

DOI: 10.13376/j.cbls/2021055

文章编号: 1004-0374(2021)04-0518-09

线粒体相关蛋白与股骨头坏死关联性研究进展

乌日莎娜¹, 何伟^{2,3}, 魏秋实^{2,3*}

(1 广州中医药大学第三临床医学院, 广州 510407; 2 广州中医药大学第三附属医院
关节骨科, 广州 510240; 3 广州中医药大学骨伤科研究所, 广州 510240)

摘要: 骨组织细胞凋亡和自噬学说越来越被认同, 逐渐成为人们研究股骨头坏死发病机制的焦点。在刺激因素作用后, 线粒体功能障碍, 影响自噬, 诱发骨组织细胞凋亡, 最终导致股骨头坏死的发生和发展。这一关联的发现对进一步阐明本病的发病机制具有重要意义, 同时检测线粒体相关蛋白可能对股骨头坏死早期诊断和预后判断有较高的参考价值, 也为未来调控相关蛋白表达进而影响股骨头坏死进展提供理论依据。

关键词: 线粒体功能障碍; 细胞凋亡; 自噬; 线粒体相关蛋白; 股骨头坏死

中图分类号: R681 **文献标志码:** A

Research progress on the correlation between mitochondrial related protein and osteonecrosis of the femoral head

WU Ri-Sha-Na¹, HE Wei^{2,3}, WEI Qiu-Shi^{2,3*}

(1 Third School of Clinic Medicine, Guangzhou University of Chinese Medicine, Guangzhou 510407, China;
2 Department of Joint Orthopaedics, the Third Affiliated Hospital of Guangzhou University of Chinese Medicine,
Guangzhou 510240, China; 3 Institute of Orthopaedics of Guangzhou University of Chinese Medicine,
Guangzhou 510240, China)

Abstract: The theory of apoptosis and autophagy in bone tissue has been recognized by more and more people and has gradually become the focus of research on pathogenesis of osteonecrosis of the femoral head (ONFH). After the action of stimulating factors, mitochondrial dysfunction induces autophagy and apoptosis of bone tissue cells, and eventually leads to the development and progression of ONFH. The discovery of correlation is of great significance to elucidate the pathogenesis of ONFH. Meanwhile, detection of mitochondrial related proteins may play an important role in early diagnosis and prognosis of ONFH. It also provides theoretical basis for regulating the expression of related proteins to affect progress of ONFH in the future.

Key words: mitochondrial dysfunction; apoptosis; autophagy; mitochondrial related protein; osteonecrosis of the femoral head

股骨头坏死(osteonecrosis of the femoral head, ONFH)是一种由于各种原因引起股骨头内血供受损或中断, 骨细胞死亡, 最终导致股骨头结构改变, 甚至塌陷的好发于青壮年的难治性、致残性骨科疾病^[1-2]。据统计, 美国新发ONFH 病例以每年1万到2万例的速度增长, 且好发于30~60岁的中青年^[3]。日本的一项调查报告显示, 每年新增ONFH病例约2 200例, 有近11.4万名患者需要治疗^[4]。而我国现患ONFH人数约800万人, 每年新发10到20万例,

多见于男性青壮年, 北方居民患病率高于南方居民^[5-6]。毫无疑问, ONFH是全球关注的公共卫生问题。骨坏死很少会自愈, 大约67%的无症状患者和85%的有症状患者的坏死股骨头会进展为塌陷^[7]。

收稿日期: 2020-09-21; 修回日期: 2020-11-18

基金项目: 国家自然科学基金面上项目(81904226, 8187-3327); 广东省自然科学基金项目(2017A030313698)

*通信作者: E-mail: weiqishi@126.com; Tel: 13602407269

一般认为股骨头塌陷患者需要采用全髋关节置换术(total hip arthroplasty, THA)进行治疗^[8]。但中青年患者作为家庭的主要劳动力, 选择THA意味着很大可能需要进行多次翻修手术, 这将给患者及其家庭带来巨大的经济压力和心理压力。因此, 本病治疗关键在于早期积极保髋治疗以预防股骨头塌陷, 延长自身髋关节使用寿命^[9]。那么研究ONFH发病机制在早期诊断和治疗时机的把握上就显得尤为重要。

