

· 专家论坛 ·

骨 吸 收 机 制

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骨吸收是破骨细胞(Osteoclast, OC)在成骨细胞(Osteoblast, OB)参与下侵蚀骨质的生物学功能,是OC发生、激活和与骨基质相互作用的吸收过程。影响因素包括由OC的产生和凋亡速度决定的细胞数量和功能状态。正常情况下,OC的骨吸收作用在维持稳定的血钙水平和骨重建中负有重要责任,而骨吸收过分活跃则是高转换型骨质疏松症的重要原因,因而阐明骨吸收机制对认识骨质疏松症的病理机制和推进防治骨质疏松药物的研究具有重要意义。目前有关骨吸收机制的研究主要涉及成骨细胞因素、破骨细胞因素和骨基质因素三个方面。

1. 成骨细胞因素

成骨细胞对OC的生长发育和功能激活有重要调控作用,并参与骨胶原的降解,在骨吸收过程中是不可缺少的因素。

(1)膜接触机制 骨髓造血干细胞与骨碎片(含OB)共培养可发育形成OC,如用半透膜分隔培养,却不能形成OC^[1-3]。经集落刺激因子(Colony-stimulating factor, CSF)预处理的骨髓干细胞与OB共培养,在1,25(OH)₂D₃刺激下可明显增加OC的成熟率^[4]。由新生鼠长骨取材经10~15 min培养细胞贴壁,去掉未贴壁细胞(含OB、基质细胞等),这种相对纯化的OC在甲状旁腺激素(Parathyroid hormone, PTH)、甲状旁腺激素相关肽(Parathyroid hormone-related protein, PTHrP)、肿瘤坏死因子(Tumor necrosis factor, TNF α)作用下在骨片上不能形成吸收小凹,而延长贴附时间(OB贴附增多)或加入一定量OB培养,在骨片上可形成吸收小凹^[5]。上述实验表明OB对OC的分化成熟和发挥骨吸收功能是一种必要的条件,这种必要的因素是通过OB-OC膜接触机制而实现的^[6]。

现已知道OB可表达一对特异分子——“破骨细胞分化因子”(Osteoclast Differentiation Factor, ODF)和“OC发生抑制因子”(Osteoclastogenesis inhibitory fac-

tor, OCIF)^[7,8]。前者整合在OB细胞膜上,通过膜接触机制与OC表面RANK(Receptor-activator of NF κ B)结合并活化NF κ B,引起胞浆和核内Ca变化或通过JNKs(Jun N-terminal Kinases)催化c-Jun磷酸化的作用,调节基因表达促进OC后期的分化成熟。而后者为ODF的可溶性受体,能特异阻断ODF对OC产生的刺激作用,又称骨保护蛋白(Osteoprotegerin, OPG)。OB细胞在无诱导条件下以OCIF表达占优势,局部增加刺激因素(包括PTH、PGE₂、1,25(OH)₂D₃、IL-1、IL-6、IL-11、TNF等)可抑制OCIF、促进ODF的表达,从而诱导OC的形成^[9]。

(2)介导机制 一些骨吸收促进因子对OC的激活通过OB的介导作用,如PTH、PGE₂、1,25(OH)₂D₃、IL-1、IL-6、IL-11、TNF等,其介导机制与调节局部IL-6、IL-11浓度增加和OPG/ODF的比值降低有关^[10-12]。IL-1、TNF刺激OB分泌IL-6、IL-11,后者诱导OB分泌ODF,从而诱导OC的分化成熟和刺激骨吸收功能。雌激素对OB介导IL-1、TNF等因子促IL-6分泌的效应有抑制作用,这种介导效应也可被IL-1拮抗剂、IL-1受体拮抗剂(IL-1ra)、TNF拮抗剂、TNF结合蛋白(TNF-bp)、IL-6抗体等阻断。IL-1、TNF在卵巢切除和绝经后诱发的骨丢失中被认为是关键致病因素,其机制与刺激OB和骨髓基质细胞生成M-CSF、GM-CSF增多以及OPG/ODF比例失衡有关。

2. 破骨细胞因素

骨吸收是破骨细胞的主要生物学功能,OC的活化包括OC向矿化骨表面聚集、附着、形成吸收器官和合成分泌酶和酸等。OC在骨基质表面的附着是骨基质蛋白成分与OC膜作用的结果,使细胞附着,形成由皱褶缘和透明带组成的骨吸收微环境区。皱褶缘内含短束状肌动蛋白细丝;透明带是一透明封闭膜,通过含长束状肌动蛋白细丝的足体与骨基质相连。OC胞浆中含丰富的囊泡,内含蛋白酶,在骨吸收时从皱褶缘转运到吸收微环境区,同时富含质子泵的囊泡膜融合到皱褶缘。

