特约综述

本实验室研究神经损伤、炎症、椎间盘突出、化疗和骨癌转移等病因引起的慢性疼痛的发生发展机制, 集中研究脊髓水平胶质细胞和神经元之间的相互调节在疼痛维持中的作用。目前的主要研究方向有: 趋化因子参与神经病理性疼痛调节的机制; microRNA和长链非编码RNA对慢性疼痛的调节。

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趋化因子介导的神经炎症反应和神经病理性疼痛

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摘要 各种疾病引起的神经系统的损伤或功能障碍致使全球数以百万计的人们患有神经性病理性疼痛。目前的方法对神经病理性疼痛的疗效不佳且有副作用, 需要开发有效的治疗方法。近年来人们逐渐认识到, 脊髓中胶质细胞(如小胶质细胞和星形胶质细胞)能通过释放强效的神经调质, 如促炎细胞因子和趋化因子, 在神经性病理性疼痛的产生和维持中起重要作用。近期的证据显示, 趋化因子是疼痛调控中的新成员。该文综述了一些趋化因子和受体(如CCL2/CCR2、CXCL1/CXCR2、CX3CL1/CX3CR1、CCL21/CXCR3)作为神经元和胶质细胞相互调控的介质参与神经病理性疼痛的调节。靶向趋化因子介导的神经炎症反应将成为治疗神经病理性疼痛的新方向。

关键词 神经病理性疼痛; 神经炎症; 胶质细胞; 趋化因子; 神经损伤; 脊髓

Chemokine-Mediated Neuroinflammation and Neuropathic Pain

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Abstract Millions of people worldwide suffer from neuropathic pain as a result of damage to or dysfunction of the nervous system under various disease conditions. Treatment of neuropathic pain is always accompanied by a poor response and undesired adverse effects. Development of effective therapeutic strategy is critical in this field. It has been increasingly recognized that spinal cord glial cells (such as microglia and astrocytes) play a critical role in the induction and maintenance of neuropathic pain by releasing powerful neuromodulators such as proinflammatory cytokines and chemokines. Recent evidence revealed chemokines as new players in pain control. In this paper, we demonstrated that different chemokines and chemokine receptors (e.g., CCL2/CCR2, CXCL1/CXCR2,

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CX3CL1/CX3CR1, and CCL21/CXCR3) serve as mediators for neuron–glia communication subsequently modulating neuropathic pain. Targeting chemokine-mediated neuroinflammation will be a new approach for treatment of neuropathic pain.

Key words neuropathic pain; neuroinflammation; glial cells; chemokines; nerve injury; spinal cord

神经病理性疼痛(neuropathic pain)是慢性疼痛的一种, 也是困扰人类健康的严重问题之一。临床上常见的炎症、外伤、手术、癌症、代谢性疾病(如糖尿病)、免疫性疾病(如艾滋病)等都可以引起神经病理性疼痛, 表现为自发痛(spontaneous pain)、触诱发痛(allodynia, 即正常情况下非伤害性刺激引起的疼痛)和痛觉过敏(hyperalgesia, 即伤害性刺激引起的疼痛增强)。神经病理性疼痛的特点是疼痛剧烈, 甚至达到"痛不欲生"的程度; 而且持续时间长, 经常在原发病治愈后, 疼痛依然存在数月、数年乃至终生。目前, 我国至少有1 600万神经病理性疼痛患者。虽然临床治疗药物和手段众多, 但资料显示, 只有50%的人可达到30%的缓解程度。近年来, 对神经病理性疼痛的机制研究取得了较多的进展。本文对趋化因子介导的神经炎症反应在神经病理性疼痛中的作用进行综述。

1 神经炎症反应

神经病理性疼痛通常可被看作是一种神经元可塑性的表现, 它包括外周敏化和中枢敏化, 前者即周围神经系统中初级感觉神经元的敏感性和兴奋性增加, 后者即中枢神经系统中伤害性感受神经元的活性和兴奋性增加, 两者均促进神经病理性疼痛的发展和维持[1-3]。近年来的研究证明, 除了神经元, 外周神经系统和中枢神经系统中的非神经细胞(如免疫细胞和胶质细胞)在慢性疼痛中也起着关键的作用[4-7]。神经损伤后, 在损伤部位、背根神经节(dorsal root ganglia, DRG)、脊髓的非神经元细胞, 释放多种炎症介质参与调节伤害性信息的传递。在受损神经, 出现免疫细胞浸润并对神经病理性疼痛的早期启动发挥重要作用[8-9]。在脊髓, 胶质细胞(如星形胶质细胞和小胶质细胞)不但发生形态上的改变, 还出现胞内信号激酶的激活、膜受体和通道蛋白表达增加以及炎症介质的表达和释放增多。胶质细胞释放的炎症介质, 如促炎性细胞因子、趋化因子、神经生长因子等能够作用于神经元或胶质细胞, 从而增强神经元的兴奋性或进一步放大胶质细胞介导的炎症反应[4,10]。因此, 靶向神经炎症反应可能成为神经病理性疼痛治疗的新方向。近年来对促炎性细胞因子如肿瘤坏死因子(TNF-α)、白介素1β(IL-1β)、白介素6(IL-6)在慢性疼痛、神经退行性疾病和肿瘤中的作用有很多研究[11]。对趋化因子在慢性疼痛, 尤其是神经病理性疼痛中的作用也取得了较多的进展。