线粒体不仅是细胞内主要的能量供应中心, 还参与调控细胞内各种信号转导过程, 如Ca²⁺稳态、细胞凋亡、活性氧(reactive oxygen species, ROS)的产生等^[10-11]。线粒体功能障碍多指线粒体通透性转变孔(mitochondria permeability transition pore, mPTP)开放, 导致线粒体通透性转变和细胞内脂肪酸蓄积, 氧化应激增加, 最终导致细胞凋亡^[12]。ONFH的发病机制尚不清楚, 但在缺血发作后24~72 h内, 骨髓坏死和骨细胞凋亡的组织学迹象十分明显^[1]。Mutijima等^[13]研究发现不同病因的ONFH坏死区周围成骨细胞和骨细胞凋亡显著升高。值得注意的是, 骨重建失衡和骨组织细胞凋亡是ONFH病理改变过程中的重要环节^[14], 成骨细胞(osteoblast, OB)、破骨细胞(osteoclast, OC)是ONFH骨重建和修复中的关键因素^[15]。研究发现骨细胞^[16]、OB^[17]和OC^[18]的作用和凋亡与线粒体途径息息相关。自噬在ONFH中亦发挥重要作用, 且自噬与凋亡存在联系^[19-21]。总的来说, 刺激因素影响线粒体自噬, 诱导骨细胞、OB和OC凋亡, 进而导致ONFH的发生和发展。为了明确这一关系, 笔者对本病与骨组织细胞凋亡、自噬及其线粒体相关蛋白的关系展开综述, 以期防治ONFH提供理论依据和新的思路。

1 股骨头坏死与线粒体途径细胞凋亡

1.1 激素性股骨头坏死

激素性股骨头缺血性坏死(steroid-induced osteonecrosis of the femoral head, SONFH)是由于长期或大剂量地应用糖皮质激素(glucocorticoid, GC)导致的股骨头缺血性坏死的疾病^[22]。有研究发现, SONFH患者骨标本内骨小梁密度不均, 空骨陷窝率明显增加^[23], 也就是说, SONFH坏死区存在骨细胞凋亡现象。动物实验结果亦说明这一现象存在的正确性^[24-25]。亦有研究证实, 使用大剂量GC可通过线粒体途径诱导OB、OC及骨细胞凋亡, 且细胞凋亡率与剂量存在关联性^[26-28]。另外, 自噬可减轻GC对骨细胞^[29]、OB^[30]的功能抑制和促凋亡作用, GC

作用下OC自噬增加^[31]。综上所述, GC的长时间大量使用会导致线粒体功能障碍, 诱导骨组织细胞的凋亡, 影响自噬, 推动SONFH的发展。

1.2 酒精性股骨头坏死

流行病学研究表明, 酒精中毒是世界范围内ONFH发生的主要危险因素之一, 20%~45%的ONFH是饮酒过度导致的^[32]。酒精性股骨头坏死(alcohol-induced osteonecrosis of the femoral head, AONFH)是我国重要的公共卫生问题, 氧化应激反应在AONFH发病机制中占有重要地位^[33]。线粒体是ROS的重要来源, 过量的ROS会诱导氧化应激反应, 改变线粒体膜通透性, 引起线粒体功能障碍, 促使细胞色素C释放, 引起细胞凋亡^[34]。有研究表明, 大量酒精摄入介导氧化应激反应, 影响OB和OC活性, 抑制骨形成^[35-37], 同时损害自噬^[38], 不利于及时清除受损线粒体。在AONFH早期, 骨组织细胞凋亡确实存在^[39]。魏秋实等^[40]研究发现AONFH患者坏死区骨小梁连续性被破坏, 骨小梁数量明显减少, 说明存在骨细胞凋亡现象。动物实验也证实, AONFH与线粒体途径骨细胞凋亡紧密联系^[41-42]。另有研究发现, 自噬在AONFH的病变过程中发挥重要的保护作用^[19]。也就是说, AONFH发病机制中的重要一环可能是大量酒精引发氧化应激反应, 导致线粒体功能障碍, 最终影响骨细胞、OB和OC凋亡, 激活自噬可能有利于防治AONFH。