(1)吸收区酸化 H⁺由皱褶缘质子泵(V-H⁺-

ATP酶)产生,使OC骨吸收区造成酸性环境,PH达1.5~4.8,甚至更低^[10,11]。细胞内离子和酸碱平衡由钠泵(Na⁺-ATP酶)和碳酸酐酶Ⅰ(Carbonic anhydrase I,CAⅠ)维持^[12]。H⁺溶解羟磷灰石,分离出Ca²⁺、C₁₀(PO₄)₆、(OH)₂+3H⁺→6HPO₄²⁻+10Ca²⁺+2H₂O)。PTH、1,25(OH)₂D₃、PGE₂等因子可促进碳酸酐酶的活性,促进骨吸收。CAⅡ基因突变是骨硬化症原因之一^[13]。降钙素对OC、CAⅠ有抑制作用,并可抑制PTH刺激OC吸收区酸度的增加,使PH值上升到7.0~7.3。乙酰唑胺、奥美拉唑(Omeprazol)等抑制CAⅡ,可以抑制骨吸收。

(2)OC合成,分泌多种溶酶体酶降解胶原等有机骨基质,形成骨吸收小凹。这些酶主要有:组织蛋白酶K(Cathepsin K,CK),属溶酶体胱氨酸蛋白酶的一种,在OC前体细胞有大量CKmRNA表达,是OC分化的标志,CK降解Ⅰ型胶原、骨粘素等,CK基因突变可致OC骨吸收功能障碍^[17,18]。基质金属蛋白酶(Matrix metalloproteinase,MMP),是一类锌依赖性内肽酶,如胶原酶(MMP-1、MMP-8、MMP-13、MMP-19)、明胶酶(MMP-2、MMP-9)、基质溶解素(MMP-3、MMP-10、MMP-11)、膜型MMP(MT1-MMP、MT2-MMP、MT3-MMP等)和其他MMP。在OC迁移和骨吸收中MMP-9、MT1-MMP起很重要的作用^[19,20]。

(3)多种细胞因子促进OC的形成和功能。目前认为ODF和CSF是OC发生的基本条件。M-CSF、GM-CSF在骨髓造血干细胞分化为OC前体细胞中起至关重要的作用,M-CSF基因突变小鼠导致OC缺乏,形成骨硬化(OP/OP小鼠),外源性M-CSF可予纠正^[21]。IL-1、TNF促进骨髓基质细胞和成骨细胞分泌M-CSF、GM-CSF,加速OC前体细胞的生成,并增加生存时间^[22]。ODF诱导OC前体细胞发育成熟^[23]。IL-6是促进OC形成和刺激OC活化的主要因子,绝经后骨质疏松妇女血清中的IL-6水平升高,IL-6抗体可抑制去卵巢造成的OC增多^[11,25]。研究发现此效应是IL-6促进ODF^[26]表达。此外,IL-11、EGF、PDGF、PTH、PTH-rP等均能诱导OC前体向OC的分化,ODF上调表达可能是其作用的基本途径。

3. 骨基质因素

骨基质对OC的活化和骨吸收功能具有诱导和调控作用,这种作用是通过OC膜整合素(Integrin)实现的。骨基质中含有多种多肽蛋白成分,如Osteocalcin(BGP)、Osteopontin(OPN)、Fibronectin(FN)、Bone Sialoprotein(BSP)、Vitronectin(VN)和TGF-β等。这些

成分由成骨细胞合成分泌,储存于骨基质中^[1,27]。BGP对OC移行有趋化作用,VD₃可减低OC对BGP的反应能力^[28]。OPN、VN、FN、BSP等分子含RGD(精氨酸-甘氨酸-天冬氨酸)序列,与OC膜αβ₃和αβ₁整合素结合。整合素是一类α和β两种亚基组成的跨膜异二聚体,较长的胞外段与细胞外基质蛋白RGD顺序或GRGD(S)(甘氨酸-精氨酸-甘氨酸-天冬氨酸)顺序结合,使细胞锚着于骨基质,较短的胞内段与细胞骨架和信号传递分子结合,形成细胞外基质-整合素-细胞骨架的信号传递分子复合物,细胞外信号通过这一复合物传递到细胞核内,影响OC基因表达及功能表达^[29]。骨基质蛋白与OC的结合是在OC透明区,透明区含许多圆锥形足突小体(Podosome),足突小体中心轴含由肌动蛋白微丝、α-actinin、凝溶胶蛋白(gelsolin)和伞蛋白(fimbrin)组成的更小的圆锥形小轴,小轴顶端被纽蛋白(Vinculin)、talin环绕。这些骨架蛋白(主要是talin和α-actinin)与整合素β亚基结合^[31]。有些因子可抑制骨基质蛋白的合成,如1,25(OH)₂D₃抑制BSP合成,而有些因子如地塞米松可促进其合成,从而影响OC的附着和功能。c-src基因在OC发生、附着和骨吸收中十分重要,c-src基因敲除小鼠的OC不能形成皱褶缘,是骨硬化发生的主要原因^[31,32]。抑制成骨细胞合成骨基质蛋白的分子和αβ₃抗体等一类药物可减弱骨基质对OC的诱导与附着,与骨质疏松防治药物研究的方向之一。

总之,围绕OC分化发育、黏附、激活和骨吸收调控等环节的骨吸收机制研究正受到广泛重视。ODF/OPG和MMP/TIMP是OB和骨基质分子作用的分子基础,相关研究的进一步深入必将推进骨吸收分子机制的阐明和骨质疏松症等代谢性骨病的防治。

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