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生殖医学

·专题报道·

槲皮素对母胎界面免疫调节的影响

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［摘　要］ 母胎界面免疫失衡与不良妊娠结局密切相关，是生殖领域亟待解决的热点问题之一。随着中医药在生殖领域中的应用，发现槲皮素在菟丝子、桑寄生等常用补肾中药中含量丰富，可起到一定的妊娠保护作用。作为常见的黄酮类物质，槲皮素具有强大的抗炎、抗氧化、类雌激素等作用，可调节母胎界面免疫细胞（如蜕膜自然杀伤细胞、蜕膜巨噬细胞、T细胞、树突状细胞及髓源性抑制细胞）、绒毛外滋养层细胞及蜕膜基质细胞的功能及各细胞因子活性，通过减弱细胞毒性、减少组织细胞过度凋亡、抑制过度炎症反应等维持母胎免疫动态平衡。本文阐述了槲皮素对母胎界面各组成成分免疫调节过程中的作用及分子机制，以期为复发性流产等不良妊娠结局的治疗提供思路。

［关键词］ 槲皮素；母胎界面；蜕膜免疫细胞；滋养层细胞；蜕膜基质细胞；母胎免疫耐受；分子机制；综述
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Effects of quercetin on immune regulation at the maternal-fetal interface

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［**Abstract**］ The imbalance of immune homeostasis at the maternal-fetal interface is

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closely related to adverse pregnancy outcomes, so it has become one of the hot research topics in the reproductive field. Quercetin is rich in common TCM kidney-tonifying herbs such as dodder and lorathlorace, and has shown pregnancy protection function. As a common flavonoid, quercetin has powerful anti-inflammatory, antioxidant, estrogen-like effects; and it can regulate the functions of maternal-fetal interface immune cells (such as decidual natural killer cells, decidual macrophages, T cells, dendritic cells and myeloid-derived suppressor cells), exovillous trophoblast cells, decidual stromal cells, and the activities of their cytokines. Quercetin maintains the dynamic balance of maternal and fetal immunity by attenuating cytotoxicity, reducing excessive apoptosis of the tissue cells and inhibiting excessive inflammatory reactions. In this article, the role and molecular mechanism of quercetin in the immunomodulatory process of the maternal and fetal interface are reviewed to provide reference for the treatment of recurrent spontaneous abortion and other adverse pregnancy outcomes.

[**Key words**] Qercetin; Maternal-fetal interface; Decidual immune cells; Trophoblast cells; Decidual stromal cells; Maternal-fetal immune tolerance; Molecular mechanism; Review

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[**缩略语**] 自然杀伤细胞(natural killer cell,NK细胞);树突状细胞(dendritic cell,DC);人类白细胞抗原(human leukocyte antigene,HLA);吲哚胺2,3-双加氧酶(indoleamine 2,3-dioxygenase,IDO);肿瘤坏死因子(tumor necrosis factor,TNF);白介素(interleukin,IL);Toll样受体(Toll-like receptor,TLR);髓样分化因子(myeloid differentiation factor,MyD);核因子κB(nuclear factor-κB,NF-κB);腺苷一磷酸活化蛋白激酶(adenosine monophosphate-activated protein kinase,AMPK);NOD样受体热蛋白结构域相关蛋白(NOD-like receptor thermal protein domain associated protein,NLRP);脂多糖结合蛋白(lipopolysaccharide binding protein,LBP);促分裂原活化的蛋白激酶(mitogen-activated protein kinase,MAPK);血红素加氧酶(heme oxygenase, HO);Kelch样环氧氯丙烷相关蛋白(Kelch-like epichlorohydrin-associated protein,Keap);核转录因子红系2相关因子2(nuclear factor-erythroid 2-related factor 2,Nrf2);抗氧化响应元件(antioxidant reaction element,ARE);蛋白激酶B(protein kinase B,Akt);辅助性T细胞(helper T cell,Th细胞);调节性T细胞(regulatory T cell,Treg细胞);免疫球蛋白样转录物(immunoglobulin-like transcript,ILT);信号传导及转录激活因子(signal transduction and activator of transcription,STAT);趋化因子CXC亚家族受体(CXC subfamily receptor,CXCR);磷脂酰肌醇3激酶(phosphoinositide 3-kinase,PI3K);血清和糖皮质激素调节蛋白激酶(serum and glucocorticoid-regulated kinase, SGK);胱天蛋白酶(cysteine aspartic acid specific protease,caspase);B细胞淋巴瘤蛋白(B cell lymphoma protein,Bcl);Bcl-2相关X蛋白(Bcl-2 associated X,Bax)

