

·学术前沿:麻醉与肿瘤专题·

右美托咪定通过抑制TLR-4/NF- κ B通路改善压力应激小鼠的乳腺肿瘤治疗效果

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摘 要:【目的】本研究旨在探讨右美托咪定(Dex)是否通过抑制TLR4/NF- κ B信号通路,改善慢性压力应激相关的小鼠乳腺肿瘤进展,并阐明其在调控焦虑-抑郁样行为及应激激素水平的作用。【方法】建立EMT6原位乳腺荷瘤小鼠模型,并采用慢性温和应激(CMS)诱导抑郁样状态。将小鼠随机分为正常对照组、荷瘤组、慢性应激组、慢性应激荷瘤组、右美托咪定治疗组及溶剂对照组。通过旷场实验与悬尾实验评估小鼠行为学变化;采用高效液相色谱法检测外周血中去甲肾上腺素、肾上腺素及皮质醇水平;通过Western blot和实时荧光定量PCR(RT-qPCR)分别检测肿瘤组织中TLR4、磷酸化NF- κ B p65(p-NF- κ B p65)、磷酸化I κ B α (p-I κ B α)及炎症因子(IL-6、IL-1 β 、TNF- α)的表达;体外实验采用皮质醇处理EMT6细胞模拟体内应激环境,并通过CCK-8、Western blot及RT-qPCR技术验证右美托咪定对TLR4/NF- κ B信号通路的调控作用。【结果】荷瘤小鼠表现出显著的焦虑-抑郁样行为($P<0.05$),且外周血中去甲肾上腺素、肾上腺素及皮质醇水平显著升高($P<0.05$)。慢性压力应激处理进一步加剧上述行为异常,并促进肿瘤生长($P<0.05$)。右美托咪定干预可显著改善应激荷瘤小鼠的焦虑-抑郁样行为($P<0.05$)并降低外周血应激激素水平($P<0.05$)。体外实验表明,皮质醇处理可激活EMT6细胞中TLR4/NF- κ B通路($P<0.001$),并上调炎症因子表达($P<0.05$),而右美托咪定可有效抑制该通路的活化($P<0.001$)。在体内模型中,右美托咪定治疗下调肿瘤组织中TLR4、p-NF- κ B p65、p-I κ B α 及促炎因子的表达($P<0.001$),并显著抑制肿瘤生长($P<0.05$)。【结论】右美托咪定可通过抑制TLR4/NF- κ B信号通路,减轻压力应激相关的炎症反应,改善焦虑-抑郁样行为并降低应激激素水平,最终抑制乳腺肿瘤进展。本研究为理解焦虑-抑郁相关乳腺肿瘤进展提供了实验依据,并提示右美托咪定及TLR4/NF- κ B通路可能成为潜在的干预靶点。

关键词:右美托咪定;TLR4/NF- κ B通路;应激;乳腺肿瘤;焦虑-抑郁样行为

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Dexmedetomidine Improves the Therapeutic Efficacy Against Mammary Tumors in Stress-exposed Mice by Inhibiting the TLR4/NF- κ B Pathway

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Abstract: 【Objective】 This study aimed to investigate whether dexmedetomidine (Dex) alleviates stress-induced mammary tumor progression in mice by inhibiting the TLR4/NF- κ B signaling pathway, and to examine its effects on anxiety-depression-like behaviors and stress hormone levels. 【Methods】 An orthotopic EMT6 mammary tumor model was established in mice, and chronic mild stress (CMS) was applied to induce anxiety-depression-like states. The mice were divided into six groups: naive, tumor, CMS, CMS+tumor, CMS+tumor+Dex, and CMS+tumor+Vehicle groups. Behavioral changes were evaluated using the open field test and tail suspension test. Plasma stress hormone levels were measured by high-performance liquid chromatography. Western blot and RT-qPCR were performed to detect the expression of TLR4, p-NF- κ B p65, p-I κ B α , and inflammatory cytokines (IL-6, IL-1 β , TNF- α) in tumor tissues. *In vitro*, EMT6 cells were treated with cortisol to mimic the stress-related microenvironment, and the effects of Dex on the TLR4/NF- κ B pathway were assessed by CCK-8, Western blot, and RT-qPCR. 【Results】 Tumor-bearing mice exhibited significant anxiety-depression-like behaviors ($P<0.05$), along with markedly elevated plasma levels of norepinephrine, epinephrine, and cortisol ($P<0.05$). Chronic stress further aggravated these behavioral abnormalities and promoted tumor growth ($P<0.05$). Dexmedetomidine intervention significantly improved anxiety-depression-like behaviors ($P<0.05$) and lowered peripheral stress hormone levels ($P<0.05$) in stress-exposed tumor-bearing mice. *In vitro* experiments demonstrated that cortisol treatment activated the TLR4/NF- κ B pathway ($P<0.001$) and upregulated the expression of inflammatory cytokines ($P<0.05$ in EMT6 cells, whereas dexmedetomidine effectively suppressed this activation ($P<0.001$). In the *in vivo* model, dexmedetomidine treatment downregulated the expression of TLR4, p-NF- κ B p65, p-I κ B α , and pro-inflammatory cytokines in tumor tissues ($P<0.001$) and significantly inhibited tumor growth ($P<0.05$). 【Conclusion】 Dexmedetomidine inhibits the TLR4/NF- κ B signaling pathway, alleviates stress-induced inflammatory responses, improves anxiety- and depression- like behaviors, reduces stress hormone levels, and suppresses mammary tumor progression. These findings provide an experimental basis for understanding anxiety- and depression- associated breast tumor progression and suggest that Dexmedetomidine and the TLR4/NF- κ B pathway may serve as potential therapeutic targets.

