

# 干扰肿瘤相关巨噬细胞的P2X4受体表达可抑制胶质瘤细胞的迁移和侵袭

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**摘要:**目的 研究P2X4受体在小鼠胶质瘤中肿瘤相关巨噬细胞(TAMs)的表达对胶质瘤细胞侵袭迁移的影响及分子机制。方法 将16只健康C57BL/6小鼠随机分为肿瘤组、对照组(8只/组),利用小鼠胶质瘤GL261细胞接种于小鼠大脑尾状核,建立小鼠脑胶质瘤模型。术后第21天处死小鼠,取荷瘤小鼠及正常小鼠脑组织,采用HE染色观察小鼠胶质瘤形态,利用免疫荧光检测Iba-1、P2X4受体的表达情况;应用GL261条件培养基将RAW264.7细胞诱导为TAMs,采用RT-qPCR检测巨噬细胞极化相关标志物及P2X4受体在TAMs的mRNA表达情况;采用Western blot检测P2X4受体在TAMs的蛋白表达情况;采用siRNA P2X4,下调TAMs中P2X4受体的蛋白表达,RT-qPCR、Western blot检测siRNA P2X4转染后TAMs中IL-1 $\beta$ 、IL-18的mRNA及蛋白表达;利用transwell侵袭及迁移实验检测siRNA P2X4转染后TAMs对GL261细胞侵袭迁移能力的影响。结果 与对照组相比,荷瘤小鼠脑胶质瘤组织中有较高数量的Iba-1阳性细胞( $P<0.0001$ ),且P2X4受体在Iba-1阳性细胞中的表达增高( $P=0.001$ );使用GL261条件培养基刺激RAW264.7细胞转化为TAMs后,M2型巨噬细胞标志基因Arg-1、IL-10表达上调( $P=0.0001, 0.001$ ),M1型巨噬细胞标志基因iNOS、TNF- $\alpha$ 表达也上调( $P=0.006, 0.001$ ),但以M2型巨噬细胞标志基因Arg-1、IL-10表达上调更为显著,同时TAMs中P2X4受体蛋白表达水平及mRNA水平均增高( $P=0.005, 0.014$ )。干扰TAMs中P2X4受体表达引起其IL-1 $\beta$ 、IL-18的mRNA( $P<0.01$ )及蛋白表达水平( $P<0.01, P<0.05$ )降低,并抑制TAMs促进胶质瘤细胞侵袭和迁移的能力( $P=0.004, 0.017$ )。结论 降低肿瘤相关巨噬细胞P2X4受体表达可能通过IL-1 $\beta$ 、IL-18影响胶质瘤细胞的迁移和侵袭。

**关键词:**胶质瘤;P2X4受体;肿瘤相关巨噬细胞;侵袭;迁移

## Interference of P2X4 receptor expression in tumor-associated macrophages suppresses migration and invasion of glioma cells

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**Abstract: Objective** To investigate the effect of interference of P2X4 receptor expression in tumor-associated macrophages (TAMs) on invasion and migration of glioma cells. **Methods** C57BL/6 mouse models bearing gliomas in the caudate nucleus were examined for glioma pathology with HE staining and expressions of Iba-1 and P2X4 receptor with immunofluorescence assay. RAW264.7 cells were induced into TAMs using conditioned medium from GL261 cells, and the changes in mRNA expressions of macrophage polarization-related markers and the mRNA and protein expressions of P2X4 receptor were detected with RT-qPCR and Western blotting. The effect of siRNA-mediated P2X4 interference on IL-1 $\beta$  and IL-18 mRNA and protein expressions in the TAMs was detected with RT-qPCR and Western blotting. GL261 cells were cultured in the conditioned medium from the transfected TAMs, and the invasion and migration abilities of the cells were assessed with Transwell invasion and migration experiment. **Results** The glioma tissues from the tumor-bearing mice showed a significantly greater number of Iba-1-positive cells, where an obviously increased P2X4 receptor expression was detected ( $P=0.001$ ), than the brain tissues of the control mice ( $P<0.001$ ). The M2 macrophage markers (Arg-1 and IL-10) and M1 macrophage markers (iNOS and TNF- $\alpha$ ) were both significantly up-regulated in the TAMs derived from RAW264.7 cells (all  $P<0.01$ ), but the up-regulation of the M2 macrophage markers was more prominent; the expression levels of P2X4 receptor protein and mRNA were both increased in the TAMs ( $P<0.05$ ). Interference of P2X4 receptor expression significantly lowered the mRNA ( $P<0.01$ ) and protein ( $P<0.01, P<0.05$ ) expression levels of IL-1 $\beta$  and IL-18 in the TAMs and obviously inhibited the ability of the TAMs to promote invasion and migration of the glioma cells ( $P<0.05$ ). **Conclusion** Interference of P2X4 receptor in the TAMs suppresses the migration and invasion of glioma cells possibly by lowering the expressions of IL-1 $\beta$  and IL-18.