复发性流产是生殖领域一直以来的研究重点,其中25%~50%为不明原因的复发性流产[1-2],母胎界面的免疫失衡是其主要诱因[3]。子宫蜕膜基质细胞、免疫细胞以及胎儿来源的滋养层细胞是母胎界面的主要组成成分,不同妊娠阶段各细胞及细胞因子之间进行有序的相互作用是维持

免疫动态平衡的前提,而细胞成分功能障碍及炎性细胞因子过度产生均会导致母胎排斥,引发流产等不良妊娠结局。目前,对于母胎界面免疫紊乱临床上常使用免疫调节药物,但其疗效及安全性仍存在争议[3]。因此,探寻更安全有效的治疗方式极为重要。中医药治疗以补肾为大法,在不良妊娠结局防治中具有广泛的应用。槲皮素是补肾安胎常用中药(如菟丝子、桑寄生等)的主要成分[4],现代药理学研究表明,其具有强大的抗炎、抗氧化、雌激素样等作用,作为天然化合物在诸多领域应用广泛,具有良好的免疫调节功能,可改善不良妊娠结局[5-6],且毒副作用较小[7]。因此,探讨槲皮素对母胎界面主要细胞的免疫调节机制,有望为临床防治母胎界面免疫异常引起的病理妊娠提供思路。

目前研究认为,胚胎着床过程与肿瘤侵袭相似[8],绒毛外滋养细胞的侵袭、子宫螺旋动脉的重塑及免疫耐受微环境的建立是构建母胎界面的关键过程[9]。研究显示,母胎界面的免疫失衡除了导致复发性流产外,还与不孕、先兆子痫、宫内发育迟缓等疾病的发生密切相关[10]。母体蜕膜组织中已发现存在多种免疫细胞,包括NK细胞、巨噬细胞、T细胞、DC等,除局部固有的免疫细胞外,滋养层细胞、蜕膜基质细胞及各种细胞因子也参与了母胎界面的免疫形成及调节过程[9]。本文围绕槲皮素对上述细胞及细胞因子的作用,阐述其参与母胎界面免疫调节的机制。

1 槲皮素调节蜕膜NK细胞

蜕膜NK细胞是母胎界面中主要的免疫细胞,以$CD56^{+}CD16^{-}$表型为主,其来源尚未明确,目前认为可能来自于外周血NK细胞、子宫内膜NK细胞及蜕膜的造血前体[10]。不同表型的NK细胞具有不同的功能特性,相较于具有高细胞毒性的$CD56^{-}$外周血NK细胞,$CD56^{+}$蜕膜NK细胞毒性较低,从而有利于维持母胎免疫耐受[11]。