1.3 创伤性股骨头坏死

创伤性股骨头坏死(trauma-induced osteonecrosis of the femoral head, TONFH)一般是由股骨颈骨折导致股骨头供血不足, 骨细胞凋亡的严重并发症^[43]。骨折伴有创伤、机械应力和炎症是已知的诱发线粒体通透性转变的条件, 这是由于mPTP开放导致的, 且其开放程度是决定细胞凋亡程度的重要因素^[44]。有研究发现, 大鼠TONFH模型的骨小梁骨细胞大量减少, 空骨陷窝率明显增大^[45], 证明TONFH中确实存在骨细胞凋亡现象。值得关注的是, 力学的重建和骨小梁的结构是相关联的^[46]。对股骨颈骨折的患者来说, 股骨颈移位较大, 骨折复位欠佳, 则股骨头的骨小梁结构就会随着它的受力负荷而发生变化, 当超出其阈值时会致使骨小梁微骨折, 诱发线粒体功能障碍, 造成骨细胞凋亡, 最终导致股骨头坏死^[47]。此外, 激活自噬有利于骨折愈合, 保护OB不受细胞凋亡的影响^[48]。因此, 改善局部微环境和干预骨组织细胞自噬和凋亡可能是促进骨重建的重要解决手段。

1.4 特发性股骨头坏死

特发性股骨头坏死(idiopathic osteonecrosis of the femoral head, IONFH)是指不明原因的非创伤性、难治性髋关节疾病^[49]。世界范围内IONFH患者数量逐年增加^[50],有研究发现IONFH的发生发展与脂质代谢紊乱息息相关^[51]。脂类是细胞膜的结构成分和信号转导的基础,脂类和脂膜是激活细胞凋亡蛋白所需的重要辅助分子^[52]。因此,脂质代谢紊乱势必导致细胞通透性改变,线粒体功能障碍,诱发骨组织细胞凋亡。有研究发现,IONFH存在骨细胞凋亡^[53-54]。另外,自噬可调节OC前体分化及其骨吸收功能^[55],可以促进OB分化和矿化^[56]。综上所述,IONFH发病机制之一可能是由于脂质代谢紊乱导致线粒体途径骨组织细胞凋亡被激活,而且自噬在其中可能也发挥重要作用。但是,目前仍缺少证据证实其关联的紧密性。

2 股骨头坏死线粒体相关蛋白

2.1 Bcl-2蛋白家族

Bcl-2蛋白家族位于线粒体外膜上,其活性与线粒体途径介导的细胞凋亡密切相关^[57]。目前发现的Bcl-2蛋白家族中有Bcl-2、Bax、Bak、Bad等蛋白。Bcl-2的表达可以延长骨细胞存活的时间,增加骨细胞的稳定性^[58];Bax、Bak、Bad等明确诱导线粒体功能障碍和细胞色素C释放,导致细胞凋亡^[59-60]。简而言之,Bcl-2基因表达抑制细胞凋亡,而Bax、Bak、Bad基因表达促进细胞凋亡。研究发现,通过抑制线粒体途径细胞凋亡,增强Bcl-2表达,降低Bax表达对SONFH有促修复作用^[61-63]。Bcl-2蛋白家族亦参与酒精诱导的骨细胞凋亡^[64]。另外,Ren等^[65]发现脂肪源性间充质干细胞可以通过上调Bcl-2/Bax的比值从而显著降低缺氧、血清不足诱导的骨细胞凋亡。综上所述,线粒体途径诱导的骨细胞凋亡在ONFH早期发挥着重要作用,其中Bcl-2蛋白家族发挥的作用需予以重视。由于不同实验动物或不同实验条件产生的Bcl-2和Bax结果可能不一致,所以Bcl-2/Bax的比值可能比单因素的大小更有参考价值。

2.2 细胞色素C

细胞色素C(cytochrome c, Cyt C)正常情况下仅存在于线粒体膜间隙(intermembrane space, IMS),当细胞受到刺激时,Cyt C会通过线粒体途径从IMS释放进入胞浆从而引起细胞凋亡^[66-67],且其释放受Bcl-2、Bax调控^[68]。Cyt C参与的骨细胞凋亡可能与

早期SONFH的发病过程有关^[69]。长期使用GC会导致Cyt C释放和线粒体分解,从而导致OB凋亡^[17,70]。另外,Wu等^[71]发现原儿茶酸通过调节线粒体膜电位改变,激活Cyt C来诱导OC凋亡。换句话说,Cyt C是因刺激因素作用于细胞线粒体而释放,影响OB、OC的凋亡从而可能对ONFH的早期进展产生影响。