Key words: dexmedetomidine; TLR4/NF- κ B pathway; stress; mammary tumor; anxiety- and depression-like behavior

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肿瘤患者常因疾病本身及治疗过程而承受严重心理负担,其焦虑与抑郁的发生率显著高于普通人群。流行病学数据显示,肿瘤患者中抑郁的患病率约为普通人群的四倍,且近30%的患者存在明显的焦虑或抑郁症状^[1-2]。此外,持续的抑郁焦虑状态不仅可促进肿瘤进展,还会削弱肿瘤治疗的疗效^[3-4],从而形成“情绪-肿瘤进展”的恶性循环^[5-6]。该循环的核心病理机制涉及慢性应激导致的神经内分泌功能紊乱,主要表现为下丘脑-垂体-肾上腺(hypothalamic-pituitary-adrenal, HPA)轴负反馈调节受损以及交感神经系统(sympathetic nervous system, SNS)持续过度激活^[7-8]。在长期或多重应激作用下,上述神经内分泌系统异常活化可导致皮质醇、肾上腺素及去甲肾上腺素等应激激素释放失衡,进而激活相关促肿瘤炎症通路,最终促进肿瘤细胞增殖与活化^[9-10]。肾上腺素能信号是HPA轴与SNS下游的关键效应通路之一^[11]。其中, α_2 肾

上腺素能受体激动剂可通过激活胆碱能抗炎通路,抑制核因子- κ B(NF- κ B)等炎症信号通路,减少肿瘤坏死因子- α (TNF- α)、白细胞介素-6(IL-6)等炎症因子释放,从而减轻炎症反应^[12-13]。慢性炎症是肿瘤发生发展的重要驱动因素,NF- κ B作为调控炎症、细胞存活与增殖的关键转录因子,在多种恶性肿瘤中均呈现异常活化状态,并促进疾病进展^[14]。研究证实,抑制NF- κ B活性可有效延缓结直肠癌^[15]、肝癌^[16]及乳腺癌^[17]等多种类型肿瘤的生长。值得注意的是,TLR4/NF- κ B信号轴可能在连接慢性应激、局部炎症与肿瘤发展的病理过程中发挥关键的枢纽作用。

右美托咪定(dexmedetomidine, Dex)是一种高选择性 α_2 肾上腺素能受体激动剂,临床上广泛用于缓解围术期应激反应^[18-20],其作用机制主要通过抑制HPA轴与SNS过度激活,从而减少皮质醇等应激激素的释放^[21]。近年研究表明,Dex能够快速

改善焦虑、抑郁等负性情绪状态,兼具神经保护作用且成瘾性低,因此在精神障碍相关疾病的治疗中展现出独特的应用潜力^[22-23]。除调节神经与情绪功能外,Dex还具有显著的抗炎与抗肿瘤效应。研究显示,在心肌缺血再灌注损伤^[24]、脓毒症休克^[25]及认知功能障碍^[26]等炎症相关疾病中,Dex可通过抑制TLR4/NF- κ B信号通路发挥保护作用^[27]。此外,Dex亦能通过表观遗传修饰、诱导细胞凋亡与铁死亡等多种机制发挥抗肿瘤效应^[28]。然而,在压力应激相关乳腺肿瘤中,Dex是否通过调控TLR4/NF- κ B通路抑制肿瘤生长,目前尚缺乏系统研究。本研究旨在通过构建体内外压力应激模型,探究右美托咪定对压力应激相关的小鼠乳腺肿瘤进展的影响,并重点阐明其抗肿瘤效应是否通过抑制TLR4/NF- κ B信号通路及其下游炎症反应来实现。本研究结果有望为抑郁焦虑相关乳腺肿瘤的防治提供新的实验依据与潜在治疗策略。

1 材料与方法

1.1 试剂与仪器

主要试剂为:鼠源抗TLR4单克隆抗体(ab22048; Abcam),鼠源抗磷酸化NF- κ B p65 (p-NF- κ B p65)单克隆抗体(sc-136548; Santa Cruz Biotechnology),鼠源抗NF- κ B p65单克隆抗体(sc-8008; Santa Cruz Biotechnology),兔源抗磷酸化I κ B α (p-I κ B α)多克隆抗体(TA2002; Abmart),鼠源抗 β -tubulin单克隆抗体(AF2835; 碧云天),CCK-8细胞增殖/毒性检测试剂盒(C0038; 碧云天),BCA蛋白浓度测定试剂盒(G2026-1000T; 碧云天),皮质醇标准品(C106; Sigma-Aldrich)。

主要仪器为:化学发光成像系统,高通量实时荧光定量PCR仪(LightCycler[®] 480; Roche, Switzerland)。

1.2 动物模型与分组

选用6~8周龄雌性SPF级BALB/c小鼠(购自常州卡文斯实验动物有限公司,实验动物许可证号:SCXK(苏)2021-0013),体质量(20 $\pm$ 2)g。动物于SPF级环境中饲养,维持12 h/12 h昼夜循环光照,自由摄食饮水,通风条件良好。所有动物实验方案均遵循机构指南,并已获得苏州大学实验动物伦理委员会批准(伦理编号:SUDA20240824A02)。

将EMT6乳腺肿瘤细胞以2.5 $\times$ 10⁵个/只的剂量

原位接种于小鼠乳腺脂肪垫以构建肿瘤模型,待肿瘤明显可触及后每2~3 d使用游标卡尺测量其长径(L)与短径(W),肿瘤体积计算公式为: $V=L \times W^2 \times 0.5$ 。

慢性温和应激(chronic mild stress, CMS)抑郁模型参照先前的研究基础并加以改进,连续4周每日随机施加两种不同的应激刺激[包括24 h光暗周期倒置、12 h鼠笼倾斜45°、4 h束缚于50 mL锥形管、4 h限制于3 cm $\times$ 3 cm $\times$ 8 cm透明容器、2 h水平振荡(80 r/min, $r=0.5$ cm)及12 h潮湿垫料],且同一刺激不连续出现3次以上。造模结束后通过旷场实验与悬尾实验等行为学检测手段评估模型效果。

本研究共设立以下6组:正常对照组(Naive, 正常饲养,无处理)、荷瘤组(Tumor, 仅接种EMT6肿瘤细胞)、慢性温和应激组(CMS, 接受慢性温和压力应激)、慢性温和应激荷瘤模型组(CMS+tumor, 接受慢性温和压力应激并接种EMT6肿瘤细胞)、右美托咪定治疗组(CMS+tumor+Dex, 接受慢性温和压力应激并接种肿瘤细胞,随后给予右美托咪定腹腔注射)及溶剂对照组(CMS+tumor+vehicle, 接受慢性温和压力应激并接种肿瘤细胞,随后给予PBS腹腔注射)。右美托咪定治疗组每日腹腔注射25 μ g/kg右美托咪定(Dex, 溶于100 μ L PBS),溶剂对照组每日腹腔注射等体积的磷酸盐缓冲液(PBS)。

1.3 悬尾试验

悬尾实验(tail suspension test, TST)在安静、无异味的环境中进行。将小鼠尾部穿过长约1.5 cm的透明橡胶环,用医用胶带固定于距尾尖约1 cm处,使小鼠倒挂于距地面约50 cm高的测试支架上,随即开始计时并记录行为数据。测试期间,各支架之间用隔板分隔,以避免小鼠间相互干扰。测试结束后,将小鼠放回原笼。静止时间(immobility time)定义为小鼠在5 min测试期内,处于被动悬挂状态且无任何可见肢体活动的累积时间。

