破骨细胞的形成、功能及细胞因子的调节

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破骨细胞为多核巨细胞，其生成及其在骨吸收中的作用受细胞因子和细胞间相互作用所调节。在许多常见病中，如骨质疏松症、骨炎性变形、无菌性移植松脱、原发性骨肿瘤和恶性肿瘤骨转移等都可见到破骨细胞的活化。本文就细胞因子对破骨细胞生成、分化及其在骨吸收中的调节作用作一综述，主要讨论 1)破骨细胞的特征；2)破骨细胞的起源；3)破骨细胞吸收骨质的过程；4)细胞因子对破骨细胞生成及骨吸收的作用。

1 破骨细胞的特征

破骨细胞(osteoclast)这一名词来源于希腊文'osteon'(骨)和'klao'(破坏)的组合。破骨细胞的特征为多核，其腺粒体呈多形性。降钙素受体，组织蛋白酶 K、碳酸酐酶 Ⅱ、抗酒石酸磷酸酶和 vitronectin 受体是破骨细胞的标志物。

降钙素受体(calcitonin receptor，CTR)

除鸟类外，各种哺乳动物的破骨细胞均有 CRT。在胚胎发生过程中，CTR 首先出现在非增殖性破骨细胞的前体细胞[1]。现在 CTR 已成为识别破骨细胞前体细胞的标记[2~4]。降钙素通过作用这两种细胞的 CTR 抑制骨吸收[5]，但却增加破骨细胞的生存期。

Vitronectin 受体(VR)

破骨细胞表达两种粘合蛋白，即 VLA-2 和 V3VR，前者为胶原和层粘连蛋白受体[20]，后者为细胞外基质受体[21]。两种抗 VR 抗体(23C6 和 13C2)能识别 VR 亚单位中不同的抗原决定簇，可将破骨细胞或其前体细胞与单核巨噬系统细胞区分开来[2,6,7]。

组织蛋白酶 K(cathepsinK，CK)

Li 等人[8]发现破骨细胞有 CK，属溶酶体胱氨酸蛋白酶的一种。破骨细胞前体细胞表达大量的 CKmRNA 和蛋白，提示这种酶的存在是破骨细胞分化的标记[9]。在破骨细胞性骨吸收中，CK 主要降解有机基质成分如胶原、骨粘素等[10~12]。

碳酸酐酶 Ⅱ (carbonic anhydrase Ⅱ，CA Ⅱ)

CA Ⅱ是一种锌金属酶，催化 CO_2 的可逆性水化反应。破骨细胞胞浆表达丰富的 CA Ⅱ[13]。应用荧光素标记 cDNA 探针和共聚焦激光显微镜原位杂交技术，发现呈吸收状态的破骨细胞表达高水平的 CA Ⅱ mRNA，而呈非吸收状态的破骨细胞几无表达[14]。Zheng 等人[22]报道降钙素能减少 CA Ⅱ mRNA 阳性的单核破骨细胞前体细胞的数量。由于 CA Ⅱ主要通过逆转 CO_2 成 H^+ 使无机基质降解，因此，CA Ⅱ基因突变会造成骨硬化症[15]。

抗酒石酸酸性磷酸酶(Tartrate resistant acid phosphatase，TRAP)

TRAP 已广泛用作破骨细胞特异性标记物。有趣的是 TRAP 缺失的小鼠只出现轻度的骨生长异常，提示 TRAP 在骨吸收中并非必需的，不是维持破骨细胞功能的活性酶[16]。

2 破骨细胞起源

一般认为破骨细胞来源于单核巨噬细胞系统的造血干细胞[17,18]。由于未能分离破骨细胞的前体细胞，因而对破骨细胞分化过程的系列细胞还未能准确确定[5]。最近研究表明破骨细胞来自 $CD34^+$ 的骨髓干细胞。Matayoshi 等人