2 趋化因子其受体

2.1 趋化因子

趋化因子是一类功能相关的小分子分泌蛋白, 分子量为8~14 kDa, 因其具有白细胞趋化性和细胞因子活性而被命名为"趋化因子"。 在人体内, 这个家族由大约50种相关分子组成, 在其他哺乳动物也有相近的同系物[12]。每一个趋化因子含有70~100个氨基酸, 20%~95%的序列包含保守的半胱氨酸残基。根据半胱氨酸的数量和间距, 趋化因子被分为四种亚型: CC, CXC, XC和CX3C亚型(图1)。CC趋化因子有28个成员, 它的特点是四个半胱氨酸残基中的前两个在相邻的位置。CC家族具有广泛的作用, 能够吸引单核细胞、嗜酸性粒细胞、嗜碱性粒细胞、自然杀伤性细胞和树突细胞。CXC类趋化因子是第二大类, 有16个成员, 结构上在前两个半胱氨酸残基中间有一个其它氨基酸间隔。XC类趋化因子家族有2个成员, 结构上只有两个半胱氨酸残基。CX3C家族只有1个成员, 在前两个半胱氨酸残基中间插入了三个氨基酸。CX3CL1有可溶型和膜结合型两种类型, 对T细胞和NK细胞具有黏附和趋化作用[13]。

趋化因子最初以它的生物功能命名。自2000年起, 一种新的趋化因子分类系统被使用, 趋化因子被认为是趋化因子配体(ligand, L)[14]。因此, 每个趋化因子被分别标记为CCL、CXCL、XCL或CX3CL。大部分的趋化因子都有两个名字, 一个以生物学功能命名, 另一个以结构命名, 比如以功能命名的单核细胞趋化因子-1(monocytes chemoattractant protein-1, MCP-1), 结构名为CCL2。本文中选择使用趋化因子的结构名称以便于更好地与相应的趋化因子受体相匹配。在中枢神经系统, 趋化因子可以

来源于包括小胶质细胞、星形胶质细胞、神经元和内皮细胞在内的不同类型的细胞。大多数趋化因子不是组成型表达, 能在神经系统疾病状态下诱导产生[15-17]。

2.2 趋化因子受体

所有的趋化因子通过激活膜表面G-蛋白偶联受体(G protein-coupled receptors, GPCRs)来发挥作用。迄今为止, 已经克隆了20多种趋化因子受体。这些受体的命名是在CC、CXC、XC、CX3C后面加上一个R, 然后加一个数字(图1)。除了部分趋化因子与趋化因子受体有一一对应关系, 例如CXCL13-CXCR5、CXCL16-CXCR6、CX3CL1-CX3CR1, 大多数趋化因子可激活多个受体。同样, 一个受体可以被不同的趋化因子激活[12](图1)。

趋化因子受体结构是一个7次跨膜的多肽链, 含有具有酸性的N-末端胞外结构域和富含丝氨酸/苏氨酸的胞内C-末端。趋化因子受体激活不同的信号通路, 如丝裂原活化蛋白激酶(mitogen-activated protein kinases, MAPK)通路、磷脂酶C通路(phospholipase C, PLC)和磷脂酰肌醇3激酶(phosphatidylinositol-3-kinase, PI3K)通路, 导致不同的功能结果, 包括黏附、极化和趋化等[15-16]。

许多趋化因子受体, 包括大多数的CCR家族、所有的CXCR家族和CX3CR1都在中枢神经系统中表达。这些趋化因子受体由星形胶质细胞、神经元和小胶质细胞表达[16-17]。除了一些趋化因子受体, 例如CCR1、CCR2、CCR3、CCR5、CXCR2、CXCR3、CXCR4和CX3CR1在中枢神经系统内持续表达外, 大多数趋化因子受体在病理条件下才能检测到[16]。

3 趋化因子和神经病理性疼痛

神经病理性疼痛中趋化因子及其受体的表达、分布和功能已经在不同的疼痛动物模型上进行了研究。常用的神经病理性疼痛模型包括坐骨神经切断[18]、坐骨神经慢性压迫(chronic constriction of sciatic nerve, CCI)[19]、部分坐骨神经结扎(partial sciatic nerve ligation, PSNL)[20]、坐骨脊神经结扎(spinal nerve ligation, SNL)[21]、坐骨神经分支性损伤(spared nerve injury, SNI)[22]和背根神经节的慢性压迫(chronic compression of the DRG, CCD)[23]。神经病理性疼痛也可由坐骨神经感染、炎症或者脱髓鞘[24], 以及化疗[25]、毒素[26]和糖尿病等诱导产生。下面介绍神经病理性疼痛中几对趋化因子和受体在DRG和脊髓的表达、分布和功能。