1.4 旷场试验

旷场实验(open field test, OFT)全程在安静、无刺激性气味及强光干扰的环境中进行。测试前3~4 h将小鼠移至旷场实验室以适应环境。使用体积分数75%乙醇彻底擦拭旷场装置(40 cm $\times$ 40 cm $\times$ 50 cm, 长 $\times$ 宽 $\times$ 高)以消除气味干扰,随后将小鼠轻置于测试箱中央,通过配套视频追踪系统连续记录5 min内小鼠的运动轨迹。每只小鼠测试结束后放回原笼,并再次使用体积分数75%乙醇彻底清洁测

试装置,之后方可进行下一只小鼠测试。分析指标包括:5 min内总运动距离(total distance)、中心区域运动距离(center distance)、中心区域停留时间(center time)及进入中心区域次数(center entries)。

1.5 高效液相色谱分析

精密称取去甲肾上腺素、肾上腺素及皮质醇标准品,分别溶于2 mL无水甲醇;其中儿茶酚胺溶液添加体积分数10%乙酸以防氧化。流动相A为含体积分数0.05%三氟乙酸(TFA)的50 mmol/L磷酸盐缓冲液(pH 6.0),流动相B为含体积分数0.05%三氟乙酸(TFA)的乙腈溶液。平衡液为含147 mmol/L NaCl、4 mmol/L KCl、2.3 mmol/L CaCl₂、1.2 mmol/L MgCl₂的乙腈溶液(pH调节至7.4)。采集小鼠外周血后,于4℃、1 500 × g条件下离心15 min,分离获得血浆。血浆样本经0.22 μm微孔滤膜过滤后,与经超声脱气20 min的流动相一同进样。采用ZORBAX SB-C18色谱柱(150 mm×4.6 mm)进行分离,流速设定为(0.5~1.0) mL/min,柱温控制在(30~40)℃。

1.6 细胞培养及体外模型构建

EMT6小鼠乳腺肿瘤细胞使用含体积分数10%胎牛血清(FBS)及体积分数1%青霉素-链霉素的RPMI-1640培养基,于37℃、体积分数5% CO₂条件下常规培养。待细胞生长至融合度60%~70%时用于细胞传代和冻存。本研究以皮质醇(Cortisol)处理构建压力应激细胞模型,皮质醇的作用浓度及时间通过CCK-8实验预先筛选确定。

1.7 CCK-8细胞活力检测

将EMT6细胞按每孔 2×10^3 的密度接种于96孔板中,每孔加入100 μL完全培养基,每组浓度设置6个复孔。待细胞贴壁生长至融合度约为40%~50%时,分别更换为含有不同浓度皮质醇(50、100、200、400、800 μmol/L)的完全培养基进行处理,并设置不含细胞的孔作为空白对照。分别于皮质醇处理12 h和24 h后,每孔加入10 μL CCK-8工作液,置于37℃、体积分数5% CO₂培养箱中避光孵育2 h,随后使用酶标仪于450 nm波长处测定各孔的吸光度值(OD值)。细胞活力根据以下算式计算:细胞活力(%) = $[(A_s - A_b)/(A_c - A_b)] \times 100\%$ 。实验结果显示,经皮质醇处理12 h后,EMT6细胞的增殖能力随浓度升高呈增强趋势,其中200 μmol/L浓度下的促增殖作用最为显著;而当浓度进一步升

至400 μmol/L时,细胞活力出现明显抑制。基于此,本研究选择200 μmol/L皮质醇处理12 h作为后续细胞模型的造模条件。

1.8 蛋白印迹实验

取小鼠肿瘤组织及肿瘤细胞样本,加入含蛋白酶抑制剂及磷酸酶抑制剂的预冷裂解液,组织样本经冰上超声匀浆处理后,于4℃、14 000 × g,离心20 min,收集上清液。采用BCA法测定蛋白浓度,加入5 × 上样缓冲液并于95℃加热10 min使蛋白变性。配制SDS-PAGE凝胶,每孔上样含25 μg蛋白样品,在120 V恒定电压下进行电泳分离,随后在4℃、100 mA恒定电流条件下转膜120~140 min。转膜结束后,用5%脱脂奶粉室温封闭2 h,依次进行一抗(4℃过夜孵育)与二抗(室温孵育2 h)孵育,采用ECL化学发光法对目标蛋白条带进行显影。最后,使用Image J软件对蛋白条带进行灰度值定量分析。

1.9 实时荧光定量PCR检测

使用Freerizol Reagent提取小鼠肿瘤组织总RNA,细胞总RNA则采用诺唯赞快速柱式RNA提取试剂盒进行提取,所有操作严格依照说明书进行。使用NanoDrop分光光度计测定RNA浓度及纯度,取等量RNA样品通过逆转录试剂盒合成cDNA。实时荧光定量PCR反应在Roche LightCycler 480系统上进行,反应体系与循环条件遵循仪器推荐程序。基因相对表达水平采用2-ΔΔCT法进行计算,并以βactin作为内参基因进行标准化。所用引物序列如下:Il6: Forward, 5'-TGATGGATGCTACCAAAGTGA-3', Reverse, 5'-GGAGAGCATTTGGAATTTGGGG-3', Il1b: Forward, 5'-TGCCACCTTTTGACAGTGATG-3', Reverse, 5'-AAGGTCCACGGGAAAGACAC-3', Tnfa: Forward, 5'-CCGATGGGTTGTACCTTGTC-3', Reverse, 5'-CGGACTCCGCAAAGTCTAAG-3'。

1.10 统计分析

所有统计分析均使用GraphPad Prism 10.0软件进行。两组间比较,对符合正态分布的数据采用独立样本t检验,多组间比较采用单因素方差分析(one-way ANOVA)。当数据涉及两个分类变量时,采用双因素方差分析(two-way ANOVA)进行检验。数据以均值±标准误(mean ± SEM)表示。统计学显著性水平设定为P<0.05。