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(1996)报道[19]CD34⁺细胞在粒细胞克隆刺激因子(G-CSF)作用下能分化形成造血细胞,并进入周围血循环。这些造血细胞在1,25$(OH)_2D_3$、白细胞介素(IL)-1、-3和粒细胞巨噬细胞克隆刺激因子(MG-CSF)的诱导下,能演变为成熟的破骨细胞,其特征包括有多核、有TRAP和$PP60^{c-src}$酪氨酸激酶活性以及CTR和VNR因基表达。此外,这些成熟的破骨细胞还表达CTR的异构体,具有在灭活骨片上形成吸收小凹的能力。CD34⁺细胞形成破骨细胞的过程不需要基质细胞的参与[19]。

3 破骨细胞吸收骨质的过程

破骨细胞有两种状态,吸收状态和非吸收状态。用共聚焦显微镜观察吸收状态的破骨细胞在二维图像中为圆型,在三维图像中为拱形。而非吸收状态的破骨细胞的二维图像为不规则状;三维图像为扁平状。吸收状态的破骨细胞在尖端部形成一边界清楚的骨吸收区,并分泌H^+和溶酶体酶进入该区使骨质降解[25]。整个骨吸收过程可分为破骨细胞的粘附,极化及随后的脱离和移至新的吸收部位[21]。在这一过程中,破骨细胞的胞浆在靠血流的一侧形成断续的底侧区,而在骨表面的一侧形成尖端区。尖端区中央部由多个指状皱折组成,称为皱折缘,内含短束状肌动蛋白细丝。破骨细胞与底物相互作用是在一称为封闭膜的特殊圆型尖端区进行行。封闭膜呈均质状,除游离的核糖体外,并无其它细胞器,因而又称透明带。封闭膜通过足体与骨表面相连,足体内含有长束状的肌动蛋白细丝[25]。肌动蛋白细丝穿越细胞膜,通过粘合蛋白与细胞外基质相连。足体的形成使细胞表面与骨质的连接稳固,破骨细胞将含蛋白酶和H^+的小泡从皱折缘转运到与骨表面之间形成的独立区内,造成该区的骨吸收。

破骨细胞分解无机骨基质主要与其产生H^+有关[57]。H^+由CA Ⅱ产生,并由H^+、Na^+ ATP酶或H^+ ATP酶转运,H^+通过下列反应式溶解矿化基质。

$Ca_{10}(PO_4)_6(OH)_2 + 8H^+ \rightarrow 6HPO_4^{-2} + 10Ca^{++} + 2H_2O$ 所形成的Ca^{++}和磷酸经细胞外液再进入血流。

破骨细胞分泌多种溶酶体酶降解有机骨基质,主要有组织蛋白酶K、L、B和N。组织蛋白酶K是降解胶原的主要成份[9]。破骨细胞还可分泌其它金属蛋白酶[23]和尿激酶型纤溶蛋白酶原激活物(uPA)等酶[24]。

在骨吸收过程中,骨的降解产物如降解的Ⅰ型胶原通过皱折缘进入破骨细胞,然后以小泡形式运送至基膜区,再通过转胞作用排出胞外[25,26]。

4 细胞因子在破骨细胞生成和骨吸收中作用

调节破骨细胞功能的局部因子主要来源于骨母细胞和基质细胞,以及骨髓中各种免疫细胞[27]。

用GM-CSF和$1\alpha,25(OH)_2D_3$处理人骨髓培养细胞能刺激破骨样多核细胞及其前体细胞的形成,但不影响其后的分化[17,28,29],提示GM-CSF可能不是破骨细胞生成的关键因子[30]。

M-CSF在小鼠骨母细胞和脾细胞共培养系统中对破骨细胞前体细胞的增殖和分化是必不可少的。抗M-CSF抗体或抗M-CSF受体抗体均能抑制破骨样细胞的形成[48]。M-CSF能使接种在灭活骨上的人骨髓细胞生成增多[50],因此认为M-CSF在破骨细胞生成中更为重要。