**Keywords:** glioma; P2X4 receptor; tumor associated macrophages; invasion; migration

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神经胶质瘤约占原发性脑肿瘤的50%,其中胶质母细胞瘤是最常见、最具侵略性的亚型,肿瘤组织广泛浸润到周围的脑实质中,从而导致肿瘤复发<sup>[1]</sup>。胶质瘤微环境中浸润大量的肿瘤相关巨噬细胞(TAMs),占肿瘤的30~50%<sup>[2]</sup>,包括小胶质细胞和外周血来源的巨噬细胞,TAMs在肿瘤来源的细胞因子和趋化因子作用下聚

集到胶质瘤肿瘤微环境中<sup>[3-6]</sup>,抗肿瘤的功能被抑制,反而支持胶质瘤的生长和侵袭<sup>[7]</sup>,TAMs的浸润往往提示胶质瘤患者预后不良<sup>[8,9]</sup>。

三磷酸腺苷(ATP)介导的嘌呤能信号已被证明可介导多种癌症相关过程,包括细胞迁移、对细胞毒性治疗的抵抗和免疫调节,近年来细胞外ATP及ATP门控P2X受体在肿瘤学领域受到越来越多的关注<sup>[10]</sup>。有研究显示,沉默P2X4受体可通过BDNF/TrkB/ATF4信号通路抑制胶质瘤细胞的生长<sup>[11]</sup>,目前关于P2X4受体是否参与胶质瘤肿瘤微环境调控的研究较少,仅见报道P2X4受体在大鼠C6胶质瘤中的巨噬细胞和小胶质细胞高表达<sup>[12]</sup>,但其所涉及的功能及机制尚未见报道。因此,本实验研究旨在了解P2X4受体在胶质瘤TAMs中的表达对胶质瘤细胞侵袭和迁移的影响,并探讨其分子机制,以期为胶质瘤治疗新药物靶点的开发提供依据。

## 1 材料和方法

### 1.1 材料

1.1.1 实验动物 16只6~8周龄C57BL/6小鼠(昆明医科大学实验动物中心),雄性,体质量18~22 g,动物及实验符合国家科学技术委员会发布的《实验动物管理条例》。

1.1.2 细胞系 鼠源巨噬细胞RAW264.7细胞系(ATCC),小鼠胶质瘤GL261细胞系(上海子实生物科技有限公司)。

1.1.3 主要试剂 DMEM高糖培养基(BI),胎牛血清(浙江天杭生物科技股份有限公司),RAPI裂解液、BCA试剂盒、SDS电泳缓冲液、转膜缓冲液、蛋白marker、5×loading buffer、QuickBlock™免疫染色封闭液、含DAPI抗荧光淬灭封片液(上海碧云天生物技术有限公司),柠檬酸组织抗原修复液(福州迈新生物技术开发有限公司),兔抗鼠P2X4受体抗体(Alomone Labs),羊抗鼠Iba-1抗体(NOVUS),兔抗鼠IL-1β抗体(Protein Tech),兔抗鼠IL-18抗体(万类生物科技公司),鼠抗鼠GAPDH抗体(成都正能生物技术有限责任公司),驴抗兔荧光二抗、驴抗羊荧光二抗(Invitrogen),羊抗兔二抗(Protein Tech),羊抗鼠二抗(Biosharp),RNAPrep Pure培养细胞/细菌总RNA提取试剂盒、FastKing cDNA第一链合成试剂盒、SuperReal 荧光定量预混试剂(天根生化科技有限公司),小干扰RNA,riboFECT™转染试剂(广州锐博生物科技有限公司),Transwell小室(耐思生物科技有限公司),Matrigel(Corning)。

1.1.4 主要仪器与设备 小鼠脑立体定位仪、小鼠颅骨骨钻(深圳瑞沃德生命科技有限公司),微量注射器(宁波镇海玻璃仪器厂),倒置显微镜(Zeiss),激光共聚焦显微镜(Nikon),凝胶成像仪(Bio-Rad),核酸蛋白检测仪(Quawell)。

### 1.2 方法

1.2.1 细胞培养 RAW264.7细胞系、GL261细胞系均采用DMEM高糖培养基,辅以10%胎牛血清和1%青霉素-链霉素,置于37℃、5%CO<sub>2</sub>培养箱内培养。

1.2.2 小鼠颅内胶质瘤模型的建立 收集对数生长长期的小鼠胶质瘤GL261细胞,经胰蛋白酶消化制成单细胞悬液,PBS清洗2次,重悬于无血清培养基中,调整细胞密度为2×10<sup>5</sup>/μL。4%水合氯醛麻醉小鼠(0.1 mL/10 g),按文献[13]方法,选取右侧小鼠脑尾状核区为接种点(前囟前1 mm,矢状缝向右2 mm,硬脑膜下3 mm),头项备毛,分离暴露颅骨,使用微量注射泵,将5 μL GL261细胞混悬液,缓慢注射至尾状核,留针3 min,缓慢拔针,缝合小鼠头部皮肤,常规饲养。

1.2.3 小鼠脑组织石蜡切片及HE染色 于种植后第21天,经4%水合氯醛(0.1 mL/10 g)腹腔麻醉,生理盐水、4%的多聚甲醛心脏灌注固定,取出脑组织,置于4%多聚甲醛中室温过夜。常规脱水、透明、浸蜡、石蜡包埋,行5 μm连续切片并常规HE染色,步骤包括烤片、脱蜡、水化、HE染色、二甲苯透明、中性树胶封片。

1.2.4 石蜡切片免疫荧光检测Iba-1和P2X4受体表达 石蜡切片常规脱蜡水化,用柠檬酸组织抗原修复液进行抗原修复,免疫染色封闭液封闭10 min,在湿盒中滴加抗Iba-1(1:200)、P2X4受体(1:200)的抗体共同孵育,4℃冰箱过夜,PBS洗3次,10 min/次;与荧光标记的二抗(1:200)室温避光孵育2 h,PBS洗3次,10 min/次;含DAPI抗荧光淬灭封片液封片,观察并拍照,用Image J分析软件分析。

1.2.5 胶质瘤TAMs的诱导 小鼠胶质瘤GL261细胞生长融合至70%,将培养基弃掉,用PBS冲洗2次,更换为新鲜的DMEM完全培养基,培养24 h后收集上清,1000 g离心10 min,去除细胞碎片,与DMEM完全培养基按照1:1配成GL261条件培养基。将对数生长长期的RAW264.7细胞接种于6孔板中,细胞数目为2×10<sup>5</sup>/孔,次日将培养基弃掉,用PBS冲洗2次,更换GL261条件培养基刺激24 h,将RAW264.7细胞诱导为TAMs<sup>[14]</sup>。