蜕膜NK细胞与滋养层细胞密切相关,其表面的免疫球蛋白样转录物2、白细胞免疫球蛋白样受体B1等多种抑制性受体[12],可与相应HLA-G、HLA-C或HLA-E等相互结合[13],对胚胎起到保护作用。自然杀伤因子蛋白46、NK细胞2D是参与蜕膜NK细胞毒性反应的激活性受体[14]。研究表明,HLA-C与蜕膜NK细胞相结合后,可刺激γ干扰素,提高IDO的表达,促使外周血NK细胞向蜕膜NK细胞转化[15]。此外,IDO可下调自然杀伤因子蛋白46、NK细胞2D的表达,抑制外周血NK细胞毒性,并维持蜕膜NK细胞处于低细胞毒性状态[16]。体外实验结果显示,槲皮素可显著上调人脐带间充质干细胞共培养系统中的IDO活性,抑制TNF-α/γ干扰素诱导下外周血单核细胞的炎症反应[17]。但有临床研究发现,健康非妊娠及哺乳的成年女性补充槲皮素12周后,其外周血NK细胞活性无显著变化[18]。上述作用的差异可能与NK细胞所处的整体免疫环境相关。

2 槲皮素调节蜕膜巨噬细胞

巨噬细胞作为母胎界面第二丰富的蜕膜免疫细胞,在维持母胎界面免疫平衡中发挥重要作用。根据其分泌的细胞因子特性及功能特征,巨噬细胞可分为经典活化巨噬细胞(M1型)和交替活化巨噬细胞(M2型),M1型巨噬细胞与促炎相关,其表面高表达CD80、CD86,分泌TNF-α、IL-12、IL-23,且具有抗原提呈能力;M2型巨噬细胞表面高表达CD206、CD209、CD163,能分泌转化生长因子-β、IL-10、IDO等抗炎细胞因子[19],具有免疫抑制特性,因此可以更多地参与凋亡细胞清除及组织重塑。研究表明,巨噬细胞不同的极化状态由周围环境决定,并在不同环境信号下"重新极化"[20]。由于巨噬细胞具有良好的可塑性,研究者对其进行了大量体内外实验研究。

2.1 调节巨噬细胞数及活性

脂多糖作为常用的促炎试剂,可通过与孕鼠体内CD14、TLR结合[21],促进IL-2、TNF-α、单核细胞趋化蛋白-1等细胞因子的分泌,募集巨噬细胞,造成局部免疫失衡引发流产,而槲皮素可降低CD14的表达、抑制巨噬细胞的功能活性[22],预先服用槲皮素可使脂多糖作用的孕鼠流产率降低,减少子宫巨噬细胞数[23]。进一步观察脂多糖诱导的巨噬细胞的超微结构,发现槲皮素不仅可抑制脂多糖诱导的巨噬细胞的增殖,还能改变其形态、减少内质网数[24]。

2.2 调节巨噬细胞能量代谢及凋亡

免疫细胞的生命活动以能量代谢为基础,代谢障碍将直接影响免疫细胞的功能。M1型巨噬细胞多呈现糖酵解为主的促炎状态,M2型则呈

现以氧化磷酸化为主的抗炎特性，并可通过改变微环境进行代谢重编程[25]。研究者通过加入脂多糖和γ干扰素体外诱导巨噬细胞M1型极化，随后加入槲皮素，发现细胞糖酵解活性显著下降，三羧酸循环重编程，从而增加了巨噬细胞的抗氧化能力[26]，提示槲皮素可通过调节能量代谢对巨噬细胞M1型极化起到一定抑制作用。细胞焦亡属于炎症性细胞死亡方式，可释放炎性因子引发炎性反应[27]。体外实验表明，槲皮素可通过TLR2/MyD88/NF-κB通路和活性氧/AMPK通路抑制NLRP3炎症小体的激活，阻止巨噬细胞焦亡[28]，避免过度炎症刺激引发流产等不良妊娠结局。

2.3 调节巨噬细胞迁移

巨噬细胞过度浸润可引起局部炎症反应，破坏免疫平衡。槲皮素可阻止孕鼠子宫巨噬细胞向子宫内膜迁移，从而调节宫内巨噬细胞数及其分布，并能通过脂多糖-LBP/CD14信号通路，抑制巨噬细胞的激活[29]。深入研究发现，槲皮素通过抑制单核细胞趋化蛋白-1可阻断巨噬细胞向内膜区迁移[30]。此外，巨噬细胞的黏附及迁移与黏着斑激酶、桩蛋白、MAPK通路密切相关，槲皮素可破坏巨噬细胞骨架纤维状肌动蛋白，通过下调黏着斑激酶–桩蛋白通路、调控MAPK通路抑制脂多糖诱导的巨噬细胞黏附和迁移[24,31]。

