MUCOSAL IMMUNOL：PRKAR2A缺失可通过增加IFN刺激的基因表达和调节肠道微生物群来保护小鼠免受实验性结肠炎的侵害

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炎症性肠病(IBD)是一组慢性、复发性胃肠道炎症性疾病，主要包括克罗恩病和溃疡性结肠炎(UC)，尽管已证明遗传易感性、上皮屏障缺陷、免疫反应失调和肠道菌群失调等诸多因素参与了IBD的发生，但其发病机制仍未完全阐明。

蛋白激酶A(PKA)是一种丝/苏氨酸蛋白激酶，通过感知环磷酸腺苷(cAMP)信号并催化下游底物蛋白的磷酸化而充当信号转导器，在非活化状态下，PKA形成具有调节亚基(PKAR)二聚体和两个催化亚基(PKAC)的四聚体蛋白。PKAR存在四种调节亚型，即PRKAR1A、PRKAR1B、PRKAR2A和PRKAR2B，每种都由单独的基因编码并且具有不同的表达模式，其中PRKAR1A和PRKAR2A普遍表达，而PRKAR1B和PRKAR2B主要在脑和脂肪组织中表达。

已有研究证明通过PKA的信号传导在调节炎症状况（包括IBD）中发挥重要作用，然而，多年来对PKA信号的研究主要集中在其催化亚基上，PKA调节亚基的作用可能被忽视了。尽管有新的证据表明PKA调节亚基的重要性，但迄今为止尚未评估它们在结肠炎症中的作用，因此，该研究假设PRKAR2A是结肠组织中最丰富的PKA调节亚基，可能参与调节结肠炎症。

图片来源：https://doi.org/10.1038/s41385-021-00426-2

该研究证明了UC患者的结肠黏膜和葡聚糖硫酸钠(DSS)诱导的结肠炎小鼠的PRKAR2A磷酸化(p-PRKAR2A)降低，并阐明了PRKAR2A缺失在改善DSS诱导的结肠炎中的功能，表明PRKAR2A可能有助于磷酸二酯酶4(PDE4)抑制剂在IBD临床试验中的不令人满意的结果。

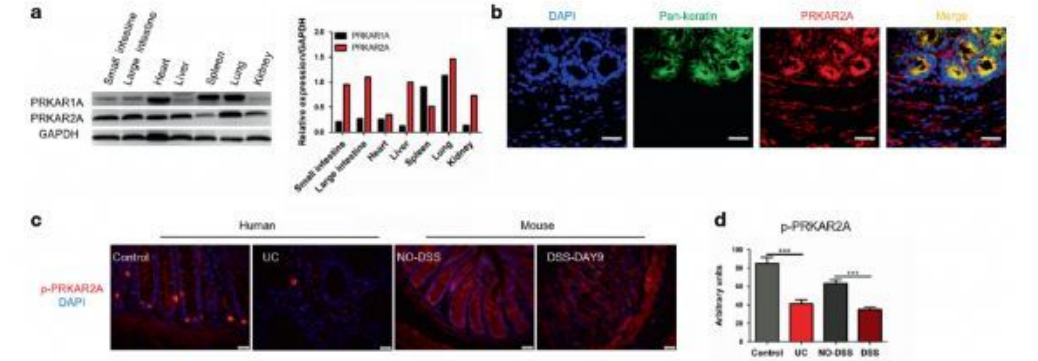

Fig. 1 Decreased phosphorylation of PRKAR2A in UC patients and mice with DSS-induced colitis. a The expression of PRKAR1A and PRKAR2A in different tissues of WT mice. **b** Colon tissues of WT mice were stained with DAPI (blue), antibody to Pan-keratin (green), and antibody to PRKAR2A (red). Representative immunofluorescent images and merged images are shown. Bar = 20 μm. **c** Representative images of immunofluorescent staining for p-PRKAR2A(ser99) in colon tissues collected from human and mice. Nuclei were counterstained with DAPI. **d** Semi-quantification of the level of p-PRKAR2A in human (UC patients ($n = 5$) and uninflamed controls ($n = 3$)) and mice (treated ($n = 5$) and untreated ($n = 5$) with DSS). Data shown in **a–d** are representative of two independent experiments. Data are presented as mean ± SEM. Student's *t* test was used to do the analysis. ***$P < 0.001$, bar = 50 μm.