1.2.6 小干扰RNA敲低TAMs中P2X4受体基因表达在TAMs模型基础上,将P2X4 siRNA(siRNA P2X4)和对照siRNA(siRNA NC)与转染试剂充分混合,转染TAMs,不做任何处理的为空白对照组,通过Western blot检测其干扰效率。P2X4 siRNA由广州锐博生物科技有限公司设计并合成,靶基因序列如下:ACACTCGGGACTTAGAACA。取转染后的siRNA P2X4组及siRNA NC组细胞,通过RT-qPCR及Western blot检测转染后IL-1β、IL-18的mRNA及蛋白表达量。

1.2.7 Western blot检测P2X4受体、IL-1β、IL-18蛋白表达水平 离心收集细胞,使用含有蛋白酶抑制剂混合物的RIPA裂解缓冲液提取细胞总蛋白,用BCA试剂盒测



定蛋白浓度,5×上样缓冲液处理并热变性蛋白质,通过10% SDS-聚丙烯酰胺凝胶电泳(SDS-PAGE)分离蛋白样品,随后转移到0.45 μm PVDF膜,5%脱脂牛奶封闭1 h,与P2X4受体(1:100)抗体、IL-1β(1:1000)抗体、IL-18(1:1000)抗体、GAPDH抗体(1:5000)室温孵育1 h,4℃冰箱过夜。随后,将膜与辣根过氧化物酶(HRP)标记的二抗(1:500),羊抗兔二抗(1:3000)(一抗为兔抗鼠IL-1β和兔抗鼠IL-18),羊抗鼠二抗(1:3000)(一抗为鼠抗鼠GAPDH);室温孵育2 h。使用ECL发光液进行显影,拍照后用Image J软件分析蛋白条带的灰度值。

1.2.8 RT-qPCR检测TNF-α、iNOS、Arg-1、IL-10、P2X4受体、IL-1β、IL-18的mRNA表达 离心收集待测细胞,提取细胞的总RNA,核酸蛋白检测仪检测RNA纯度,将RNA逆转录为cDNA,以cDNA为模板,进行聚合酶链扩增反应,根据 $2^{-\Delta\Delta Ct}$ 方法对结果进行定量分析。引物序列如下:TNF-α(上游:CCCTCACACTCAGATCATCTTCT,下游:GCTACGACGTGGGCTACAG);iNOS(上游:GTTCTCAGCCCAACAATACAAGA,下游:GTGGACGGGTTCGATGTCAC);Arg-1(上游:CTCCAAGCCAAAGTCCTTAGAG,下游:AGGAGCTGTCATTAGGGACATC);IL-10(上游:GCTCTTACTGACTGGCATGAG,下游:CGCAGCTCTAGGAGCATGTG);P2X4受体(上游:CTGGTGTGCCAACGAGGATA,下游:AGACGGAATATGGGGCAGAAG);IL-1β(上游:GCAACTGTTCTGAACTCAACT,下游:ATCTTTGGGGTCCGTCAACT);IL-18(上游:GACTCTTGCGTCAACTTCAAGG,下游:CAGGCTGTCTTTTGTC AACGA);GAPDH(上游:AGGTCGGTGTGACGGATTTG,下游:TGTAGACCATGTAGTTGAGG TCA)。

1.2.9 GL261细胞Transwell侵袭及迁移实验 分别收集siRNA P2X4组和siRNA NC组TAMs的细胞培养液上清,与DMEM完全培养基按照1:1配成TAMs条件培养基(CM),分别为CM-siRNA P2X4组及CM-siRNA NC组。选择对数生长期的GL261细胞,待细胞生长至

70%融合时,将培养基弃掉,用PBS冲洗2次,更换上述条件培养基,继续培养24 h。分别使用涂覆及未涂覆有Matrigel基质胶的Transwell小室进行侵袭及迁移实验,取CM-siRNA P2X4组及CM-siRNA NC组各200 μL( $2 \times 10^5$ )细胞悬液加入上室,下室加入含10%FBS的DMEM培养基600 μL,培养24 h;固定染色,于倒置显微镜下观察,高倍镜(×200)下随机选取5个视野计数穿膜细胞数量。

### 1.3 统计分析方法

采用GraphPad Prism 8进行统计学分析。计量资料以均数±标准差表示,组间比较采用两独立样本的*t*检验及单因素方差分析,两两比较行LSD-*t*检验。 $P < 0.05$ 为差异有统计学意义。

## 2 结果

### 2.1 荷瘤小鼠脑胶质瘤形态学观察

取接种后第21天脑组织观察,见小鼠脑右侧尾状核区有肿瘤形成,色白,肿瘤呈实性,不规则,无包膜,侵入周围脑组织,肿瘤中心见出血和坏死,正中中线因受肿瘤挤压向对侧移位(图1A)。光镜下,可见肿瘤病灶形态不规则,边界不清,周围正常脑组织中可见成团肿瘤细胞浸润,肿瘤组织中血管丰富,可见坏死和出血区域。胶质瘤细胞排列紊乱,细胞呈不规则形或短梭形,核大深染,异型性明显(图1B、C)。