2.4 调节细胞因子水平

在小鼠子宫组织中，脂多糖可诱导巨噬细胞产生TNF-α和一氧化氮，从而杀伤内皮细胞，抑制蜕膜血管的形成，引发不良的妊娠结局[30]。槲皮素可通过降低γ干扰素、TNF-α、一氧化氮水平，升高IL-4水平，发挥抗流产作用[22-23]，但其具体机制尚未明确。槲皮素作为阿司匹林补充剂可显著降低先兆子痫大鼠中NLRP3炎症小体表达，缓解先兆子痫症状[32]。体外实验表明，槲皮素可通过下调TLR4/MyD88[33]、TLR2/MyD88[28]抑制NF-κB发挥抗炎作用。HO-1具有免疫调节特性，Nrf2是细胞抗氧化过程中的关键转录因子，槲皮素可通过激活Keap1/Nrf2/ARE信号通路，诱导HO-1表达，抑制一氧化氮合酶及一氧化氮的产生，从而抑制炎症反应[34]。AMPK在维持细胞能量平衡中至关重要，且与炎性通路关系密切，槲皮素可通过提高脂多糖诱导的RAW264.7细胞内AMPK蛋白磷酸化水平[35]发挥抗炎功效。此外，槲皮素可通过调节MAPK通路[24]、下调Akt信号通路等抑制巨噬细胞的炎症反应[36]。

3 槲皮素调节蜕膜T细胞

T细胞根据其细胞膜表面标志物不同，主要可分为$CD8^+$T细胞及$CD4^+$T细胞，其中$CD8^+$效应T细胞可以直接通过滋养层细胞上的HLA-C或间接通过母体抗原提呈细胞识别胎儿抗原[37]；在$CD4^+$T细胞中，Th1细胞/Th2细胞/Th17细胞/Treg细胞的平衡是母胎界面免疫功能研究的热点之一，Th1细胞、Th17细胞通过分泌炎性因子促进炎症反应，而Th2细胞、Treg细胞则通过抗炎细胞因子维持母体对胎儿的耐受[38]。此外，滤泡Th细胞与滤泡Treg细胞间具有平衡关系，前者在B细胞的增殖分化中具有重要的作用，同时可抑制Treg细胞而促进Th1细胞、Th17细胞的发育；后者则对滤泡Th细胞过度增殖、B细胞反应和抗体产生具有抑制作用[39]。

3.1 调节T细胞成熟、分化

协同刺激信号对Th细胞的分化起到决定性作用，$CD80^+$和$CD86^+$是刺激Th0细胞向Th1细胞/Th2细胞转化的协同刺激因子[40]，阻断CD80、CD86有助于维持母胎界面的免疫耐受[41]，槲皮素还可通过降低$CD80^+/CD86^+$比值使母胎界面趋向于Th2细胞，从而有利于保护胚胎免受母体的攻击[40]。

3.2 调节细胞因子水平

循环中Th1细胞因子水平过高可导致早期妊娠丢失及植入期流产，T-bet是Th1细胞主要的转录因子，泛素特异性蛋白酶10可介导T-bet的去泛素化，并增加T-bet的表达，而槲皮素处理后该过程被抑制，有助于T-bet降解，进而抑制Th1型炎症反应[42]。妊娠大鼠的免疫应答会诱导炎性细胞因子如γ干扰素、IL-2、IL-6的产生，槲皮素可降低多氯联苯类化合物Aroclor1254所致的子宫内胚胎毒性，维持Th1细胞/Th2细胞的平衡[43]。槲皮素还可抑制子宫内膜组织分泌TNF-α，对子宫内膜细胞起到保护作用[44]。此外，槲皮素可降低血TNF-α和一氧化氮水平、升高IL-10水平，抑制TLR4/NF-κB信号通路，发挥抗炎作用，促进Th1细胞/Th2细胞的平衡趋于稳定，同时升高血清超氧化物歧化酶、谷胱苷肽过氧化酶水平，降低丙二醛水平，发挥抗氧化作用，从而保护胚