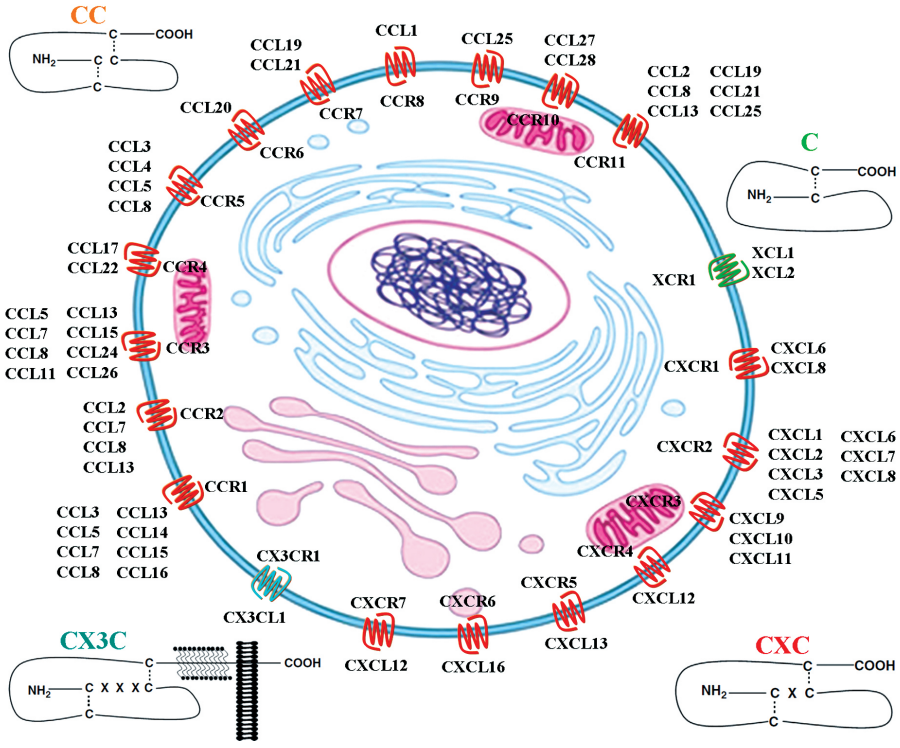


图1 趋化因子分类和它们的受体

Fig.1 Classification of chemokines and their receptors

3.1 CCL2/CCR2和神经病理性疼痛

CCL2属于CC趋化因子家族, 功能上称单核细胞趋化因子-1(MCP-1), 可以特异性地使单核细胞向炎症、外伤感染、毒素暴露和缺血部位聚集。CCL2能够识别多个受体, 包括CCR1、CCR2和CCR4, 而CCR2是CCL2首选的受体[13,27]。CCR2可以被多个趋化因子识别, 如CCL7、CCL8和CCL13。在小鼠组织内, CCR2特异性结合CCL2的亲和力比结合CCL7高10倍[28]。

3.1.1 *神经损伤后CCL2/CCR2的分布和调控* 在正常状态下, CCL2表达于DRG; 神经损伤诱导CCL2在DRG的表达增加。研究显示, 坐骨神经损伤(PSNL)后DRG神经元中的CCL2在4 h内快速上调[29]。而且, 小神经元和大神经元都能表达CCL2, 这些神经元同时也表达轴突损伤的标志物——转录因子ATF-3, 表明CCL2主要在损伤神经元中增多[30]。然而Thacker等[31]证明在腰5脊神经结扎切断模型中, 损伤的(L5)和未损伤的(L4)DRG神经元都产生CCL2。在坐骨神经脱髓鞘、慢性压迫DRG、神经切断、CCI、SNL等神经病理性疼痛模型中也出现CCL2在DRG神经元的表达增多[32-35]。而且在DRG神经元中, CCL2与P物质(substance P, SP)、降钙素基因相关肽(calcitonin gene-related peptide, CGRP)以及辣椒素受体TRPV1(transient receptor potential vanilloid 1)共表达[36]。CCL2在包绕DRG神经元的卫星细胞中也能被诱导表达[33]。因此在DRG中, CCL2能在神经元和卫星细胞中诱导产生。

CCR2在DRG中也有表达。部分CCR2与CCL2在DRG神经元共存, 说明DRG内的CCL2/CCR2信号可能具有自分泌/旁分泌的作用[37]。原位杂交结果显示, 慢性压迫DRG诱导受压迫的(L4/L5)DRG和相邻的非压迫(L3/L6)的DRG中的神经元和非神经元细胞*CCR2* mRNA的表达[34]。在坐骨神经脱髓鞘后, DRG中的CCR2也上调[33-34,37]。因此, CCL2可作用于DRG神经元和胶质细胞中的CCR2来调节疼痛敏感性。