### 2.2 P2X4受体在小鼠胶质瘤TAMs中的表达增加

免疫荧光染色结果显示,在小鼠脑胶质瘤中,Iba-1(标记小胶质细胞及巨噬细胞)阳性细胞数量明显增多,为活化的小胶质细胞及巨噬细胞,细胞体积增大,突起增多,与正常脑组织相比,差异有统计学意义( $P < 0.0001$ ,图2),且P2X4受体在胶质瘤Iba-1阳性细胞中的表达较正常脑组织中增高,差异有统计学意义( $P = 0.001$ ,图3)。

### 2.3 GL261条件培养基可诱导RAW264.7细胞向M2型TAMs方向转化及P2X4受体表达增加

收集小鼠胶质瘤细胞GL261的上清,作为肿瘤细胞条件培养基,刺激RAW264.7细胞转化为TAMs,结

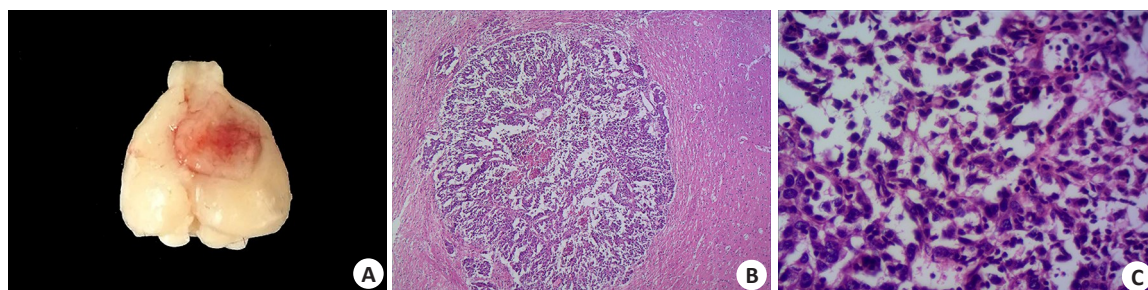


图1 接种21 d后小鼠脑内肿瘤的病理特点

Fig.1 Pathological characteristics of gliomas in mouse brain 21 days after inoculation. A: Anatomy of brain of tumor-bearing mice. B: HE detection of brain tumors in mice (Original magnification: ×50). C: HE detection of brain tumors in mice (×400).

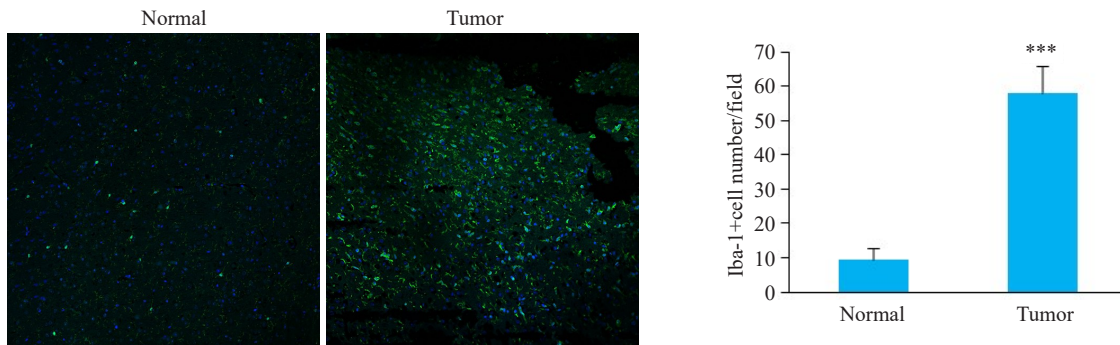


图2 Iba-1在小鼠胶质瘤中的表达

Fig.2 Expression of Iba-1 in glioma of the mice ( $n=8$ ) (Green: Iba-1 positive cell; Blue: resting cell nucleus; Immunofluorescence staining:  $\times 200$ ). \*\*\* $P<0.001$  vs normal.

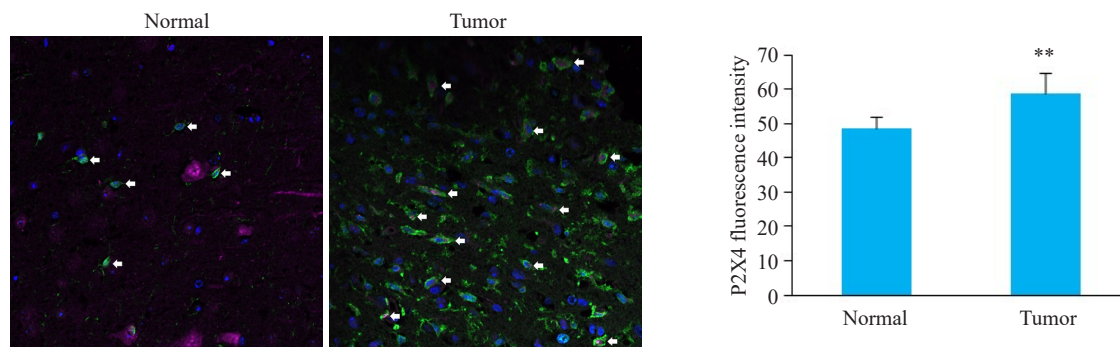


图3 P2X4受体在胶质瘤Iba-1阳性细胞中的表达

Fig.3 Expression of P2X4 receptor in Iba-1 positive cells in glioma of mice ( $n=8$ ) (Purple: P2X4 receptor positive cell; Green: Iba-1 positive cell; Blue: resting cell nucleus; Immunofluorescence staining:  $\times 600$ ). \*\* $P<0.01$  vs normal.