2.3 凋亡蛋白酶活化因子1

凋亡蛋白酶活化因子1(apoptotic protease activating factor-1, Apaf-1)主要参与线粒体凋亡途径的信号转导,被Cyt C激活后进一步活化半胱氨酸天冬氨酸蛋白酶家族(cysteiny aspartate specific proteinase, Caspase),从而启动细胞凋亡^[72-73]。Chen等^[74]发现,香兰素可通过诱导Cyt C、Caspase-3、Bax和Apaf-1在mRNA和蛋白质水平上的表达,激活线粒体依赖性凋亡,从而显著抑制OC发挥的骨吸收作用。Liu等^[75]发现,MLN2238作用后,Bax/Bcl-2、Apaf-1及促凋亡蛋白的相对表达率上调,改变线粒体外膜通透性,最终促使细胞凋亡。也就是说,刺激因素下的细胞线粒体发生功能障碍,Cyt C释放入胞浆,Apaf-1被激活,最终诱导了骨组织细胞凋亡,影响ONFH进程。

2.4 Caspase

Caspase是细胞凋亡的核心成分^[76]。在细胞受到刺激后,线粒体膜通透性发生改变,线粒体功能障碍,导致凋亡诱导因子释放和Caspase活化,最终激活细胞凋亡^[77]。Caspase-9是线粒体凋亡途径中Cyt C下游的重要作用元件,参与了细胞凋亡的启动^[78],而Caspase-3是细胞凋亡各途径的共同下游通路,是各种凋亡刺激因子中的关键蛋白酶和直接介导凋亡的效应分子^[79]。GC诱导caspase-3升高,OB凋亡是SONFH的重要发病原因^[26,80]。黎金焕和陈跃平^[64]认为酒精刺激Caspase高表达可以加速骨细胞的凋亡,在AONFH的发病机制中起着重要的作用。此外,某些药物单体通过诱导Caspase-3表达促进OC凋亡^[74]。综上所述,Caspase作用的发挥是通过线粒体途径诱导骨细胞、OB和OC凋亡,因此调控Caspase的表达可能对ONFH的发病和骨重建有影响。

2.5 PTEN诱导激酶1和Parkin

PTEN诱导激酶1(PTEN-induced kinase 1, PINK1)含有线粒体靶向序列,允许其在线粒体外膜定位。正常条件下,细胞内PINK1水平很低且难以被检测到,只有在一定应激条件下,PINK1在线粒

体外膜积累从而可被检测^[81]。Parkin是一种E3泛素连接酶, 正常状态下处于抑制状态, 线粒体受损后会被激活^[82]。PINK1作为Parkin的上游分子, 不仅参与调控Parkin, 而且二者作为线粒体自噬中的关键作用因子共同维持线粒体的形态和功能^[81]。Zhang等^[83]上调BMSCs中Parkin后发现, 受损线粒体在细胞中的累积减少, 有效抵抗BMSCs凋亡的发生。Yang等^[84]研究发现, 辛伐他汀可以减轻牙根尖周炎的骨丢失和缺氧引起的PINK1和Parkin累积和线粒体功能障碍, 抑制局部OB凋亡。另有研究发现, OPG可以显著上调OC线粒体PINK1和Parkin的表达从而促进线粒体自噬^[81]。综上所述, PINK1和Parkin的表达对维持线粒体的稳态和调节细胞凋亡具有重要作用, 这种作用可能对防治ONFH有影响。

2.6 其他相关蛋白

电压依赖性阴离子通道蛋白1 (voltage-dependent anion-selective channel protein 1, VDAC1)、线粒体融合蛋白2 (mitofusin 2, Mfn2)和线粒体外膜转位酶20 (translocase of outer mitochondrial membrane 20, TOMM20)是已知的Parkin下游底物, 可介导线粒体自噬以促进受损线粒体的降解, 在维持正常线粒体功能上发挥重要作用^[85-87]。

2.6.1 VDAC1

VDAC存在于所有真核生物的线粒体外膜上, 是mPTP的主要组分之一, 其三种亚型中VDAC1 转录表达最强^[88-90]。VDAC1与促凋亡和抗凋亡蛋白相关, 在线粒体介导的细胞凋亡中发挥关键作用^[91-93]。Rosenberg等^[94]研究TSPO配体蛋白Ro5-4864对体外培养的人OB作用, 发现VDAC1的转录和表达被降低, 其防止细胞凋亡的作用是通过减少线粒体膜电位崩溃, 稳定线粒体发挥的。此外, Parkin自身可以对VDAC1进行泛素化标记, 促进线粒体自噬, 降解受损的线粒体^[81]。简而言之, ONFH患者的VDAC1水平与正常人不同, 且这种变化可以反应出患者的线粒体受损情况。