CCL2在正常脊髓有少量表达。脊神经损伤后, 脊髓中CCL2从第3 d开始表达增加, 持续21 d以上。脊髓中的CCL2不仅分布在初级传入末梢中[30,36], 还存在于星形胶质细胞[38]。脊神经损伤和脊髓损伤都能引起星形胶质细胞中CCL2表达增多[38-39]。神经脱髓鞘损伤[40-41]、机械损伤[42]、内嗅区轴突横断[43]和局灶性脑出血[44]诱导CCL2在脑组织的星形胶质细胞中表达。

脊髓中CCR2表达的细胞定位有些争议。早期的免疫组化研究显示, CCR2在脊髓小胶质细胞中表达[45]。近年来的研究结果显示, CCR2表达在星形胶质细胞和神经元中[38]。正常状态下, CCR2在脊髓神经元中组成型表达, 在神经病理性疼痛中表达增加。原位杂交结果显示, 在正常动物的脊髓中检测不到*CCR2* mRNA, 在脊神经结扎后3 d *CCR2* mRNA信号出现在深层背角神经元和运动神经元中, 表明在神经损伤后*CCR2* mRNA的上调[38]。在脊髓损伤后CCR2在星形胶质细胞中表达上调[39]。

3.1.2 *脊髓CCL2/CCR2介导胶质细胞–神经元相互作用* 如上所述, SNL诱导脊髓星形胶质细胞中CCL2的表达, CCR2组成型表达于背角神经元中并在神经病理性疼痛中表达增加[38,46], 提示CCL2和CCR2可作为星形胶质细胞和神经元之间的信号分子促进神经损伤后的中枢敏化。

电生理实验结果支持CCL2对脊髓神经元有直接的作用。用CCL2灌流离体的脊髓切片可诱导脊髓背角II层神经元自发性突触后电流(spontaneous excitatory postsynaptic currents, sEPSC)的频率和幅度均增高[38], 表明CCL2既通过突触前机制促进了谷氨酸的释放, 又通过突触后机制增强谷氨酸受体的功能[47]。而且, CCL2迅速增强了N-甲基-D-天门冬氨酸(N-methyl-D-aspartic acid, NMDA)和α-氨基-3-羟基-5-甲基-4-异恶唑丙酸(α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid, AMPA)诱导的II层神经元的内向电流[38]。Gosselin等[46]研究显示, CCL2抑制培养的脊髓神经元中γ-氨基丁酸 (γ-aminobutyric acid, GABA)诱导的电流。这些结果支持CCL2对脊髓背角神经元兴奋性的直接调解作用。与电生理学结果一致, 行为学上脊髓注射CCL2诱导了快速的热痛觉过敏[38]。此外, CCL2孵育脊髓切片或鞘内注射CCL2诱导浅层背角神经元中枢敏化的标记物——细胞外信号调节蛋白激酶(extracellular-regulated protein kinases, ERK)快速磷酸化[38,48]。这些结果证明CCL2/CCR2通过介导星形胶质细胞–神经元的相互作用参与中枢敏化和慢性疼痛调节(图2)。

另外, CCL2也表达于脊髓背角浅层的SP阳性初级传入神经和CGRP阳性初级传入神经中, 表明DRG神经元中的CCL2可以被转运到脊髓中枢末端[36,38-49]。Zhang等[50]报道脊髓注射CCL2中和抗体

或者在*CCR2*敲除小鼠中, 神经损伤导致的脊髓小胶质细胞增生受到抑制。脊髓给予高剂量CCL2可诱导野生型小鼠的小胶质细胞增生, 但不能诱导*CCR2*基因敲除小鼠中小胶质细胞的增生。Thacker等[31]报道, 脊髓内注射CCL2诱导同侧脊髓背角小胶质细胞广泛激活。这些结果提示, 在周围神经损伤后, 脊髓内CCL2/CCR2信号也参与神经元–小胶质细胞的相互作用, 并促进脊髓小胶质细胞的激活(图2)。

3.1.3 CCL2/CCR2在神经病理性疼痛中的作用 行为学实验结果从几个方面支持了CCL2/CCR2对神经病理性疼痛的促进作用。第一, CCR2缺失小鼠中坐骨神经部分结扎后诱导的机械性痛触诱发痛显著降低[45,50]。第二, 鞘内注射CCR2拮抗剂逆转了周围神经轴突脱髓鞘或周围神经gp120/hCD4损伤诱导的触觉异常性疼痛行为[51-52]; 脑池内注射CCR2拮抗剂缓解三叉神经损伤诱导的面部痛觉过敏[53]。第三, 星形胶质细胞中CCL2过表达小鼠表现出疼痛敏感性增强[54]。第四, 鞘内注射CCL2增强了痛觉过敏[31]。第五, CCL2中和抗体减轻了SNL或CCI诱导的机械性触诱发痛[31,38]。这些结果说明, 增强CCL2使疼痛增强, 而抑制CCL2/CCR2能够有效缓解神经病理性疼痛。