2 结果

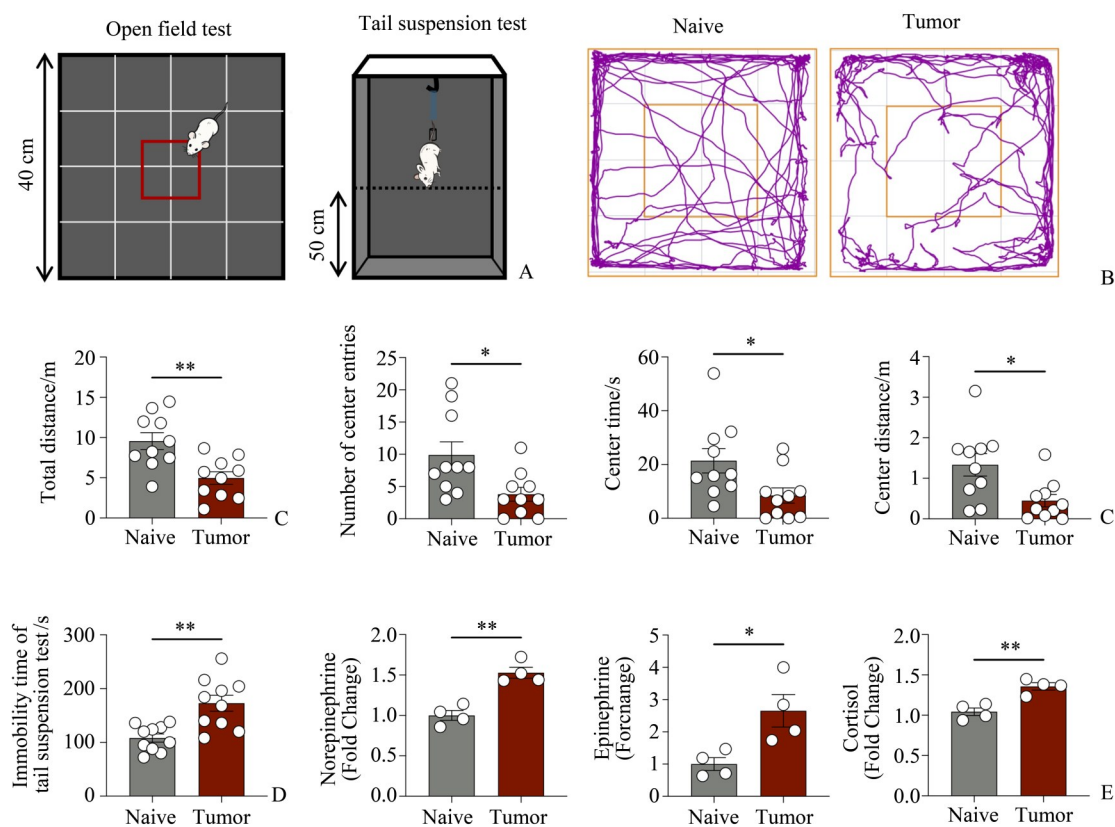
2.1 荷瘤小鼠焦虑-抑郁样行为与应激激素水平升高

为明确肿瘤负荷与焦虑-抑郁样行为之间的相互作用,本研究建立了EMT6原位乳腺肿瘤荷瘤小鼠模型,并通过旷场实验与悬尾实验进行行为学评估(图1A)。结果显示,与空白对照组(Naive)相比,荷瘤组(Tumor)小鼠在旷场实验中表现出自发运动与探索行为普遍减少(图1B),其总活动距离显著缩短($P=0.003$)。此外,荷瘤小鼠呈现出明显的“趋边性”行为特征,表现为进入中心区域的次数($P=0.015$)、在中心区域的停留时间($P=0.027$)及活动距离均较对照组显著减少($P=0.012$;图1C)。悬尾实验进一步显示,荷瘤组小鼠的静止时间较Naive

组有所延长($P=0.002$,图1D),上述行为学变化共同提示荷瘤小鼠存在焦虑-抑郁样表型。通过液相色谱法检测小鼠外周血中应激激素水平发现,荷瘤小鼠外周血中去甲肾上腺素($P=0.001$)、肾上腺素($P=0.039$)及皮质醇($P=0.003$)含量均较对照组显著升高(图1E),提示其核心应激激素水平整体上调。以上结果表明,EMT6乳腺肿瘤负荷可诱发小鼠出现焦虑-抑郁样行为,并激活下丘脑-垂体-肾上腺轴及交感神经系统,促进应激激素的释放。

2.2 焦虑-抑郁状态促进小鼠肿瘤进展

本研究在既往慢性应激模型基础上进行改进,通过连续4周每日随机施加两种不同应激源的方式建立小鼠模型。旷场实验结果显示,慢性应激组(CMS)较空白对照组(Naive)表现出更明显的“趋边性”行为,而慢性应激荷瘤组(CMS+tumor)的“趋



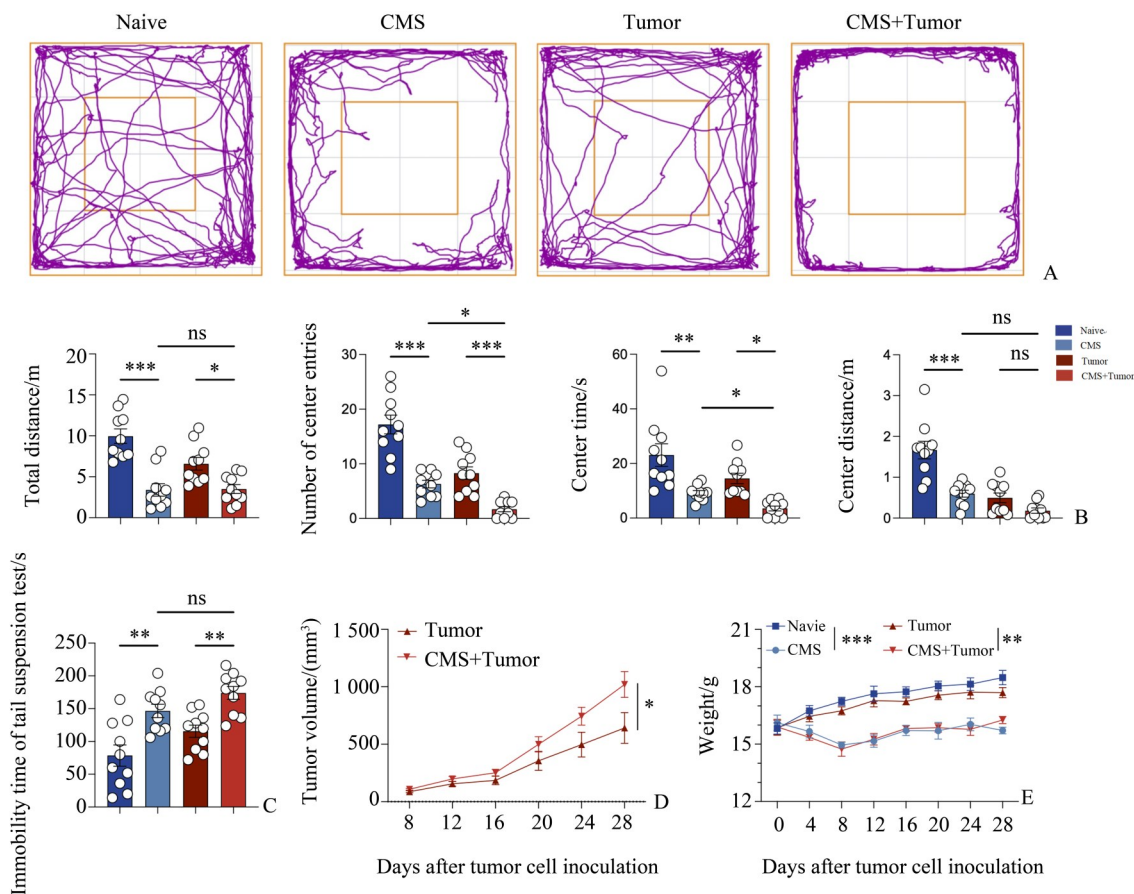
A: Schematic diagram of the open field and tail suspension tests. B: Representative movement traces of individual mice in the open field test. C: Quantitative analysis of open field behaviors: total distance ($t=3.488$, $P=0.003$), number of center entries ($t=2.662$, $P=0.015$), center time ($t=2.415$, $P=0.027$), and center distance ($t=2.785$, $P=0.012$, $n=10$). D: Immobility time in the tail suspension test ($t=3.877$, $P=0.002$, $n=10$). E: Plasma levels of epinephrine ($t=3.050$, $P=0.039$), norepinephrine ($t=5.793$, $P=0.001$), and cortisol ($t=4.800$, $P=0.003$) measured by high-performance liquid chromatography ($n=4$). * $P < 0.050$, ** $P < 0.010$, *** $P < 0.001$.

