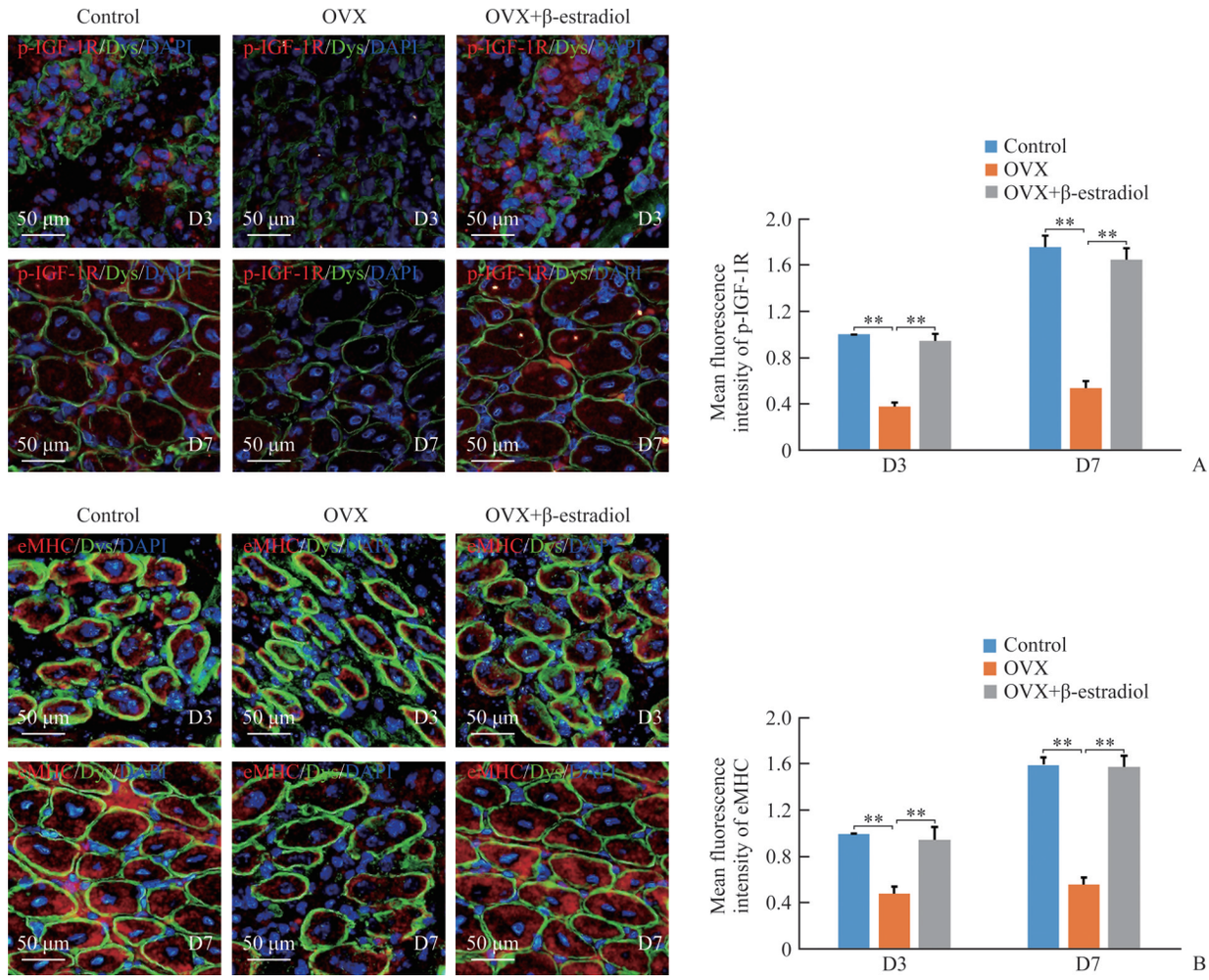


图10　雌激素信号影响雌鼠损伤肌内p-IGF-1R和eMHC的表达(*n*=5)

Figure 10　Estrogen signaling affects the expression of p-IGF-1R and eMHC in injured female mouse muscle (*n*=5)

A: Immunofluorescence detection of p-IGF-1R expression in muscle fibers of injured female mouse muscle. B: Immunofluorescence detection of eMHC expression in muscle fibers of injured female mouse muscle. Data are represented with mean±standard deviation. **P<0.01. Tukey's HSD test was used for statistical analysis. eMHC: Embryonic myosin heavy chain.