UC患者和DSS诱导的结肠炎小鼠中PRKAR2A的磷酸化降低

图片来源：https://doi.org/10.1038/s41385-021-00426-2

该研究评估了不同小鼠组织中两个广泛表达的PKA调节亚基（PRKAR1A和PRKAR2A），PRKAR2A主要在肠、心、肝和肺中表达，而PRKAR1A主要在心、脾和肺中表达，表明PRKAR2A 是肠中主要的PKA调节亚基。为了表征结肠黏膜中PRKAR2A的细胞起源，用4',6-二脒基-2-苯基吲哚(DAPI)、抗泛角蛋白和抗PRKAR2A抗体对结肠组织进行免疫染色，发现PRKAR2A主要在结肠黏膜上皮区被检测到，并且UC患者结肠黏膜中PRKAR2A的磷酸化水平低于未发炎供者结肠黏膜。由于PRKAR2A在Ser99（第99位丝氨酸）上能被磷酸化，为了证实这一结果，向野生型(WT)小鼠施用2%DSS以诱导实验性结肠炎，DSS诱导的结肠炎是一种常用的模拟IBD临床病理的小鼠模型，研究者观察到磷酸化的PRKAR2A(p-PRKAR2A)在DSS治疗小鼠的结肠黏膜中比在未经治疗的同窝小鼠的黏膜中下调。这些数据表明p-PRKAR2A在UC患者和DSS诱导的结肠炎小鼠中下调。