图1 荷瘤小鼠焦虑-抑郁样行为与应激激素水平升高

Fig. 1 Anxiety- and depression-like behaviors and elevated stress hormone levels in tumor-bearing mice

边性”表现较CMS组更为突出(图2A)。对各行为学指标的定量分析发现,CMS+tumor组进入中心区域的次数($P=0.029$)及停留时间($P=0.011$)均少于CMS组(图2B)。悬尾实验结果表明,CMS+tumor组的静止时间有升高趋势,但与CMS组相比差异未达到统计学意义($P=0.256$,图2C)。对肿瘤生长曲线的监测进一步表明,慢性应激状态显著促进了EMT6肿瘤的生长($P=0.048$,图2D)。体质量降低

作为评估模型构建成功的重要生理指标,对小鼠体质量进行了动态监测发现,结果显示,随着压力应激的施加,应激组荷瘤小鼠体质量较单纯荷瘤小鼠明显下降($P=0.004$,图2E)。以上研究结果提示,荷瘤状态与慢性应激之间存在相互促进的恶性循环关系:荷瘤状态显著加剧慢性应激诱导的行为异常,与此同时,慢性应激亦进一步促进肿瘤进展。



A: Representative movement traces in the open field test for mice from the Naive, CMS, Tumor, and CMS+Tumor groups. B: Quantitative analysis of open field behaviors: total distance ($F=17.220$, $P=0.999$), number of center entries ($F=34.110$, $P=0.029$), center time ($F=12.380$, $P=0.011$), and center distance ($F=23.170$, $P=0.145$, $n=10$). C: Immobility time in the tail suspension test ($F=12.330$, $P=0.256$, $n=10$). D: Tumor growth curve in the EMT6 orthotopic breast cancer mouse model ($t=2.165$, $P=0.048$, $n=8$). E: Body weight of mice in each group ($F=22.95$, $P=0.004$, $n=8$). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

图2 焦虑-抑郁状态促进小鼠肿瘤发展

Fig. 2 Anxiety and depression-like states promote tumor progression in mice

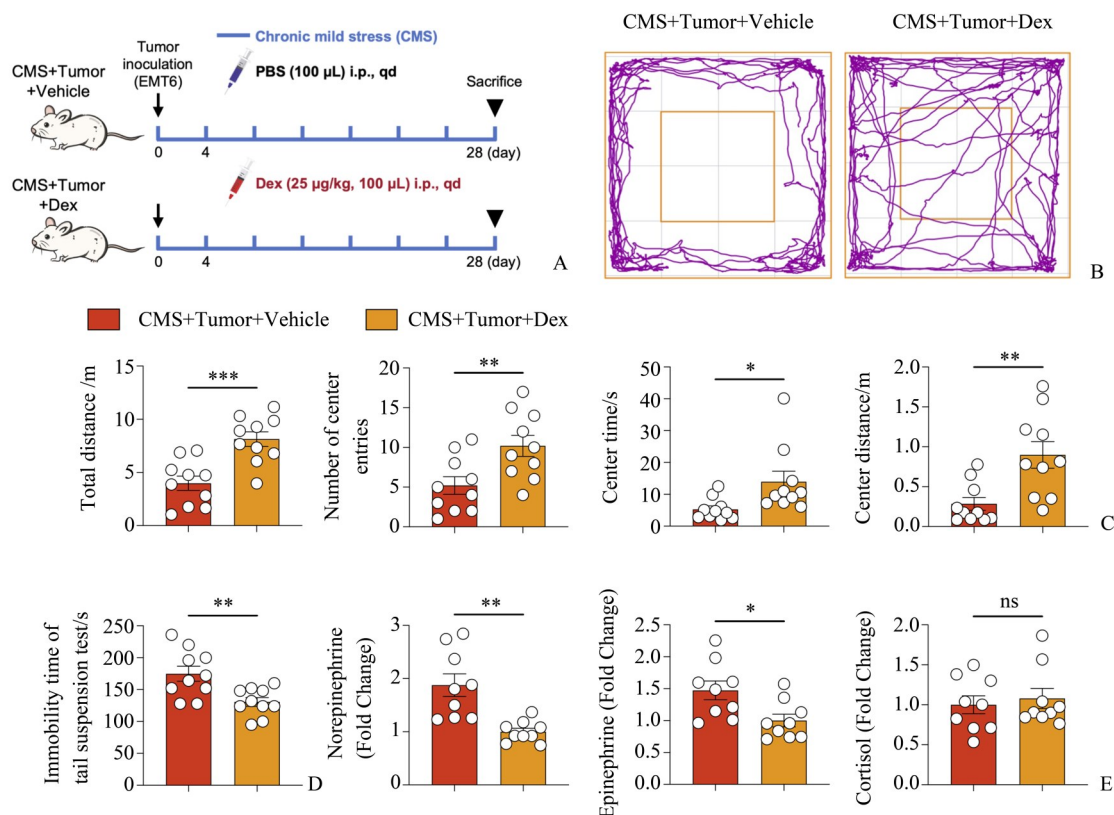
2.3 右美托咪定缓解焦虑-抑郁症状并降低外周血中应激激素水平

为探究右美托咪定作为高选择性 α_2 肾上腺素能受体激动剂,是否可通过抑制应激激素释放而改善抑郁焦虑样行为,本研究在慢性应激荷瘤(CMS+tumor)小鼠模型中对其进行腹腔注射干预(图3A)。