2.6.2 Mfn2

Mfn2是定位于线粒体外膜的跨膜蛋白, 主要功能是介导线粒体融合, 稳定线粒体外膜通透性, 保护线粒体结构和调节细胞凋亡^[95]。此外, Mfn2与Bcl-2蛋白家族存在相互作用。研究显示, Mfn2表达与Bcl-2表达呈正相关, 与Bax表达呈负相关^[95]。翟启明等^[97]研究发现, 炎症微环境下牙周膜干细胞的Mfn2表达量升高, 成骨分化能力下降。有研究发现, 淫羊藿苷能促进Mfn2表达, 增加Cyt C蛋白从

细胞质到线粒体的转运, 保护铁超载引起的BMSCs功能障碍^[98]。Jung等^[99]研究发现, 抑制骨髓基质中Mfn2的表达时, OC分化受到抑制, 成熟OC活性被降低。Gao等^[100]发现Mfn2通过介导线粒体转移, 将正常骨细胞转移到邻近的应激骨细胞, 恢复骨细胞的代谢功能。因此, 鉴于Mfn2对BMSCs、OB、OC和骨细胞的影响, 笔者推测Mfn2与ONFH之间亦存在联系。

2.6.3 TOMM20

TOMM20属于线粒体外膜蛋白, 其表达直接影响线粒体功能, 并且参与细胞凋亡^[101]。李金堂等^[102]发现, 异基因造血干细胞移植后ONFH患者行THA疗效良好, 这项病例报道从侧面反证重建血运对ONFH预后的重要性。自噬是造血干细胞(haematopoietic stem cells, HSCs)进行长期自我更新和正常分化的关键^[103], 有研究发现促使HSCs自噬后, TOMM20显著减少, 影响HSCs正常分化和血运重建^[104]。白胜超^[105]发现, 大负荷运动后针刺干预骨骼肌细胞, 胞浆中Cyt C的含量明显下降, 线粒体的片段化现象减少, TOMM20/DRP1的共定位百分数显著降低, 在一定程度上促进线粒体功能的恢复。

综上所述, 笔者推测一定程度上干预VDAC1、Mfn2和TOMM20表达, 可能对稳定线粒体结构和功能以及调节ONFH骨组织细胞凋亡有重要意义。

3 讨论

ONFH的发病机制至今尚未明确, 其共同病理机制是骨组织缺血基本得到认可。在骨组织缺血早期, 存在骨细胞凋亡, 且这种细胞凋亡与线粒体功能相关。激素、酒精、骨折等因素从不同程度上影响线粒体膜通透性和线粒体结构完整性从而造成线粒体功能障碍, 影响线粒体自噬, 诱发骨细胞、OB和OC凋亡, 最终导致ONFH的发生和发展。但是, IONFH与线粒体功能障碍之间的紧密联系还无法得到有效证明, 这可能是IONFH病因不明和病例数相对较少的缘故。