3.2 CXCL1/CXCR2和神经病理性疼痛

CXCL1属于CXC趋化因子家族, 功能上称为生长相关癌基因(growth-related oncogene, GRO), 在鼠类称为角质细胞起源趋化因子(keratinocyte-derived chemokine, KC)或中性粒细胞趋化因子-1(cytokine-induced neutrophil chemoattractant-1, CINC-1)。它是一个8 kDa的分泌蛋白, 被认为是人类IL-8的功能类似物。在外周组织, CXCL1主要由激活的单核细胞、内皮细胞、成纤维细胞产生。而且, CXCL1通过其主要受体CXCR2参与早期炎症中中性粒细胞的趋化和脱颗粒, 并介导炎症性疼痛和急性术后痛[55-56]。在脑组织, CXCR2表达于神经元、小胶质细胞和少突胶质细胞[57-59]。

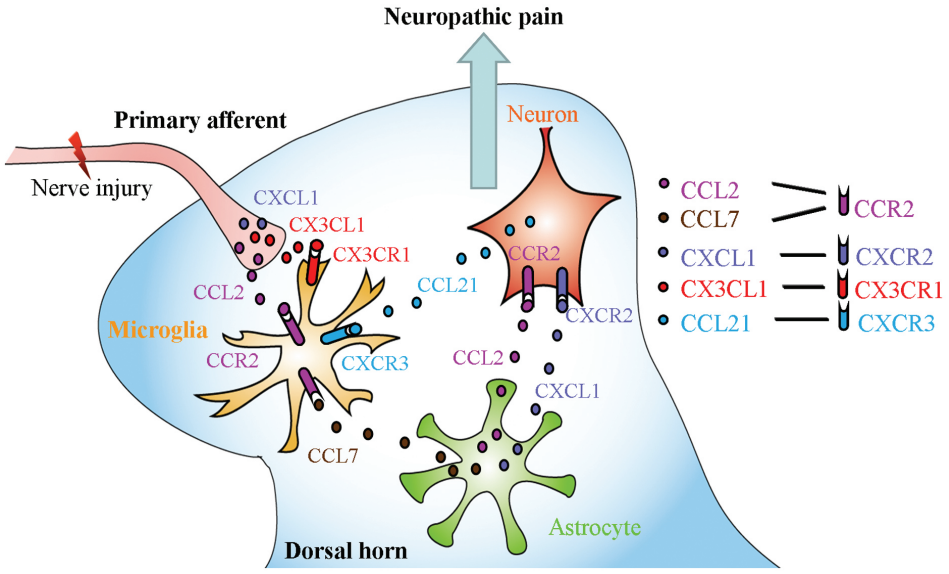
3.2.1 神经损伤后CXCL1/CXCR2的分布和调控 CXCL1表达于DRG神经元中, 并在神经损伤和DRG局部炎症状态下出现快速表达上调[60-61]。CXCL1能促进DRG神经元内钙离子的内流和CGRP的释放[62]; CXCL1还能增加DRG内中、小型神经元的钾电流和钠电流以及TRPV1受体的功能[63-65]。受体CXCR2也表达于DRG的神经元, 并且在大、中、小神经元中都有表达, 与CGRP、IB4和NF200共存。

在脊髓, CXCL1表达于正常动物, 并在神经损伤后增多。SNL后脊髓中CXCL1的mRNA和蛋白的表达从第3 d开始增多, 在第10 d达到高峰, 第21 d时有所下降但仍高于正常动物[66]。PSNL也能诱导CXCL1在损伤神经和脊髓的表达增多, 但从1 h开始增多, 到2 d时恢复正常[67]。原位杂交和免疫组化研究显示, CXCL1 mRNA和蛋白均表达于脊髓星形胶质细胞, 而不表达于神经元或小胶质细胞[66]。Pineau等[68]报道在脊髓损伤后, *CXCL1* mRNA在星形胶质细胞中表达; 神经元损伤或脑室注射ED-1诱导脑内星形胶质细胞表达CXCL1[69-70]。在多发性硬化症病人的发病区域出现CXCL1选择性地表达在星形胶质细胞[71-72]。但也有结果显示癫痫大鼠CXCL1表达于脑内的神经元[73]。这些结果表明, CXCL1在星形胶质细胞表达并在病理状态下表达上调。

CXCR2在正常动物脊髓也有表达, 并且在神经损伤诱导的神经病理性疼痛中表达增加[66]。在前列腺癌细胞诱导的骨癌痛中, 脊髓CXCR2也出现长期的(>21 d)表达上调(待发表资料)。而且, CXCR2表达于脊髓神经元, 并分布于整个脊髓背角[66]。

