



急性期蛋白CRP表达模式的建立依赖于远端增强子

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The accurate expression pattern of acute phase marker C-reactive protein depends on the distal enhancer

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人C反应蛋白(C-reactive protein, CRP)作为可溶性模式识别受体在宿主防御中发挥重要作用^[1-4]。人CRP主要由肝脏表达分泌,其在循环系统中的浓度,在感染或组织损伤时可迅速增加到数百倍,因此被认为是一种典型的炎症标志物^[5-7]。而对于人CRP在急性期内为什么会出现表达的暴发,已有研究普遍认为主要是受转录水平的调控,尤其是响应IL-6和IL-1 β 细胞因子的下游转录因子STAT3、NF- κ B和C/EBP- β ,可以协同诱导人CRP的急性期表达^[8-13]。由于这些急性期关键的转录因子结合位点主要集中在人CRP的近端启动子^[8,9,11,12],因此长期以来,研究者普遍认为CRP的急性期表达模式主要受近端启动子的调控。近年来兰州大学吉尚戎课题组发现CRP近端启动子DNA的去甲基化,可以增强C/EBP- β 的募集^[14],进一步地证实人CRP近端启动子对急性期表达的关键作用。

然而,一项人CRP在小鼠中的转基因研究暗示远端调节元件参与了CRP的急性期表达调控^[15]。增强子作为基因组远端调控元件,在调节基因时空表达中起关键作用^[16,17]。事实上已有报道发现,在CRP基因下游邻近区存在一个可以响应TNF- α 诱导的增强子^[18];然而这个增强子的作用并不很强,似乎也难以响应急性期典型的诱导因子IL-6和IL-1 β 的刺激。近期吉尚戎课题组发表在*J Biol Chem*的研究工作^[19],进一步揭示了一个命名为E1,位于CRP上游的远端增强子可以很好地响应IL-6和IL-1 β 的诱导,为明确CRP的急性期表达提供了重要的理论支持;同时,物种间E1的变异也可能是导致CRP不同表达模式的关键因素。

研究者首先利用3C技术检查了与CRP启动子相互作用的远端元件(图1(a)),并通过双荧光素酶报告基因、表观遗传修饰分析、染色质免疫沉淀等技术,初步表明远端元件E1可能是与CRP表达相关的增强子。进一步通过细胞系在原位水平对E1增强子进行敲除(图1(b))、压制(图1(c))以及激活(图1(d))实验,证实E1增强子是CRP急性期表达的必要元件。研究者通过序列删除以及染色质免疫沉淀实验表明,E1增强子的调节作用是由STAT3、C/EBP- β 和USF1/2共同介导的(图1(e))。前两个是由IL-6和IL-1 β 激活的经典转录因子,并且近端启动子同样具有它们的结合位点。相比之下,USF1/2在急性期会选择性地被招募到E1增强子而非启动子,预示E1增强子对CRP急性期表达的关键作用。

此外,E1似乎也决定了进化中CRP的表达差异。众所周知,与人相比,小鼠CRP只是一种非典型的急性期反应物,其循环水平在响应炎症损伤时仅增加2~3倍^[20,21]。通过E1增强子的进化分析,研究者发现源于小鼠的E1增强子显著缺乏增强子活化所必需的H3K27ac修饰(图1(f)),正好解释了为什么鼠源CRP表现较弱的急性期反应。有些出乎意料的是,小鼠E1和小鼠CRP启动子的调节功能似乎基本完整,因为它们与人源CRP启动子或人源E1一起作用时,它们仍然可以单独驱动明显但受损的急性期反应。研究者预测,小鼠CRP急性期的严重弱化,应该是E1和启动子突变丧失了二者间互作的结果。