胎[45]。研究者将槲皮素与醋酸冰片联用,证实其能通过调节CD4⁺T细胞/CD8⁺T细胞平衡和γ干扰素/IL-4平衡发挥抗流产作用[46]。先兆子痫也与异常母胎免疫的激活密切相关,先兆子痫女性血清及胎盘的炎性因子水平高于正常妊娠女性[47],常规阿司匹林治疗中加入槲皮素能显著降低IL-6、TNF-α水平,从而加强对先兆子痫引起炎症反应的调节作用,并提高妊娠大鼠的活产率及后代体重[48]。此外,对于妊娠期在PM2.5中暴露的小鼠,槲皮素干预可以降低IL-6、IL-8等炎性因子水平,升高HO-1水平,恢复母胎界面免疫动态平衡[49]。

4 槲皮素调节蜕膜DC

DC虽只占蜕膜免疫细胞的1%~2%,但在免疫调节中具有重要作用[50]。经典DC通常分为两个主要亚型:髓样DC亚群和浆细胞样DC亚群,髓样DC能产生IL-12诱导Th1细胞分化,而浆细胞样DC可使优势转向Th2细胞,自然流产患者的蜕膜组织中髓样DC亚群比值显著增加[51]。此外,未成熟DC与成熟DC亦是常见的分类方式,前者更利于母胎界面免疫耐受的维持[52]。研究表明,槲皮素能有效减少成熟浆细胞样DC和髓样DC中炎性细胞因子、趋化因子的分泌[53],其强大的抗炎作用显著抑制了促炎细胞因子IL-1β、IL-6、γ干扰素、TNF-α和IL-12p70等分泌,维持免疫稳态[54-55]。此外,槲皮素处理后的DC可调节相关铁代谢基因,使细胞内铁含量下降,从而表现出较低的炎症特性[56]。

5 槲皮素调节蜕膜髓源性抑制细胞

随着对母胎界面的深入探索,发现髓源性抑制细胞包括未成熟的粒细胞和单核细胞前体细胞组成的异质性细胞群在小鼠子宫蜕膜中的细胞数仅次于子宫NK细胞。雌激素、孕激素、缺氧、HLA-G/ILT4、STAT3、CXCR2是促进髓源性抑制细胞在母胎界面分化和聚集的主要因素[57]。髓源性抑制细胞可通过抑制DC和T细胞增殖,促进Treg细胞产生,从而维持母胎界面免疫耐受[58]。研究表明,槲皮素可上调人脐带血来源髓源性抑制细胞的水平,促进体外T细胞抑制因子分泌,从而负向调节免疫反应,其机制与雌激素受体信号通路相关[59]。

6 槲皮素调节蜕膜绒毛外滋养层细胞

绒毛外滋养层细胞是母胎界面中胚胎来源的细胞,具有固定胚胎于母体蜕膜之上、参与血管重塑、免疫调节等重要作用。HLA-G和HLA-C是表达于绒毛外滋养细胞上的重要成分,可抑制NK细胞、T细胞的细胞毒性;前者还可增强Treg细胞的功能,诱导免疫耐受,且在滋养细胞的侵袭中起到关键作用[60-61]。上述过程的免疫失衡及滋养细胞功能障碍均会导致胚胎植入异常,从而引起流产、早产、先兆子痫等不良妊娠结局[61]。