3.2.2 脊髓CXCL1/CXCR2介导星形胶质细胞和神经元的相互作用 由于CXCL1和CXCR2分别表达于脊髓星形胶质细胞和神经元, 提示它们可能参与星形胶质细胞和神经元的相互作用(图2)。我们最近的研究表明, 鞘内注射CXCL1诱导CXCR2依赖的热痛觉过敏, 并诱导CXCR2依赖的磷酸化ERK、磷酸化cAMP反应元件结合蛋白(cAMP-response element binding protein, CREB)和c-Fos在脊髓神经元的表达增加, 表明CXCL1能够在功能上激活神经元[66]。电生理结果显示, CXCL1灌流脊髓薄片II层神经元, 快速增加了sEPSC和NMDA诱导的电流, 并能被CXCR2拮抗剂阻断(待发表资料), 进一步支持CXCL1/CXCR2介导星形胶质细胞和神经元的相互作用。

虽然CCL2/CCR2和CXCL1/CXCR2都参与神经病理性疼痛中星形胶质细胞和神经元的相互作用, 但它们在表达时程上存在差异。神经损伤后, CCL2表达高峰在第3 d, CXCL1的表达高峰在第10 d。而且, 在SNL后1 d脊髓中CCL2和CXCL1与正常动物相比无显著性差异[38,66], 提示它们可能不参与神经病理性疼痛的产生。相比之下, SNL后1 d *TNF-α* mRNA的表达升高达到高峰, 增加在5倍左右, 而在



神经损伤诱导脊髓水平趋化因子和受体表达增加。CCL2可从初级传入末梢释放作用于小胶质细胞上的CCR2受体。CCL2也表达于星形胶质细胞, 释放后作用于神经元上的CCR2受体。CXCL1和CXCR2分别表达于星形胶质细胞和神经元。全长的CX3CL1表达于初级传入末梢和脊髓神经元。剪切后的CX3CL1作用于小胶质细胞上它的唯一受体CX3CR1。CCL21表达于神经元, 作用于小胶质细胞上的CXCR3受体。趋化因子和受体介导的胶质细胞和神经元的相互作用参与神经病理性疼痛。

Nerve injury increases the expressions of chemokines and their receptors in the spinal cord. CCL2 is released from primary afferents and acts on CCR2 on microglia. CCL2 can also be expressed and released from astrocytes and act on CCR2 on neurons. CXCL1 and its major CXCR2 are distributed on astrocytes and neurons, respectively. The full length CX3CL1 is expressed in primary afferents and spinal neurons. CX3CL1 can be cleavaged and act on its sole receptor CX3CR1 on microglia. CCL21 is expressed on neurons and its receptor CXCR3 is expressed on microglia. The glial-neuronal interactions mediated by chemokines and their receptors contribute to neuropathic pain.

图2 趋化因子介导脊髓背角胶质细胞和神经元的相互作用并参与神经损伤诱导的神经病理性疼痛

Fig.2 Chemokines mediate glial-neuronal interactions in spinal dorsal horn and involve in nerve injury-induced neuropathic pain

3~10 d增加不到2倍。另外, 鞘内注射TNF-α能快速上调CCL2和CXCL1在脊髓星形胶质细胞的表达。SNL术前鞘内注射TNF-α的拮抗剂能部分抑制神经病理性疼痛的产生并抑制CXCL1的表达上调[66]。这些结果表明, 神经病理性疼痛中CCL2和CXCL1在星形胶质细胞的表达上调继发于TNF-α的表达上调。而TNF-α主要来源于小胶质细胞, TNF受体1(tumor necrosis factor receptor 1, TNFR1)表达于星形胶质细胞[10], 它们可能在神经病理性疼痛早期介导小胶质细胞和星形胶质细胞的相互作用。

3.2.3 CXCL1/CXCR2在神经病理性疼痛中的作用

CXCL1在外周组织参与疼痛的产生。爪底注射角叉菜胶能诱导爪底皮肤产生CXCL1[55]。爪底直接注射CXCL1可诱发机械性痛觉过敏[74], 小鼠踝关节注射CXCL1也能诱发机械性痛觉过敏[75]。

抑制CXCL1能有效缓解神经病理性疼痛。在神经损伤后10 d, 鞘内注射CXCL1中和抗体, 短暂和部分缓解了SNL诱导的热痛觉过敏和机械性触诱发痛。脊髓内注射表达*CXCL1* shRNA的慢病毒以长时间抑制CXCL1的表达, 有效阻断了SNL诱导的神经病理性疼痛。而且在术后3 d注射这种慢病毒长时间(2周以上)缓解了神经病理性疼痛[66]。另外, PSNL术前静脉注射CXCL1抗体延迟了PSNL诱导的热痛觉过敏和机械性触诱发痛的产生; 在术后4 d静脉给药或鞘内给药均能缓解PSNL诱导的神经病理性疼痛[67]。

SB225002是CXCR2的特异性拮抗剂。鞘内注射SB225002能阻断CXCL1诱导的热痛觉过敏。在SNL后3 d鞘内注射SB225002剂量依赖性地缓解SNL诱发的热痛觉过敏和机械性触诱发痛[66]。SB225002还能翻转小鼠后爪足底切开诱导的机械性触诱发痛[76]。重复腹腔注射SB225002长时间缓解PSNL诱导的神经病理性疼痛[77]。