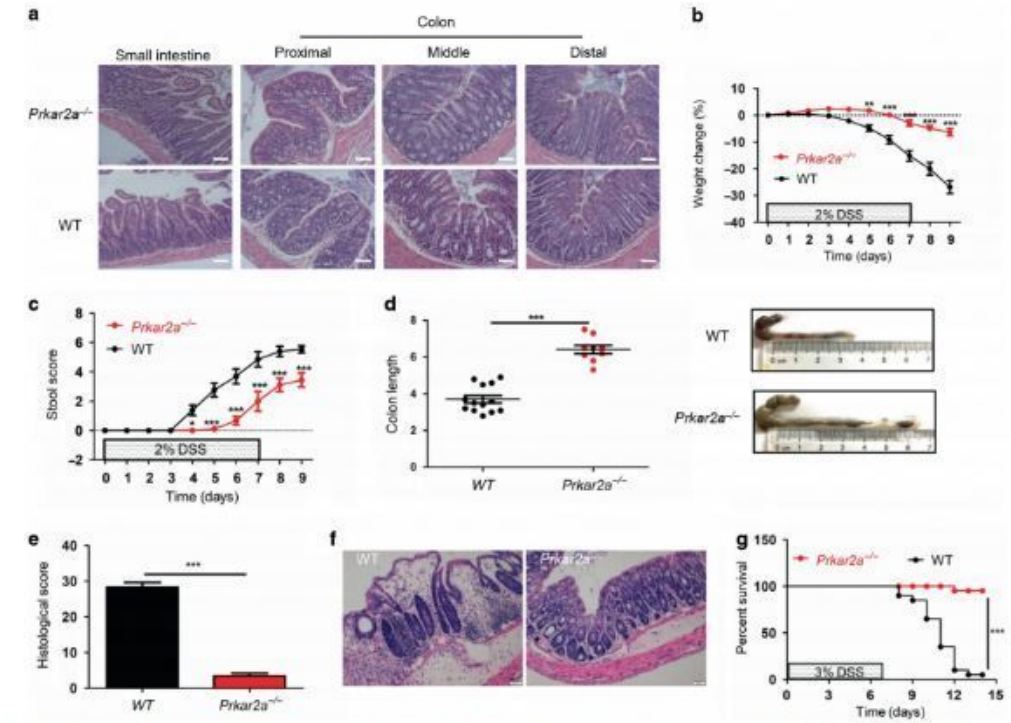

Fig. 2 Prkar2a deficiency provides protection against DSS-induced colitis. a Representative images of H&E-stained small and large intestine of *Prkar2a*$^{-/-}$ and WT mice. Scale bar = 50 μm. **b**–**f** Colitis was induced in WT ($n = 13$) and *Prkar2a*$^{-/-}$ ($n = 9$) mice by adding 2% DSS in the drinking water for 7 days, followed by 2 days of regular water. **b** Body weight was monitored over 9 days. Graph shows the percentage of body weight relative to initial body weight. **c** Stool score of DSS-treated WT and *Prkar2a*$^{-/-}$ mice were measured every day during colitis development. **d** Colons were removed and colon lengths were determined at day 9. **e** Histological scores of colitis. **f** Representative microscopic images of H&E-stained colons at day 9. Scale bar = 50 μm. **g** Survival curve of the WT ($n = 20$) and *Prkar2a*$^{-/-}$ ($n = 16$) mice challenged with 3% DSS. Log-rank (Mantel–Cox) test was used to do the analysis. $^{***}P < 0.001$. Data shown in **a**–**g** are representative of three independent experiments. Data are presented as mean ± SEM. Student's *t* test (**d**, **e**) or two-way ANOVA (**b**, **c**) was used to compare experimental groups. $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$.

PRKAR2A缺失可防止DSS诱导的结肠炎

图片来源：https://doi.org/10.1038/s41385-021-00426-2

为了阐明PRKAR2A是否能够调节结肠炎的发展，研究者使用了PRKAR2A缺陷型(Prkar2a-/-)小鼠，对WT和Prkar2a-/-小鼠接受DSS治疗并监测胃肠道疾病的临床症状，如体重减轻、腹泻和直肠出血，将粪便稠度和直肠出血作为粪便评分。用2% DSS治疗7天然后单独给予普通水2天后，相比于WT小鼠，Prkar2a-/-小鼠的体重减轻显著减少、大便评分较低以及和更少的结肠缩短，同时，用苏木精和伊红(H&E)染色的结肠组织的组织学分析显示，Prkar2a-/-小鼠的组织学评分显著低于WT小鼠，Prkar2a-/-小鼠出现了上皮/隐窝损伤和白细胞浸润减少。为了进一步确定Prkar2a缺失对DSS诱导的结肠炎的保护作用，该研究将DSS的浓度增加到3%并将观察时间延长到14天，结果表明Prkar2a-/-小鼠的死亡率在3% DSS处理后显著低于WT小鼠。这些结果表明PRKAR2A的缺失可以保护小鼠免受DSS诱导的结肠炎的侵害。