果显示,处理后RAW264.7中M2型巨噬细胞标志基因Arg-1、IL-10表达增高( $P<0.001$ ),M1型巨噬细胞标志基因TNF- $\alpha$ 、iNOS的表达也增高( $P=0.006,0.001$ ,图4),但以M2型巨噬细胞标志基因Arg-1、IL-10的表达增高更为明显。

为进一步探讨P2X4受体在GL261条件培养基诱导TAMs中的表达情况,我们提取细胞的mRNA和蛋白检测P2X4受体表达情况。实验结果显示,无论在蛋白水平还是mRNA水平,GL261细胞培养上清能够诱导RAW264.7细胞中P2X4受体表达上调( $P=0.005,0.014$ ,图5)。

#### 2.4 干扰P2X4后对TAMs中P2X4受体蛋白表达的影响

Western blot检测P2X4受体的表达,结果显示:与空白对照组及siRNA NC组相比,干扰P2X4能够降低TAMs中P2X4受体的蛋白表达水平( $P<0.0001$ ,图6A,B)。

#### 2.5 干扰P2X4引起TAMs中IL-1 $\beta$ 、IL-18的表达水平降低

进一步检测IL-1 $\beta$ 、IL-18的表达,结果显示,与空白对照组及siRNA NC组相比,干扰P2X4降低TAMs中IL-1 $\beta$ 、IL-18的mRNA( $P<0.01$ )及蛋白表达水平( $P<0.01, P<0.05$ ,图7)。

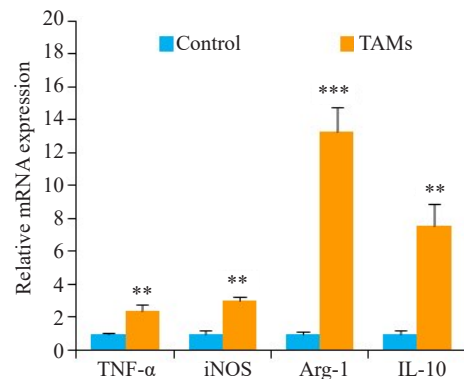


图4 TAMs中M1/M2型巨噬细胞表面标志物表达情况

Fig.4 Expression of surface markers of M1/M2 macrophages in TAMs ( $n=3$ ). \*\* $P<0.01$ , \*\*\* $P<0.001$  vs control.

#### 2.6 干扰TAMs中P2X4受体表达能抑制胶质瘤细胞侵袭和迁移的能力

Transwell侵袭实验结果显示,CM-siRNA P2X4组GL261细胞的侵袭能力低于CM-siRNA NC组( $P=0.004$ ,图8)。Transwell迁移实验结果显示,CM-siRNA P2X4组GL261细胞的的迁移能力低于CM-siRNA NC组( $P=0.017$ ,图9)。

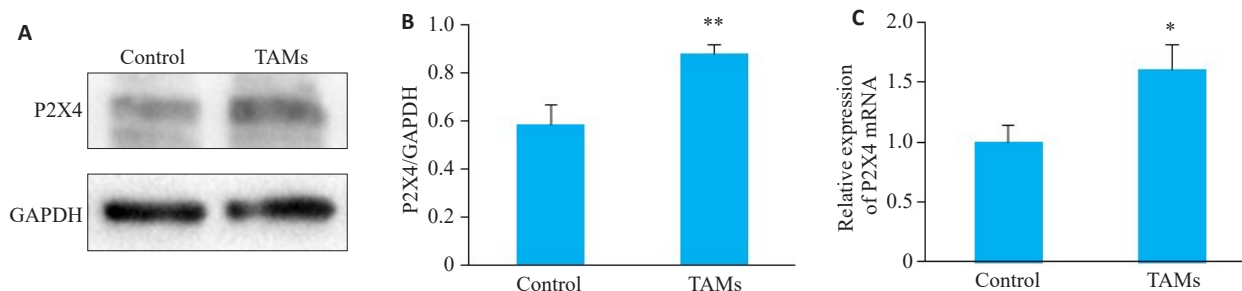


图5 P2X4受体在TAMs中的表达情况

Fig.5 Expression of P2X4 receptor in TAMs. **A, B:** Western blotting for detecting P2X4 in TAMs. **C:** Expressions of P2X4 mRNA in TAMs detected using RT-qPCR ( $n=3$ ). \* $P<0.05$ , \*\* $P<0.01$  vs control.

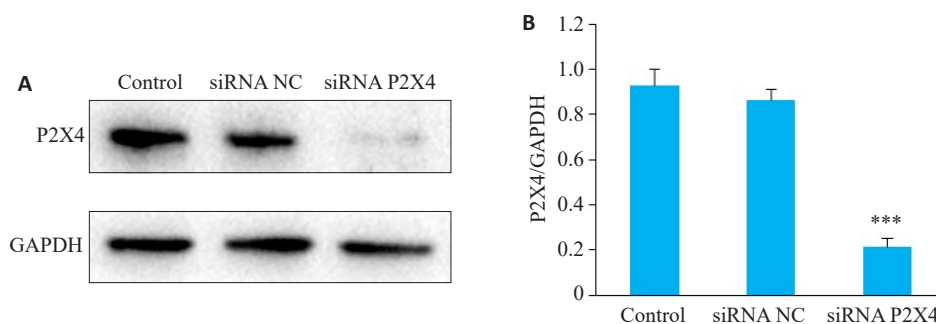


图6 转染siRNA P2X4后对TAMs中P2X4受体蛋白表达的影响

Fig.6 Effect of siRNA P2X4 transfection on P2X4 receptor protein expression in TAMs. **A, B:** Western blotting for P2X4 in TAMs after siRNA P2X4 transfection ( $n=3$ ). \*\*\* $P<0.001$  vs control/siRNA NC.