3.3 CX3CL1/CX3CR1和神经病理性疼痛

3.3.1 神经损伤后CX3CL1/CX3CR1的分布和调控

CX3CL1是CX3CL家族的唯一成员, 有两种不同特性的形式: 一个是呈黏附特性的膜结合形式, 另一个是从细胞膜剪切下来并且有趋化性的可溶形式。两

种不同形式的CX3CL1能够以不同的空间特性和功能存在[78]。

在神经系统, 全长的CX3CL1表达于脊髓和DRG神经元[79-80]。神经损伤和炎症后, DRG和脊髓中的*CX3CL1* mRNA的总表达并没有改变[80]。相反, 脊神经结扎诱导DRG中的膜结合形式的CX3CL1显著减少[81], 表明神经损伤可能会导致该趋化因子的裂解和释放。在改良的脊神经结扎手术后, CX3CL1也在脊髓星形胶质细胞中被诱导产生[79]。

CX3CR1是CX3CL1唯一的受体, 在DRG表达于神经元周围的胶质细胞, 在脊髓主要存在于小胶质细胞[80]。而且, 在神经损伤和神经炎症诱导的神经病理性疼痛条件下, CX3CR1在小胶质细胞中表达上调[79-82]。

3.3.2 脊髓中CX3CL1/CX3CR1介导神经元和小胶质细胞相互作用 与CCL2/CCR2和CXCL1/CXCR2在脊髓的分布不同, CX3CL1和CX3CR1分别在脊髓神经元和小胶质细胞中表达, 提示CX3CL1和CX3CR1可能参与神经元和小胶质细胞之间的信息传递(图2)。

体外研究表明, 用谷氨酸刺激培养的皮层神经元诱导CX3CL1与细胞膜的裂解。另外, 谷氨酸处理3 h后的神经元的培养基对小胶质细胞具有显著的趋化活性, 而且被CX3CR1受体的中和血清所抑制, 表明裂解的CX3CL1通过CXCR1产生对小胶质细胞的募集作用[83]。而且一些蛋白酶, 如组织蛋白酶S(cathepsin S)、金属蛋白酶(metalloprotease, MMP) MMP-9和MMP-2都能裂解CX3CL1, 从而激活小胶质细胞[84-86]。

体内实验进一步证实了CX3CL1参与小胶质细胞的激活。首先, 神经损伤诱导脊髓小胶质细胞中CX3CR1的上调[79-81]。其次, 鞘内注射CX3CL1诱导的热痛觉过敏被小胶质细胞功能抑制剂米诺环素(minocycline)阻断[87]。第三, 鞘内注射CX3CL1激活了有丝分裂原激活的蛋白激酶P38, 而磷酸化的P38主要表达在大鼠脊髓的小胶质细胞中[81]。最后, 鞘内注射CX3CR1的中和抗体阻止了P38的激活[81]。这些结果支持CX3CL1通过结合其唯一受体CX3CR1激活P38, 从而诱导小胶质细胞的激活。

3.3.3 CX3CL1/CX3CR1和神经病理性疼痛行为 行为研究表明, CX3CL1诱导大鼠和小鼠产生显著的机械性痛觉过敏和热痛觉过敏[81,87-88]。在*CX3CR1*敲除小鼠中, CX3CL1不能诱导痛觉过敏的产生[89]。另外, CX3CR1或CX3CL1的中和抗体能够减弱CCI和SNL神经病理性疼痛模型的机械性痛觉过敏[81,87-88]。

3.4 其它趋化因子和受体

除了上述三对在神经病理性疼痛中研究较多的趋化因子和受体之外, 还有其它一些趋化因子和受体也参与神经病理性疼痛的调节。

CCL21, 又称二级淋巴组织趋化因子(secondary lymphoid-tissue chemokine, SLC), 外周神经损伤后表达于受损的DRG小神经元并转运到脊髓背角初级传入末梢。鞘内注射CCL21中和抗体能缓解SNL诱导的机械性触诱发痛。在CCL21缺失动物中, SNL不能诱导触诱发痛, 而且小胶质细胞中P2X4受体的上调受到抑制。相反, CCL21能诱导小胶质细胞P2X4的表达升高[90]。CCL21可识别受体CCR7和CXCR3, CCL21通过受体CXCR3诱导小胶质细胞趋化。在脊髓损伤后, CCL21能够通过激活丘脑的小胶质细胞来调节丘脑对疼痛的处理。对脊髓丘脑束进行电刺激可诱导丘脑CCL21水平上升。而且, 向丘脑注射CCL21能够瞬时激活小胶质细胞并且诱导产生疼痛相关行为[91]。因此, CCL21可能是中枢神经病理性疼痛中的另一个神经元–小胶质细胞间的信息传递分子(图2)。