焦虑-抑郁样行为检测结果显示,与溶剂对照组(CMS+tumor+vehicle)相比,右美托咪定治疗组(CMS+tumor+Dex)小鼠在旷场实验中的“趋边性”行为明显改善(图3B),具体表现为进入中心区域的次数($P=0.01$)、停留时间($P=0.024$)及中心区域活动距离($P=0.004$)均显著增加(图3C);悬尾实验

中静止时间亦显著缩短($P=0.006$;图3D)。外周血应激激素检测结果进一步表明,治疗组小鼠血浆中去甲肾上腺素($P=0.003$)和肾上腺素($P=0.019$)水平较溶剂对照组显著降低(图3E)。上述结果表

明,右美托咪定干预可有效缓解慢性应激荷瘤小鼠的焦虑-抑郁样症状并降低其外周血应激激素水平。这为进一步在体外细胞模型中探究其具体作用机制提供了重要的在体证据。



A: Experimental animal grouping and study design schematic. B: Representative movement traces of mice from the CMS+Tumor+Vehicle and CMS+Tumor+Dex groups in the open field test. C: Quantitative analysis of open field behaviors: total distance ($t=4.294$, $P<0.001$), number of center entries ($t=2.891$, $P=0.01$), center time ($t=2.471$, $P=0.024$), and center distance ($t=3.308$, $P=0.004$, $n=10$). D: Immobility time measured in the tail suspension test ($t=3.239$, $P=0.006$, $n=10$). E: Plasma levels of epinephrine ($t=2.658$, $P=0.019$), norepinephrine ($t=3.937$, $P=0.003$), and cortisol ($t=0.469$, $P=0.645$) measured by liquid chromatography ($n=9$). * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

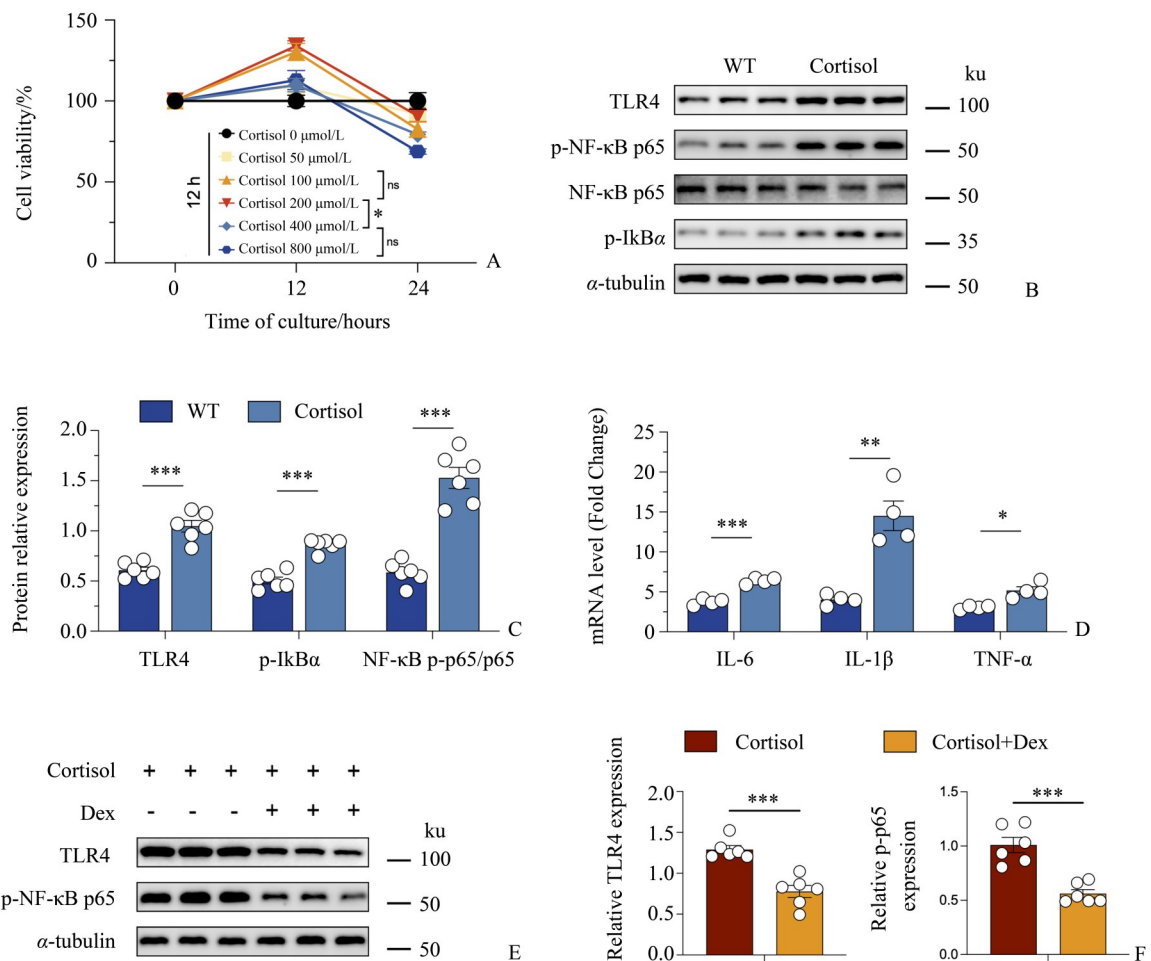
图3 右美托咪定缓解应激荷瘤小鼠焦虑-抑郁样行为及应激激素水平

Fig. 3 Dexmedetomidine reduces anxiety- and depression-like behaviors and stress hormone levels in stress-exposed tumor-bearing mice