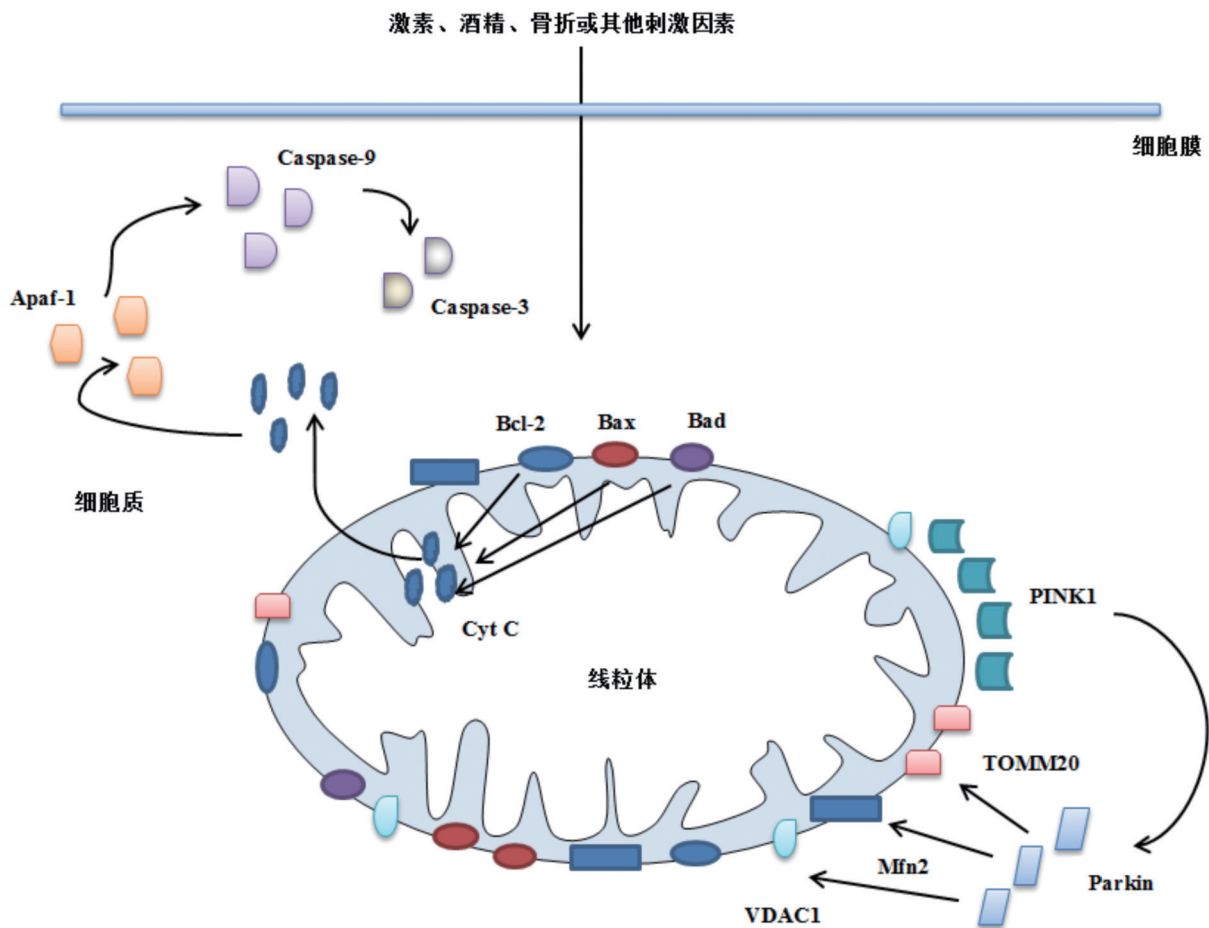
在ONFH发生发展中, 细胞凋亡与自噬发挥不可替代的作用。细胞凋亡与自噬作为维持细胞稳态的重要机制, 通过线粒体途径调节OB、OC和骨细胞的增殖、分化和功能^[16-18, 106-108]。Wang等^[21]发现低氧预处理联合姜黄素可通过上调PINK1和Parkin的表达显著提高线粒体质量, 同时通过改变线粒体嵴的形态显著抑制线粒体Cyt C释放, 从而抑制细胞凋亡信号, 最终增强BMSCs移植后的适应性, 改善

其治疗性能。Menk等^[109]发现慢性酒精摄入损害了细胞自噬功能,并导致细胞凋亡。上述研究证实细胞凋亡与自噬存在紧密联系。一般而言,在刺激因素作用下,细胞线粒体结构和功能改变,自噬启动以清除受损线粒体,同时诱发细胞凋亡,最终维持细胞正常功能。

正常情况下,OB和OC相互作用,维持骨吸收与骨形成的动态平衡,影响骨重建。同时,正常骨细胞对骨吸收和骨形成都有影响,是维持成熟骨新陈代谢的主要细胞。一些与线粒体功能相关的蛋白在ONFH发病中发挥着重要作用。长期受到GC、酒精、其他刺激因素影响,或者创伤骨折后的骨组织细胞线粒体外膜上Bcl-2蛋白家族被激活,从而调控Cyt C从IMS释放到细胞质,促使Apaf-1与Caspase-9结合,形成Cyt C-Apaf-1-Caspase-9,活化的Caspase-9

继续激活Caspase-3,启动Caspase级联反应,最终促使细胞凋亡。另一方面,PINK1会在线粒体外膜累积,诱导Parkin发生转位^[110]。PINK1泛素化或磷酸化修饰Parkin,并且Parkin自身也对线粒体表面的蛋白进行泛素化标记,包括VDAC1、Mfn2和TOMM20等,继而通过泛素-蛋白酶体途径降解,维持线粒体形态和功能(图1)。