3 讨　论

雌二醇及其受体在多数组织损伤中主要通过限制损伤程度发挥保护作用[18-19]，但在富含卫星细胞的骨骼肌中调控机制具有根本差异[20-21]。急性损伤后，骨骼肌依赖卫星细胞的激活、增殖与分化完成修复。本研究发现，CTX致急性骨骼肌损伤后，雌、雄鼠血清雌二醇均显著升高，且雌鼠高于雄鼠；文献[22]显示相同损伤下雄鼠炎性浸润更重，提示雌二醇可能参与调控炎症与再生，但其具体途径尚不明确。

本研究及文献[23-24]证实，损伤区再生肌纤维主要表达ERβ，且其信号活性与修复质量呈正相关。据此提出核心论点：雌二醇-ERβ信号轴在骨骼肌急性损伤修复中的首要功能是直接创造并优化适于肌卫星细胞激活与分化的局部微环境。本研究采用OVX模型降低雌鼠雌二醇水平，并以β-estradiol和PHTPP干预[25-27]。

损伤后CK和炎症因子可从肌细胞释放，Mac被募集至局部[15-16]。本研究发现，与control组相比，OVX组血清CK、肌内促炎因子及单核/Mac浸润均显著升高，β-estradiol可逆转；单独PHTPP亦可增加炎性浸润。OVX组M1型Mac增多、M2型减少，β-estradiol矫正此失衡，且CK和MCP-1趋势与M1一致。上述结果表明，雌二醇-ERβ通过促进M2型Mac浸润、下调CK和MCP-1，加速炎症缓解[28-29]。

炎症消退是肌纤维再生的前提，eMHC为评估再生的关键指标[30-31]。OVX组eMHC表达降低，β-estradiol可使其恢复。M2型Mac分泌的IGF-1

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通过p-IGF-1R促进肌卫星细胞增殖[5-6]。本研究发现，OVX组表达IGF-1的M2比例及p-IGF-1R均下降，β-estradiol可逆转这一变化。这提示雌二醇-ERβ可能通过促进M2表达IGF-1推动再生。

此外，M2型Mac分泌的HGF亦可经c-Met促进肌卫星细胞增殖[7-8]。为排除体内干扰，开展体外实验。单独给予C2C12肌管β-estradiol(无Mac)不改变c-Met、p-IGF-1R及Pax7表达，提示雌二醇-ERβ不能直接调控上述分子，其促增殖依赖其他细胞介导。将C2C12与腹腔Mac共培养后发现，β-estradiol显著提高M2数量及表达IGF-1的M2比例，PHTPP则相反；但表达HGF的M2比例无显著差异，说明雌二醇-ERβ对M2功能调控具有分泌因子特异性[32-33]。加入Mac后，β-estradiol组C2C12中p-IGF-1R和Pax7显著上调，而c-Met无变化，表明该促进作用依赖Mac介导，且依赖IGF-1而非HGF旁路。

本研究主要存在2个局限性。其一，所有干预均为全身性或局部给药，未能实现肌纤维特异性*ERβ*敲除，因此不能完全排除Mac自身ERβ的直接贡献。体外结果提示肌纤维ERβ不可或缺，但仍是间接证据。其二，未设置雄鼠给药组，结论尚不能外推至雄性群体。后续研究需结合肌纤维特异性*ERβ*敲除小鼠及雄鼠实验进一步验证。

综上，本研究揭示雌二醇-ERβ信号轴在雌鼠急性骨骼肌损伤修复中的完整通路：该轴促进M2型Mac浸润及IGF-1分泌，IGF-1与肌纤维p-IGF-1R结合，驱动肌卫星细胞活化与再生。该发现提示雌激素不仅减轻即刻损伤，还主动塑造再生微环境，为理解性别差异提供依据。临床上，靶向ERβ的选择性调节剂有望加速运动损伤、创伤或术后肌功能恢复。下游通路及表观遗传/代谢调控Mac机制仍需深入探索。

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