2.4 右美托咪定抑制皮质醇诱导的EMT6肿瘤细胞TLR4/NF- κ B通路激活及下游炎症反应

小鼠体内实验表明,右美托咪定可缓解应激荷瘤小鼠的焦虑-抑郁样行为并降低其应激激素水平。为深入探究右美托咪定在压力应激相关肿瘤生长中发挥重要作用的具体机制,本研究进一步开展了体外细胞实验。实验通过皮质醇处理EMT6乳腺癌细胞,以模拟体内应激环境。通过CCK-8实验确定皮质醇最佳刺激浓度为200 μ mol/L, 12 h (Cortisol 200 μ mol/L vs. 400 μ mol/L, $P=0.011$;图4A)。Western

blot及定量分析表明,皮质醇处理可显著激活TLR4/NF- κ B信号通路(均为 $P<0.001$;图4B,C),并促进EMT6细胞的炎症反应($P_1<0.001$, $P_2=0.009$, $P_3=0.018$;图4D)。为进一步明确右美托咪定的作用机制,在皮质醇处理的EMT6细胞中加入右美托咪定进行干预。结果显示,右美托咪定可有效逆转皮质醇引起的TLR4($P<0.001$)及NF- κ B p65磷酸化水平升高($P<0.001$;图4E,F)。体外实验结果表明,右美托咪定能够抑制皮质醇诱导的TLR4/NF- κ B信号通路激活及促炎因子释放。



A: Viability of EMT6 cells treated with cortisol (0–800 μmol/L) for 12 h and 24 h, assessed by CCK-8 assay. B, C: Representative Western blots and quantitative analysis of TLR4 ($t=6.66$, $P<0.001$), phospho-NF-κB p65 (p-p65, $t=8.198$, $P<0.001$) and phospho-IκBα (p-IκBα, $t=8.524$, $P<0.001$) expression in EMT6 cells ($n=6$). D: mRNA levels of *Il6* ($t=9.480$, $P<0.001$), *Il1b* ($t=5.582$, $P=0.009$) and *Tnfα* ($t=4.302$, $P=0.018$) in EMT6 cells detected by RT-qPCR ($n=4$). E, F: Representative Western blots and quantitative analysis of TLR4 ($t=5.711$, $P<0.001$) and phospho-NF-κB p65 (p-p65, $t=5.621$, $P<0.001$) expression in EMT6 cells ($n=6$). * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

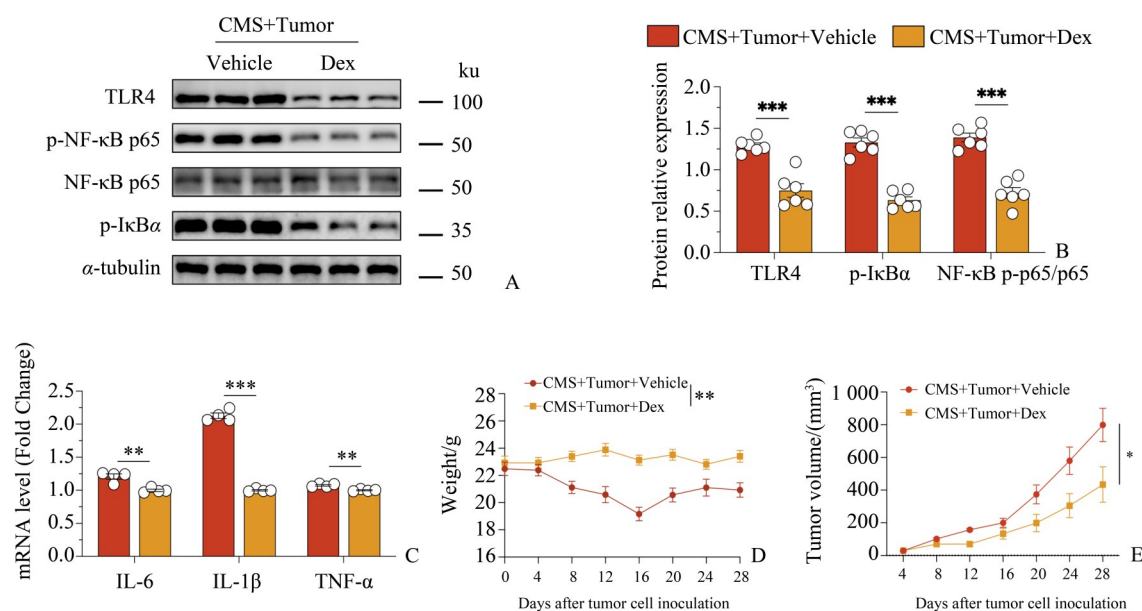
图4 右美托咪定缓解皮质醇诱导的EMT6细胞TLR4/NF-κB通路激活及炎症反应

Fig. 4 Dexmedetomidine alleviates cortisol-induced activation of the TLR4/NF-κB pathway and inflammatory response in EMT6 cells

2.5 右美托咪定抑制慢性压力应激乳腺荷瘤小鼠的肿瘤生长

体外研究提示,右美托咪定可有效抑制皮质醇诱导的TLR4/NF-κB信号通路活化及促炎因子释放。为验证上述体外实验结果,本研究进一步在慢性压力应激荷瘤小鼠的肿瘤组织中进行了相关检测。Western blot检测结果显示,与溶剂对照组(CMS+tumor+vehicle)相比,右美托咪定治疗组(CMS+tumor+Dex)肿瘤组织中TLR4($P<0.001$)、磷酸化NF-κB p65($P<0.001$)及磷酸化IκBα($P<0.001$)表达水平均显著下调(图5A,B)。RT-qPCR分析进一步表明,肿瘤组

织内促炎因子IL-1β($P<0.001$)与IL-6($P=0.008$)的mRNA表达显著降低,TNF-α($P=0.003$)亦呈现下降趋势(图5C)。值得注意的是,肿瘤生长曲线结果显示,右美托咪定治疗可显著抑制慢性压力应激相关乳腺肿瘤的生长($P=0.028$,图5D)。此外,在焦虑-抑郁小鼠模型的构建中,我们发现给予右美托咪定治疗的焦虑-抑郁小鼠体重质量相对稳定,进一步验证了右美托咪定可以有效缓解焦虑-抑郁状态($P=0.003$,图5E)。上述结果表明,右美托咪定可能通过抑制TLR4/NF-κB信号通路及其下游促炎因子的表达,从而发挥抗肿瘤作用。