缺氧/复氧可诱导人绒毛膜滋养层细胞HTR-8/SVneo处于氧化应激状态,谷胱甘肽水平及滋养细胞侵袭显著下降,加入3 μmol/L槲皮素后可明显增加还原型谷胱甘肽,抑制p38 MAPK及c-Jun氨基末端激酶活化,从而缓解HTR-8/SVneo细胞凋亡,对滋养细胞的生长及侵袭起到有益的作用[62-63]。此外,胎盘缺氧诱导的氧化应激与线粒体代谢障碍关系密切[64],槲皮素可在缺氧条件下改善线粒体功能,增强滋养细胞活性并抑制其凋亡,对改善复发性流产的结局有积极作用[6]。胎盘滋养细胞的侵袭高度依赖于糖酵解供能,槲皮素可抑制HTR-8/SVneo细胞对葡萄糖的摄取[65],并增加子宫中转化生长因子-β1 mRNA表达,从而防止滋养细胞过度浸润及淋巴细胞增殖,同时降低Th1型细胞因子含量,利于母胎免疫耐受的维持(见杨倩, 史万玉, 钟秀会. 中国畜牧兽医学会2010年学术年会交流"槲皮素对细菌脂多糖诱导流产孕小鼠子宫组织中转化生长因子β1的影响")。

7 槲皮素调节蜕膜基质细胞

蜕膜基质细胞作为母胎界面蜕膜细胞的主要组成成分,可分泌多种细胞因子调节免疫[66];在母胎界面免疫调节过程中主要发挥营养支持、抗原提呈及分泌细胞因子等作用[9]。研究表明,蜕膜基质细胞可通过分泌多种趋化因子及细胞因子,促进Th2细胞、Treg细胞、蜕膜NK细胞的分化,维持免疫耐受[9,67]。雌二醇可通过PI3K/SGK1信号通路抑制脂多糖/TLR4诱导的细胞凋亡,并促进Th2型抗炎反应以维持免疫耐受[68]。研究证明,槲皮素能增加妊娠小鼠的雌二醇水平,并可通过增加胰岛素样生长因子1、整合素

αvβ3和环氧合酶2的基因表达,降低caspase-3活性,提高妊娠糖尿病小鼠的子宫容受性、减少子宫组织细胞凋亡,且能激活Wnt/β-连环蛋白通路,改善妊娠结局[69]。

衰老蜕膜基质细胞的及时清除对维持免疫耐受十分重要,因为其积累后可通过刺激炎症及传播衰老导致不明原因的复发性流产[70],槲皮素可减少蜕膜基质细胞中衰老相关的β半乳糖苷酶阳性细胞数和衰老标志物的表达,同时增加蜕膜标志物胰岛素样生长因子结合蛋白1、催乳素和叉头框蛋白O1表达,诱导蜕膜化过程顺利进行[71]。此外,槲皮素可通过调控Bcl-2/Bax蛋白的表达抑制子宫内膜细胞凋亡,从而有利于胚胎着床[72]。但亦有研究提示,皮下注射50 $mg\cdot kg^{-1}\cdot d^{-1}$槲皮素会干扰子宫液体量及容受性,不利于胚胎着床[73]。

8 结 语

槲皮素作为天然黄酮类化合物,可作用于母胎界面的免疫细胞、蜕膜滋养层细胞及基质细胞等多种细胞,通过对细胞增殖、分化、代谢、凋亡、迁移以及各细胞因子的调节,维持母胎界面免疫动态平衡,且较少产生不良反应,抗炎、抗氧化、类雌激素等作用是其发挥疗效的机制。但对于槲皮素的研究,大多集中于心血管、肝脏、神经系统疾病及肿瘤等方面,其在生殖领域的研究仍有较大的探索空间,继续进行深入的临床及实验研究十分必要。目前,生殖医学领域相关研究主要以妊娠大鼠为模型,人鼠模型之间存在的差异是需要解决的难点之一[74]。总之,深入开展槲皮素在母胎界面免疫稳态调节中的作用研究或许对不明原因的复发性流产等病理妊娠的防治具有重要意义。

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