IL-1α和β都是骨吸收的强刺激剂[31~33],能增加骨髓培养中的破骨细胞形成[34]。用IL-1受体拮抗物阻断IL-1,能防止去卵巢大鼠的骨质丧失,其作用与使用雌激素相似[35]。另外,抑制IL-1活性后,可使从绝经期妇女获取的单核细胞上清液的骨吸收能力降低[36]。在骨髓细胞和基质细胞株(ST2)共培养时,细胞因子中只有M-CSF和IL-1能延长TRAP阳性的破骨样多核细胞的寿命,说明这两种因子对破骨细胞的存活是十分重要的[37]。

IL-6 为另一种细胞生长因子，其功能与 IL-1 相似。IL-6 能刺激正常人和骨 Paget's 病人长期骨髓培养中的破骨样细胞的形成[38,39]，而抗 IL-6 抗体则能防止因去卵巢造成的破骨细胞增多[40]。用 IL-6 反义寡核苷酸阻止 IL-6 mRNA 转录，也可使人骨巨细胞瘤中的多核巨细胞骨吸收作用受到抑制[41]。

IL-11 由间叶来源的骨髓细胞产生。它也具有诱导破骨细胞形成的能力。抗 IL-11 抗体能中和 IL-11，使由 $1\alpha,25(OH)_2D_3$、PTH、IL-1 或 TNF 引起的破骨细胞生成作用受阻[42]。

IL-10 是一种细胞因子合成抑制因子，抑制破骨细胞的分化和减少其生成[43]。IL-4 和 IL-13 通过抑制骨母细胞合成环氧合酶依赖性前列腺素(PG)而起到抑制骨吸收的作用[44]。

IL-17 最初从 T 细胞杂交瘤克隆所得，在小鼠造血细胞和原代骨母细胞混合培养中，用 IL-17(1pg-10ng/ml)处理 6 天，能诱导多核破骨细胞形成，这些细胞表达 TRAP 活性、CTR，并能在牙片中形成吸收小凹。用 IL-17 处理后，破骨细胞形成的数量与培养细胞释放 PGE2 的量成正比，PGE2 是由骨母细胞释放的，因而，IL-17 作用于骨母细胞合成 PGE2，后者促使破骨细胞前体分化形成成熟的破骨细胞[59]。

IL-18 由骨母细胞产生，重组 IL-18 抑制破骨样细胞形成。用抗 GM-CSF 抗体中和，则能解除 IL-18 对破骨样细胞的抑制，说明在脾或骨髓造血细胞与骨母细胞共培养的早期，IL-18 是通过使培养细胞产生 GM-CSF 而抑制破骨样细胞的形成[58]。

EGF 和 PDGF 能使破骨细胞前体细胞的数量增加，致使破骨细胞数量增加而刺激骨吸收[45,46]。在小鼠颅骨培养中，TGFβ 诱导 PG 合成而使破骨细胞生成增加，但在长期骨髓培养中，当 PG 的作用变得不重要时，TGFβ 则通过阻断破骨细胞前体细胞的融合，抑制破骨细胞生成[47]。应用上下两个小室的实验模型证实，TGFβ 对破骨细胞具有趋化作用，能吸引破骨细胞从上室通过微孔滤膜移动至含 TGFβ 的下室[49]。

甲状旁腺素相关蛋白(PTHrp)的 N-末端与甲状旁腺素(PTH)结构同源，能诱导破骨细胞前体细胞分化形成破骨细胞[51]，对 PTHrp 的 C 末端的作用则有所争论。一些研究表明 PTHrp 的 C 末端是破骨细胞性骨吸收的强抑制剂[52,53,55]，而有报道观察到 PTHrp 的 C 末端诱导小鼠骨髓培养中的破骨细胞生成[54]。

骨形成蛋白(BMP)由成骨细胞产生并保留在骨组织中。BMP 能刺激破骨细胞的形成及其骨吸收活性[56]，其作用可能是间接通过基质细胞作用于成熟的破骨细胞或直接刺激破骨细胞前体细胞。

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