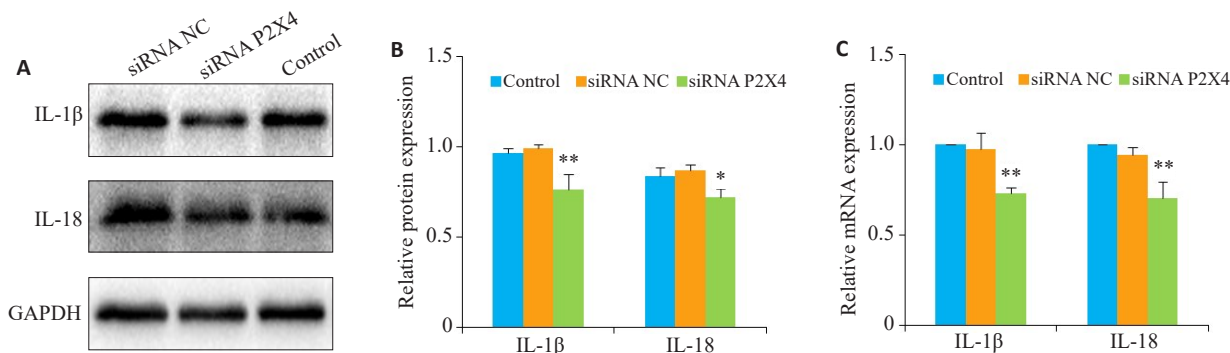


图7 转染P2X4 siRNA对TAMs中IL-1β、IL-18表达的影响

Fig.7 Effect of P2X4 siRNA transfection on expressions of IL-1β and IL-18 in TAMs. **A, B:** Western blotting for IL-1β and IL-18 proteins in TAMs after P2X4 siRNA transfection. **C:** Expressions of IL-1β and IL-18 mRNA in TAMs detected using RT-qPCR after P2X4 siRNA transfection ( $n=3$ ). \* $P<0.05$ , \*\* $P<0.01$  vs control/siRNA NC.

### 3 讨论

高水平的细胞外ATP是肿瘤微环境的特征之一<sup>[15]</sup>,胶质瘤细胞或胶质瘤肿瘤组织中浸润的炎症细胞可在机械损伤、缺氧、坏死及缺血等刺激下,通过胞吐、细胞膜通道形成和细胞溶解等方式向细胞外释放大量ATP,促进肿瘤细胞的增殖和生长<sup>[16]</sup>。高水平的细胞外ATP还可刺激巨噬细胞向肿瘤区域募集,并促进炎症介质的分泌,从而启动和维持肿瘤的发展<sup>[17,18]</sup>。ATP通过激活

嘌呤受体P2X离子通道或P2Y蛋白偶联受体介导其信号作用<sup>[19]</sup>。以往对P2X受体在肿瘤中的作用研究多集中于P2X7受体,近年来发现P2X4受体在肝癌<sup>[20]</sup>、胶质瘤<sup>[11]</sup>、前列腺癌<sup>[21]</sup>、胃癌<sup>[22]</sup>肿瘤细胞上表达和发挥作用。但对P2X4受体在肿瘤相关免疫细胞中作用的研究报道较少,有研究发现在转移性非小细胞肺癌患者支气管肺泡灌洗液的炎症细胞中P2X4水平升高<sup>[23]</sup>,P2X4受体在前列腺癌大多数CD68<sup>+</sup>巨噬细胞中表达<sup>[21]</sup>,P2X4



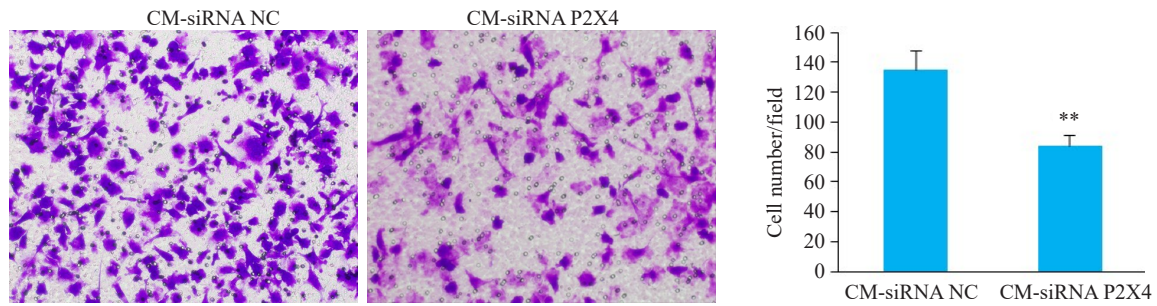


图8 不同条件培养基对GL261细胞侵袭能力的影响

Fig.8 Effects of different conditioned media on invasion of GL261 cells ( $n=3$ ) (Crystal violet staining:  $\times 200$ ). \*\* $P<0.01$  vs CM-siRNA NC.

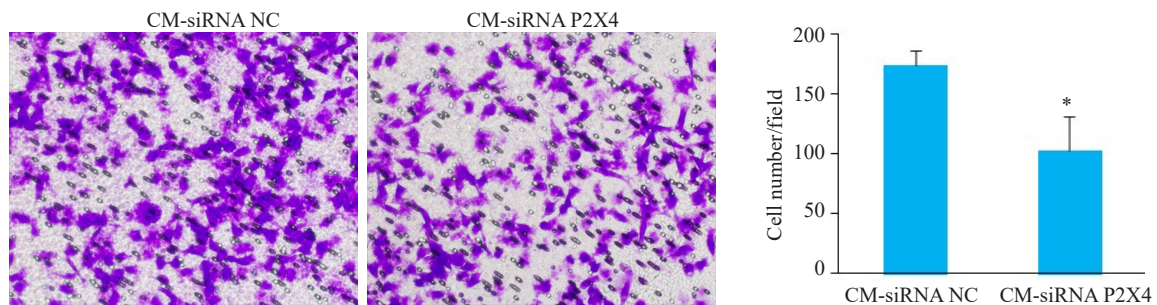


图9 不同条件培养基对GL261细胞迁移能力的影响

Fig.9 Effects of different conditioned media on migration of GL261 cells ( $n=3$ ) (Crystal violet staining:  $\times 200$ ). \* $P<0.05$  vs CM-siRNA NC.