CCL3又称巨噬细胞炎症因子(macrophage inflammatory protein-1alpha, MIP-1α), 在体外和体内均能调节巨噬细胞的功能。它能被免疫细胞和胶质细胞释放, 通过受体CCR1和CCR5调节神经系统炎症[92]。CCR5也是CCL4(MIP-1β)的受体。足底注射CCL3或CCL4诱导热痛觉过敏[93-94]。在DRG, CCL3与TRPV1共存于小神经元。而且, CCL3诱导DRG神经元钙离子内流、敏化TRPV1通道[94]。另外, CCL3在DRG与μ受体共存, 并使μ受体脱敏[94]。坐骨神经部分结扎可诱导损伤的神经内CCL3、CCL4、CCR1和CCR5的mRNA和蛋白表达升高, 它们表达于神经内的巨噬细胞和雪旺氏细胞。而且, 神经内注射*CCR1*和*CCR5*的siRNA缓解PSNL诱导的疼痛[92]。在脊髓, 坐骨神经部分结扎诱导*CCL3* mRNA在术后3~14 d上调, *CCR1* mRNA在12 h~7 d上调, 但*CCR5* mRNA表达没有变化[95]。坐骨神经部分结扎后鞘内注射CCL3中和抗体缓解热痛觉过敏和机械性触诱发痛。反之, 鞘内注射CCL3剂量依赖性地诱导热痛觉过敏和机械性触诱发痛[92,95]。

CXCL12, 又称基质细胞衍生因子-1(stronal cell-derived factor-1, SDF-1), 它的受体是CXCR4。CXCL12和CXCR4组成型地表达于DRG的神经元和卫星细胞, 并在抗病毒治疗引起的神经病理性疼痛中表达上调[96]。在单侧神经损伤后, 双侧DRG中CXCL12和CXCR4上调[97]。注射CXCR4拮抗剂能缓解病毒引起的神经病理性疼痛[96]。而且, 脊髓损伤诱导CXCL12和CXCR4在脊髓的表达增加[98]。

除此之外, CCL5和CCL7也被报道参与神经病理性疼痛。*CCL5*基因敲除的动物, 坐骨神经部分结扎诱导的痛觉过敏减轻, 而且损伤的神经中巨噬细胞浸润和炎症因子(TNF-α、IL-1β、IL-6、IFN-γ)表达减少, 而抗炎因子(IL-4和IL-10)表达增多[99]。坐骨神经部分结扎诱导CCL7在脊髓星形胶质细胞表达增多, 通过受体CCR2调节神经病理性疼痛[100](图2)。

4 结论与展望

周围神经系统和中枢神经系统中, 神经元可塑性在神经病理性疼痛的发展和维持中发挥重要作用。因此, 神经病理性疼痛治疗的发展一直以神经元作为靶点, 尤其是阻断神经传递。虽然某些药物, 如NMDA受体拮抗剂、选择性五羟色胺/去甲肾上腺素重吸收抑制剂、阿片类镇痛药、钠通道阻滞剂和三环类抗抑郁药, 它们在一些患者中起到镇痛的作用, 但往往只能短暂减轻疼痛。此外, 这些药物产生与中枢神经系统有关的副作用, 如恶心、镇静、嗜睡、眩晕以及镇痛耐受性等, 这些副作用极大限制了它们的广泛应用[101]。

近年来的研究发现, 一些胶质细胞功能调节剂和神经炎症的抑制剂对实验动物能产生镇痛作用, 如星形胶质细胞抑制剂(氟代柠檬酸)、小胶质细胞抑制剂(如米诺环素)、细胞因子抑制剂(如TNF-α抑制剂依那西普)、ATP受体拮抗剂(如P2X4和P2X7拮抗剂)、TLR拮抗剂(如TLR2和TLR4拮抗剂)、大麻素CB2受体激动剂, 以及抗炎细胞因子(如IL-10)等[102]。同时, 靶向趋化因子或趋化因子受体的药物, 包括单克隆抗体、趋化因子突变体和小分子拮抗剂也显示出镇痛作用。近年来, 制药公司一直致力于针对不同的趋化因子受体非肽类小分子拮抗剂的研究。10多种趋化因子受体拮抗剂如CCR1-5、CCR8、CCR9、CXCR1-4已经被开发。但在临床试验中, 大多没有显示出任何疗效, 其可能原因在于种属差异性、药代动力学性质和药物的组织分布差异。目前仅有一种小分子趋化因子受体拮抗剂, 抗CCR5分子已经获得FDA批准, 可结合其他抗逆转录病毒药物治疗HIV-1感染。仍有一些趋化因子受体拮抗剂应用于临床试验中, 将来可能成为有效的治疗方法[103]。

总之, 神经病理性疼痛的治疗是临床面临的挑战。越来越多的证据表明, 趋化因子(如CCL2、CXCL1和CX3CL1)通过调节神经元–胶质细胞的相互作用在神经病理性疼痛的产生和维持中起重要的作用。因此, 靶向趋化因子信号通路将可能成为治疗神经病理性疼痛的新方法。由于趋化因子系统的复杂性以及中枢内趋化因子的重要性, 开发具有中枢神经系统穿透性的受体拮抗剂, 并能靶向多种趋化因子受体将成为疼痛治疗的有效手段。

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