A, B: Representative Western blots and quantitative analysis of TLR4 ($t=6.078$, $P<0.001$), phospho-NF-κB p65 (p-p65, $t=7.981$, $P<0.001$) and phospho-IκBα (p-IκBα, $t=10.09$, $P<0.001$) expression in tumor tissue ($n=6$). C: mRNA levels of *IL6* ($t=4.733$, $P=0.008$), *IL1β* ($t=25.78$, $P<0.001$) and *TNFα* ($t=5.322$, $P=0.003$) in tumor tissues detected by RT-qPCR ($n=4$). D: Tumor growth curve in the EMT6 orthotopic breast cancer mouse model ($t=2.456$, $P=0.028$, $n=8$). E: Body weight of mice in each group ($t=3.585$, $P=0.003$, $n=8$). * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

图5 右美托咪定在压力应激荷瘤小鼠中抑制TLR4/NF-κB通路并发挥抗肿瘤作用

Fig. 5 Dexmedetomidine inhibits the TLR4/NF-κB pathway and exerts anti-tumor effects in tumor-bearing mice under chronic stress

3 讨论

本研究结果表明,在抑郁-焦虑背景下,右美托咪定通过抑制TLR4/NF-κB介导的炎症反应,在改善压力应激小鼠抑郁-焦虑样行为的同时,亦能遏制EMT6乳腺癌生长。荷瘤小鼠表现出加重的抑郁-焦虑样行为,并伴有外周血应激激素水平升高;慢性压力应激还可进一步促进肿瘤生长,印证了临床研究中提示的“情绪-肿瘤”恶性循环。体外实验表明,皮质醇处理可促进EMT6乳腺肿瘤细胞增殖并激活TLR4/NF-κB信号通路,而右美托咪定干预能显著抑制该通路关键分子的磷酸化水平、减轻炎症反应,进而发挥抗肿瘤效应。更重要的是,在慢性应激荷瘤小鼠体内,腹腔注射右美托咪定不仅能改善其抑郁-焦虑样行为表型,还能明显抑制肿瘤生长,为其作为抑郁焦虑相关肿瘤的潜在治疗策略提供了有力支持。

流行病学研究表明,重度抑郁症可能增加女性乳腺癌的患病风险并影响其临床预后^[29]。慢性应激作为抑郁症的重要病理特征,已被证实可影响机体抗肿瘤免疫、驱动肿瘤代谢重编程并干扰激素合

成^[30-31],从而促进肿瘤转移进程^[32]。本研究的动物实验结果进一步证实,慢性压力应激不仅能加剧荷瘤小鼠的抑郁-焦虑样行为,还可显著促进肿瘤生长。

右美托咪定作为一种临床常用麻醉镇静药物,其抗抑郁疗效已得到研究证实^[33]。该药物主要作用于中枢及外周α₂肾上腺素能受体,通过抑制HPA轴与SNS的过度激活,进而缓解机体的应激反应^[18, 34]。除改善情绪障碍外,右美托咪定亦展现出明确的抗肿瘤潜力。多项研究表明,在多种小鼠肿瘤模型中,该药物能够有效抑制肿瘤生长、提高动物生存率并改善肿瘤免疫微环境^[35]。与我们前期的发现一致,腹腔注射右美托咪定可显著增加肿瘤组织中免疫细胞的比例,并上调细胞毒性相关基因的表达,从而增强机体抗肿瘤反应^[36]。值得注意的是,右美托咪定还可通过抑制TLR4活性减轻术后认知功能障碍^[37-39],提示其对TLR4/NF-κB通路具有调控潜力。TLR4作为一种重要的模式识别受体,其激活能够促进NF-κB磷酸化及核转位,进而诱导IL-1β、IL-6、TNF-α等促炎因子释放,该机制在抑郁及肿瘤发生发展中均起关键作用^[40]。

NF- κ B是调控炎症反应的关键信号通路,通过激活下游效应基因,在肿瘤发生发展中发挥重要作用^[41]。在经典通路中,p65/p50复合物与I κ B结合并滞留于胞质;促炎细胞因子与其受体结合可启动信号级联,诱导IKK磷酸化。活化的IKK进而磷酸化I κ B,促使其泛素化降解,释放p65/p50并转入细胞核内,协同其他转录因子调控靶基因表达^[42]。该通路常在多种实体肿瘤中异常活化,由肿瘤微环境中高表达的炎症因子或关键蛋白异常磷酸化所驱动^[43]。值得注意的是,尽管糖皮质激素通常发挥抗炎作用,但慢性应激下持续升高的皮质醇可能通过诱导代谢紊乱和免疫细胞功能重塑,间接维持甚至增强TLR4介导的炎症信号,从而促进抑郁-焦虑症状及肿瘤进展。此机制与既往研究相符,即TLR4/NF- κ B信号在小胶质细胞中激活可加剧神经炎症并参与抑郁发生发展^[44-45]。我们先前研究表明,慢性压力应激通过激活S100A8/A9促进心房组织中中性粒细胞胞外诱捕网(NETs)形成及炎症反应^[46]。本研究进一步揭示,皮质醇处理能显著提高EMT6肿瘤细胞中I κ B与NF- κ B p65的磷酸化水平,而右美托咪定在慢性应激荷瘤小鼠中则能有效抑制该通路的激活,提示TLR4/NF- κ B信号通路的异常活化可能是压力应激促进肿瘤局部炎症反应及生长的重要中间环节。

本研究仍然存在局限性。CMS+tumor模型的稳定性受应激强度、作用周期及肿瘤类型等多因素影响,可能导致行为学检测结果的波动,后续需在多种肿瘤细胞模型中进一步验证模型的可靠性。DEX除激动 α 2-AR外,还涉及多种非受体效应途径;同时, α 2-AR在不同免疫细胞亚群中的表达和功能可能存在差异,DEX的具体作用机制仍有待深入探究。TLR4在焦虑抑郁荷瘤小鼠肿瘤组织中表达上调,但其上游调控机制尚不明确,这限制了对TLR4与焦虑抑郁相互关系的深入理解。

本研究通过行为学、外周血激素、细胞与分子生物学等多角度实验,系统阐释了右美托咪定在慢性应激荷瘤小鼠模型中的作用。结果表明,该药物可通过抑制TLR4/NF- κ B通路,降低应激激素与肿瘤局部炎症因子水平,从而改善小鼠抑郁-焦虑样行为并抑制乳腺肿瘤生长。上述结果提示,靶向TLR4/NF- κ B信号通路为理解焦虑-抑郁相关乳腺肿瘤进展提供了实验依据,并提示Dex及TLR4/NF- κ B通路可能成为潜在干预靶点。未来研究可进一步探究该通路在右美托咪定抗肿瘤作用中的具体调控网络及其与免疫、代谢途径的交互关系,以深化机制认识,推动临床转化。

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