受体在大鼠C6胶质瘤中的巨噬细胞和小胶质细胞高表达<sup>[12]</sup>,但其在肿瘤相关免疫细胞中表达的具体作用及其机制尚有待于进一步研究。

本研究通过颅内原位注射小鼠胶质瘤GL261细胞建立小鼠胶质瘤模型,并经Iba-1、P2X4受体免疫荧光检测,结果显示,正常组小鼠脑组织中Iba-1阳性细胞数量较少,而肿瘤组胶质瘤组织中有较多数量的Iba-1阳性细胞浸润,P2X4受体在小鼠胶质瘤Iba-1阳性细胞中的表达上调,提示P2X4受体可能在肿瘤微环境中发挥一定的作用。

TAMs具有高度可塑性,根据环境不同可极化为M1型和M2型,M1型TAMs表达高水平的促炎性因子(如TNF- $\alpha$ )和诱生型一氧化氮合酶(iNOS)等,具有促进Th1的应答以及杀肿瘤能力;M2型TAMs具有促进组织修复和重塑、促进Th2的免疫应答及肿瘤进展的作用,并产生Arg-1、IL-10等。神经胶质瘤中的巨噬细胞多表现出M2样表型<sup>[24]</sup>。本研究使用GL261条件培养基刺激RAW264.7细胞转化为TAMs,检测巨噬细胞极化相关标志物基因表达,结果显示,处理后RAW264.7中M2型巨噬细胞标志基因Arg-1、IL-10表达明显上调,M1型巨噬细胞标志基因iNOS、TNF- $\alpha$ 表达也上调,但以M2型巨噬细胞标志基因Arg-1、IL-10表达上调更

为显著,同时P2X4受体表达上调,说明在肿瘤中,巨噬细胞经肿瘤驯化后,主要向M2型TAMs转化,P2X4受体也随之明显增高。使用P2X4 siRNA转染下调TAMs中P2X4受体的蛋白表达后,发现TAMs促进胶质瘤侵袭和迁移的能力也随之减弱,提示TAMs上P2X4受体的表达可能参与了胶质瘤的侵袭和迁移行为的调控。

胶质瘤中肿瘤相关巨噬细胞作用的发挥与其表面多种受体密切相关,其中嘌呤受体对于识别ATP信号起重要作用。细胞外ATP能够通过激活P2X4受体导致NLRP3炎性小体的活化,引起炎症级联反应以及IL-1 $\beta$ 、IL-18的成熟和分泌<sup>[25]</sup>。有研究显示,IL-1 $\beta$ 可促进神经胶质瘤细胞的增殖、迁移和侵袭<sup>[26,27]</sup>。TAM释放IL-1 $\beta$ ,通过上调甘油3-磷酸脱氢酶的表达来促进神经胶质瘤细胞的代谢,从而促进糖酵解,加速肿瘤的增殖和生长<sup>[28]</sup>。研究发现C6胶质瘤细胞可以分泌纤维粘连蛋白和玻璃粘连蛋白,从而促进小胶质细胞的激活,激活的小胶质细胞又能分泌大量的IL-18,后者作为IL-1家族的一员,又能进一步促进C6胶质瘤细胞迁移<sup>[29]</sup>。本研究用P2X4 siRNA干扰导致TAMs中P2X4受体表达下调,同时IL-1 $\beta$ 、IL-18的表达也下调。因此,我们推测,TAMs中的P2X4受体有可能通过IL-1 $\beta$ 、IL-18调控胶质瘤肿瘤微环境,进一步影响胶质瘤的发生发展。

由于肿瘤相关巨噬细胞从多方面参与肿瘤微环境的调控,对肿瘤相关巨噬细胞调控机制更精确的把控将有利于针对肿瘤相关巨噬细胞的肿瘤免疫治疗的开展。P2X4受体对于调控胶质瘤肿瘤微环境具有一定的作用,为胶质瘤的靶向治疗提供了新的可能。