然而,人体是一个复杂的、不断变化平衡的系统,机体细胞处在一个复杂的微环境中,不能以单因素去简单判断细胞凋亡或自噬处于何种状态,需要动态把握,综合评估。例如,Toscano等^[111]发现尽管Bcl-2/Bax比值增加,但颗粒状神经元和神经胶质细胞显示出活跃的Caspase-3表达。骨细胞、OB和OC无论在坏死前期还是修复过程中都发挥重要作用。在危险因素长期持续刺激下,细胞线粒体形



*Cyt C: 细胞色素C; Apaf-1: 凋亡蛋白酶活化因子1; Bcl-2: B淋巴细胞瘤-2基因; Bax: B淋巴细胞瘤-2相关X蛋白; Bad: bcl-X_L/bcl-2相关死亡启动子同源物; Caspase-3: 半胱氨酸天冬氨酸蛋白酶-3; Caspase-9: 半胱氨酸天冬氨酸蛋白酶-9; PINK1: PTEN诱导激酶1; Parkin: E3泛素连接酶; VDAC 1: 电压依赖性阴离子通道蛋白1; Mfn2: 线粒体融合蛋白2; TOMM20: 线粒体外膜转位酶20

图1 线粒体相关蛋白通路

态被直接或间接破坏, 影响线粒体功能, 启动自噬, 诱发细胞凋亡。具体表现在骨小梁稀疏、紊乱, 空骨陷窝增多, 此时轻微外力作用下股骨头发生细微损伤甚至骨折, 最终导致ONFH的发生, 同时通过影响OB和OC凋亡影响ONFH骨重建。

本文对ONFH线粒体相关细胞凋亡及自噬的进行综述, 以期对未来研究骨组织内线粒体能量代谢对ONFH发生发展的影响做出理论指导。简而言之, 线粒体功能障碍与ONFH发生发展有紧密联系, 其中涉及细胞凋亡、线粒体自噬等过程, 此中线粒体相关蛋白环环相扣、作用突出。但其具体机制需要进一步临床研究和实验去验证, 如何平衡细胞凋亡与自噬的作用也需要进一步研究。

[参 考 文 献]