吉尚戎课题组于今年早期发表的结果表明,尽管人类和小鼠中CRP的急性期反应表现出强烈的差异,但它们在急性炎症中的功能表型仍然是一致的^[22]。这种表达与功能不匹

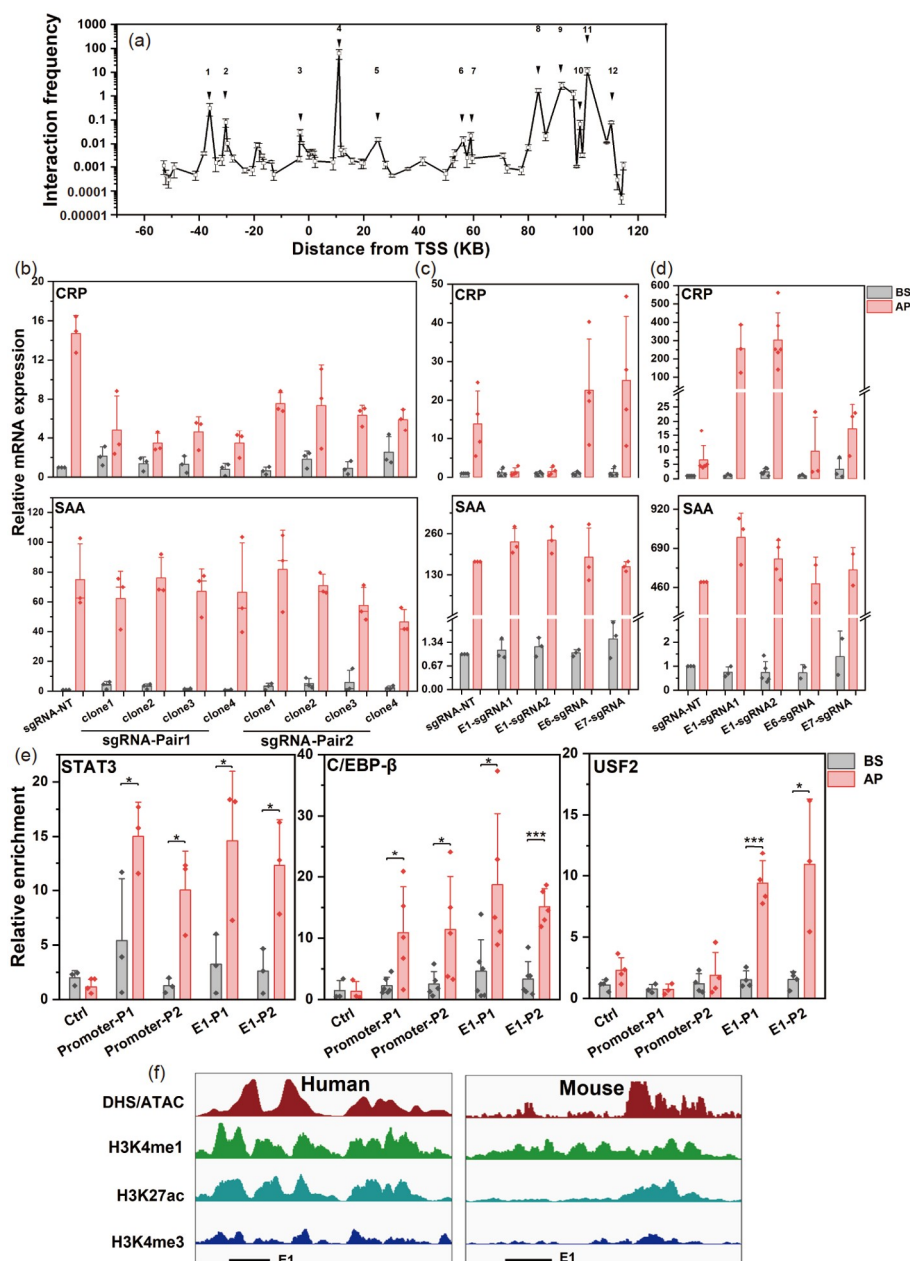


图 1 (网络版彩色)E1增强子的鉴定^[23]. (a) 染色质构象捕获3C技术检测与启动子互作的远端元件; (b~d) 在Hep3B细胞中, 利用Crispr-Cas9技术对E1增强子进行原位删除(b), CRISPRi对E1增强子进行压制(c), 以及CRISPRa对E1增强子进行激活(d), qPCR检测CRP以及急性期蛋白SAA的表达; (e) 染色质免疫沉淀检测关键转录因子在基线期(BS)和急性期(AP)时, 启动子和E1增强子的结合情况; (f) 人和小鼠中E1增强子表观遗传学的修饰情况

Figure 1 (Color online) Identification of the E1 enhancer^[23]. (a) Chromatin conformation capture (3C) to detect distal elements interacting with CRP promoter. (b~d) In Hep3B cells, *in situ* deletion of E1 enhancer using Crispr-Cas9 (b), active E1 enhancer through CRISPRi (c) and repressive E1 through CRISPRa (d) were performed. The mRNA expression of CRP and SAA was detected by qPCR. (e) Chromatin immunoprecipitation was performed to detect key transcription factors in baseline state (BS) and acute phase (AP). (f) Crucial epigenetic modifications of the E1 enhancer in humans and mice

配, 似乎很容易通过CRP隐藏活性的相应变化来解释^[23]. 由于在进化过程中非编码调控序列比编码序列受限制更少, 研究者提出调控序列中的非编码突变可能先于编码突变, 通过

优先改变物种间CRP的表达, 进而调整CRP隐藏活性的选择. 这些结果共同暗示了分子进化的级联反应, 其中远端调节元件中的突变导致表达改变, 进而启动了随后蛋白稳定性以及

功能选择。该研究对于CRP参与急性炎症反应给出了新的调控机制, 为未来针对CRP靶点进行临床干预提供了理论基础。

同时对于非编码区突变导致蛋白表达差异, 进而引发蛋白结构的改变以及功能的选择进化, 提供了有力的实证。

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