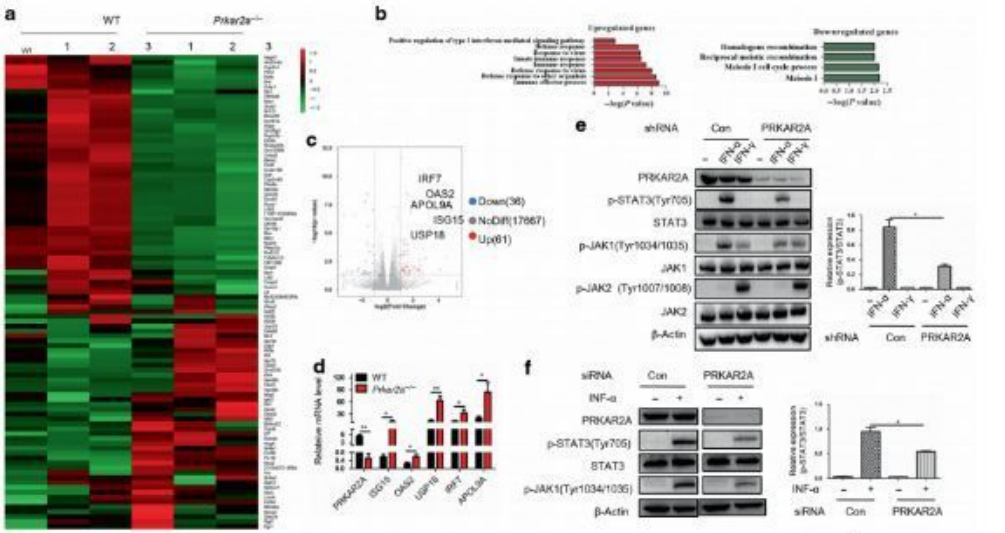

Fig. 7 PRKAR2A orchestrates type I IFN-induced signaling pathway. a–c Colon tissues from WT ($n = 3$) and *Prkar2a*$^{-/-}$ mice ($n = 3$) were subjected to RNA-seq. **a** Heatmap of RNA-seq data shows at least twofold upregulated or downregulated expression of genes in colon tissues from WT and *Prkar2a*$^{-/-}$ mice. **b** GO analysis of the upregulated and downregulated genes in colon tissues in *Prkar2a*$^{-/-}$ mice. **c** Volcano plot of the RNA-seq data. **d** Quantitative PCR analysis of PRKAR2A, OAS2, APOL9A, ISG15, IRF7, and USP18 mRNA levels in colon tissues from *Prkar2a*$^{-/-}$ ($n = 3$) and WT mice ($n = 3$). **e** PRKAR2A expression was stably knocked down in SW480 cells. The cells were then stimulated with IFN-α (200 ng/mL) or IFN-γ (50 ng/mL) for 30 min, and the indicated proteins were detected by western blot. **f** Expression of PRKAR2A was knocked down by siRNA in CCD841 cells. The cells were stimulated with or without IFN-α (200 ng/mL) for 30 min, and the indicated proteins were detected by western blot. Data shown in **d–f** are representative of three independent experiments conducted in duplicate. Error bars represent mean ± SEM. Student's *t* test was used to compare the experimental groups. $^{*}P < 0.05$, $^{**}P < 0.01$.

PRKAR2A协调I型干扰素(IFN-1)诱导的信号通路

图片来源：https://doi.org/10.1038/s41385-021-00426-2

为了阐明PRKAR2A消融后防止DSS诱导的结肠炎的潜在途径，该研究使用RNA测序(RNA-seq)对来自WT和Prkar2a-/-小鼠的结肠组织进行了全转录组分析。热量图显示了Prkar2a-/-和WT 小鼠之间不同的基因表达谱，而基因本体论分析发现，PRKAR2A失导致IFN-1介导的信号通路基因上调，与减数分裂和同源重组相关的基因下调。在17764个检查的表达标签中，61个基因的表达水平上调了两倍以上，而Prkar2a-/-小鼠中36个基因的表达水平比WT小鼠下降了两倍多，此外，在Prkar2a-/-小鼠中观察到IFN-1诱导的干扰素刺激基因(ISG)表达显著上调。随后，研究者采用定量PCR验证RNA的测序结果发现，与WT对照组相比，Prkar2a−/−小鼠结肠中ISGs的表达显著增加，这些数据表明PRKAR2A消融激活了结肠组织中经典的IFN-1信号通路。

该研究首次报道了PRKAR2A缺乏通过促进ISG表达和调节肠道微生物群来保护小鼠免受DSS诱导的实验性结肠炎，提供了PRKAR2A、IFN-1、肠道微生物群和肠道炎症之间的联系，并表明抑制PRKAR2A可能是治疗人类IBD的潜在治疗策略。

来源：生物谷

 京ICP备14047746号 京公网安备11010502026480
地址：北京市朝阳区潘家园南里5号（100021） 电话：010 - 67776816 传真：010 - 67781534 E-mail: calas@cast.org.cn
技术支持：山东瘦课网教育科技股份有限公司