#### 参考文献:

- [1] Ostrom QT, Cioffi G, Gittleman H, et al. CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2012-2016 [J]. *Neuro-Oncology*, 2019, 21 (Supplement\_5): v1-100.
- [2] Hambardzumyan D, Gutmann DH, Kettenmann H. The role of microglia and macrophages in glioma maintenance and progression [J]. *Nat Neurosci*, 2016, 19(1): 20-7.
- [3] Coniglio SJ, Eugenin E, Dobrenis K, et al. Microglial stimulation of glioblastoma invasion involves epidermal growth factor receptor (EGFR) and colony stimulating factor 1 receptor (CSF-1R) signaling [J]. *Mol Med*, 2012, 18(1): 519-27.
- [4] Held-Feindt J, Hattermann K, Mürköster SS, et al. CX3CR1 promotes recruitment of human glioma-infiltrating microglia/macrophages (GIMs)[J]. *Exp Cell Res*, 2010, 316(9): 1553-66.
- [5] Okada M, Saio M, Kito Y, et al. Tumor-associated macrophage/microglia infiltration in human gliomas is correlated with MCP-3, but not MCP-1[J]. *Int J Oncol*, 2009, 34(6): 1621-7.
- [6] Wang SC, Hong JH, Hsueh C, et al. Tumor-secreted SDF-1 promotes glioma invasiveness and TAM tropism toward hypoxia in a murine astrocytoma model[J]. *Lab Invest*, 2012, 92(1): 151-62.
- [7] Wang QH, Hu BL, Hu X, et al. Tumor evolution of glioma-intrinsic gene expression subtypes associates with immunological changes in the microenvironment[J]. *Cancer Cell*, 2017, 32(1): 42-56.e6.
- [8] Sørensen MD, Dahlrot RH, Boldt HB, et al. Tumour-associated microglia/macrophages predict poor prognosis in high-grade gliomas and correlate with an aggressive tumour subtype [J]. *Neuropathol Appl Neurobiol*, 2018, 44(2): 185-206.
- [9] Zeiner PS, Preusse C, Golebiewska A, et al. Distribution and prognostic impact of microglia/macrophage subpopulations in gliomas[J]. *Brain Pathol*, 2019, 29(4): 513-29.
- [10] Draganov D, Lee PP. Purinergic signaling within the tumor microenvironment [M]//*Advances in Experimental Medicine and Biology*. Cham: Springer International Publishing, 2021, 1270: 73-87.
- [11] Huo JF, Chen XB. P2X4R silence suppresses glioma cell growth through BDNF/TrkB/ATF4 signaling pathway [J]. *J Cell Biochem*, 2019, 120(4): 6322-9.
- [12] Guo LH, Trautmann K, Schluesener HJ. Expression of P2X4 receptor in rat C6 glioma by tumor-associated macrophages and activated microglia[J]. *J Neuroimmunol*, 2004, 152(1/2): 67-72.
- [13] Kramp TR, Camphausen K. Combination radiotherapy in an orthotopic mouse brain tumor model[J]. *JoVE*, 2012(61): 3397-405.
- [14] Cui X, Morales RTT, Qian WY, et al. Hacking macrophage-associated immunosuppression for regulating glioblastoma angiogenesis[J]. *Biomaterials*, 2018, 161: 164-78.
- [15] Fang WG, Tian XX. Identification of a new pro-invasion factor in tumor microenvironment: progress in function and mechanism of extracellular ATP[J]. *J Beijing Uni(Health Sci)*, 2017, 49(2): 188-95.
- [16] Braganhol E, Wink MR, Lenz G, et al. Purinergic signaling in glioma progression [M]//*Advances in Experimental Medicine and Biology*. Cham: Springer International Publishing, 2020: 87-108.
- [17] Jantarototai N, Choi HB, McLarnon JG. ATP stimulates chemokine production *via* a store-operated calcium entry pathway in C6 glioma cells[J]. *BMC Cancer*, 2009, 9: 442-50.
- [18] Verkhratsky A, Krishtal OA, Burnstock G. Purinoceptors on neuroglia [J]. *Mol Neurobiol*, 2009, 39(3):190-208 .
- [19] Suurväli J, Boudinot P, Kanellopoulos J, et al. P2X4: a fast and sensitive purinergic receptor[J]. *Biomed J*, 2017, 40(5): 245-56.
- [20] Asif A, Khalid M, Manzoor S, et al. Role of purinergic receptors in hepatobiliary carcinoma in Pakistani population: an approach towards proinflammatory role of P2X4 and P2X7 receptors [J]. *Purinergic Signal*, 2019, 15(3): 367-74.
- [21] Maynard JP, Lu JY, Vidal I, et al. P2X4 purinergic receptors offer a therapeutic target for aggressive prostate cancer[J]. *J Pathol*, 2022, 256(2): 149-63.
- [22] Reyna-Jeldes M, de la Fuente-Ortega E, Cerda D, et al. Purinergic P2Y2 and P2X4 receptors are involved in the epithelial-mesenchymal transition and metastatic potential of gastric cancer derived cell lines[J]. *Pharmaceutics*, 2021, 13(8): 1234-41.
- [23] Schmid S, Kübler M, Ayata CK, et al. Altered purinergic signaling in the tumor associated immunologic microenvironment in metastasized non-small-cell lung cancer[J]. *Lung Cancer*, 2015, 90 (3): 516-21.
- [24] Komohara Y, Ohnishi K, Kuratsu J, et al. Possible involvement of the M2 anti-inflammatory macrophage phenotype in growth of human gliomas[J]. *J Pathol*, 2008, 216(1): 15-24.
- [25] Han SJ, Lovaszi M, Kim M, et al. P2X4 receptor exacerbates ischemic AKI and induces renal proximal tubular NLRP3 inflammasome signaling[J]. *FASEB J*, 2020, 34(4): 5465-82.
- [26] Fathima Hurmath K, Ramaswamy P, Nandakumar DN. IL-1 $\beta$  microenvironment promotes proliferation, migration, and invasion of human glioma cells[J]. *Cell Biol Int*, 2014, 38(12): 1415-22.
- [27] Wang T, Cao LH, Dong X, et al. LINC01116 promotes tumor proliferation and neutrophil recruitment *via* DDX5-mediated regulation of IL-1 $\beta$  in glioma cell[J]. *Cell Death Dis*, 2020, 11(5): 302-10.
- [28] Lu J, Xu ZY, Duan HB, et al. Tumor-associated macrophage interleukin- $\beta$  promotes glycerol-3-phosphate dehydrogenase activation, glycolysis and tumorigenesis in glioma cells[J]. *Cancer Sci*, 2020, 111(6): 1979-90.
- [29] Yeh WL, Lu DY, Liou HC, et al. A forward loop between glioma and microglia: Glioma-derived extracellular matrix-activated microglia secrete IL-18 to enhance the migration of glioma cells [J]. *J Cell Physiol*, 2012, 227(2): 558-68.

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