- [1] Petek D, Hannouche D, Suva D. Osteonecrosis of the femoral head: pathophysiology and current concepts of treatment. *EFORT Open Rev*, 2019, 4: 85-97
- [2] Wang G, Li Y, Sun T, et al. BMSC affinity peptide-functionalized β -tricalcium phosphate scaffolds promoting repair of osteonecrosis of the femoral head. *J Orthop Surg Res*, 2019, 14: 204
- [3] Narayanan A, Khanchandani P, Borkar RM, et al. Avascular necrosis of femoral head: a metabolomic, biophysical, biochemical, electron microscopic and histopathological characterization. *Sci Rep*, 2017, 7: 10721
- [4] Kubo T, Ueshima K, Saito M, et al. Clinical and basic research on steroid-induced osteonecrosis of the femoral head in Japan. *J Orthop Sci*, 2016, 21: 407-13
- [5] Cui L, Zhuang Q, Lin J, et al. Multicentric epidemiologic study on six thousand three hundred and ninety five cases of femoral head osteonecrosis in China. *Int Orthop*, 2016, 40: 267-76
- [6] Zhao D, Zhang F, Wang B, et al. Guidelines for clinical diagnosis and treatment of osteonecrosis of the femoral head in adults (2019 version). *J Orthop Translat*, 2020, 21: 100-10
- [7] Pepke W, Kasten P, Beckmann NA, et al. Core decompression and autologous bone marrow concentrate for treatment of femoral head osteonecrosis: a randomized prospective study. *Orthop Rev*, 2016, 8: 6162
- [8] Sodhi N, Acuna A, Etcheson J, et al. Management of osteonecrosis of the femoral head. *Bone Joint J*, 2020, 102-B: 122-8
- [9] 何伟, 刘予豪, 周驰, 等. 非手术保髋治疗非创伤性股骨头坏死的临床研究. *中国中西医结合杂志*, 2020, 40: 176-81
- [10] Gavish M, Veenman L. Regulation of mitochondrial, cellular, and organismal functions by TSPO. *Adv Pharmacol*, 2018, 82: 103-36
- [11] Vafai SB, Mootha VK. Mitochondrial disorders as windows into an ancient organelle. *Nature*, 2012, 491: 374-83
- [12] 申屠路媚, 牟艳玲. 线粒体功能障碍机制及其相关疾病研究进展. *生命科学*, 2018, 30: 87-93
- [13] Mutijima E, De Maertelaer V, Deprez M, et al. The apoptosis of osteoblasts and osteocytes in femoral head osteonecrosis: its specificity and its distribution. *Clin Rheumatol*, 2014, 33: 1791-5
- [14] Narayanan A, Khanchandani P, Borkar RM, et al. Avascular necrosis of femoral head: a metabolomic, biophysical, biochemical, electron microscopic and histopathological characterization. *Sci Rep*, 2017, 7: 10721-36
- [15] 魏秋实, 李子祺, 袁颖嘉, 等. “标本兼治”理论在股骨头坏死中医药治疗中的指导作用. *中医正骨*, 2020, 32: 56-9
- [16] Hirata H, Ueda S, Ichiseki T, et al. Taurine inhibits glucocorticoid-induced bone mitochondrial injury, preventing osteonecrosis in rabbits and cultured osteocytes. *Int J Mol Sci*, 2020, 21: 6892
- [17] Nie Z, Deng S, Zhang L, et al. Crocin protects against dexamethasone-induced osteoblast apoptosis by inhibiting the ROS/ Ca^{2+} -mediated mitochondrial pathway. *Mol Med Rep*, 2019, 20: 401-8
- [18] Arnett TR, Orriss IR. Metabolic properties of the osteoclast. *Bone*, 2018, 115: 25-30
- [19] 陶振宇, 张月雷, 陈华, 等. 细胞自噬在酒精性股骨头坏死中的作用及相关机制研究. *中医正骨*, 2018, 30: 4-11
- [20] Wang XY, Gong LJ, Huang JM, et al. Pinocembrin alleviates glucocorticoid-induced apoptosis by activating autophagy via suppressing the PI3K/Akt/mTOR pathway in osteocytes. *Eur J Pharmacol*, 2020, 880: 173212
- [21] Wang X, Shen K, Wang J, et al. Hypoxic preconditioning combined with curcumin promotes cell survival and mitochondrial quality of bone marrow mesenchymal stem cells, and accelerates cutaneous wound healing via PGC-1 α /SIRT3/HIF-1 α signaling. *Free Radic Biol Med*, 2020, 159: 164-76
- [22] Liu Y, Zong Y, Shan H, et al. MicroRNA-23b-3p participates in steroid-induced osteonecrosis of the femoral head by suppressing ZNF667 expression. *Steroids*, 2020, 163: 108709
- [23] 段玮轩, 程亮亮, 赵德伟. 激素性股骨头坏死与股骨颈骨折患者股骨头内骨小梁形态学对比研究. *中华损伤与修复杂志(电子版)*, 2020, 15: 84-9
- [24] 曾晓会, 卓俊城, 杨帆, 等. 羟基红花黄色素A联合扁桃苷对激素性股骨头坏死大鼠的作用研究. *中药新药与临床药理*, 2019, 30: 1284-90
- [25] 李涛, 江蓉星, 王敏, 等. 活血通络汤在家兔激素性股骨头坏死模型中对AKP、BGP、TNF- α 影响的研究. *中华中医药学刊*, 2018, 36: 641-4
- [26] Xue XH, Feng ZH, Li ZX, et al. Salidroside inhibits steroid-induced avascular necrosis of the femoral head via the PI3K/Akt signaling pathway: *in vitro* and *in vivo* studies. *Mol Med Rep*, 2018, 17: 3751-7
- [27] Weinstein RS, Hogan EA, Borrelli MJ, et al. The pathophysiological sequence of glucocorticoid-induced

- osteonecrosis of the femoral head in male mice. *Endocrinology*, 2017, 158: 3817-31
- [28] 伍龙果, 蔡劲薇, 潘吉铭, 等. 地塞米松通过线粒体途径诱导成骨细胞凋亡的研究. *中国骨质疏松杂志*, 2019, 25: 380-4+403
- [29] Han Y, Zhang L, Xing Y, et al. Autophagy relieves the function inhibition and apoptosis-promoting effects on osteoblast induced by glucocorticoid. *Int J Mol Med*, 2018, 41: 800-8
- [30] Zhu L, Chen J, Zhang J, et al. Parathyroid hormone (PTH) induces autophagy to protect osteocyte cell survival from dexamethasone damage. *Med Sci Monit*, 2017, 23: 4034-40
- [31] Fu L, Wu W, Sun X, et al. Glucocorticoids enhanced osteoclast autophagy through the PI3K/Akt/mTOR signaling pathway. *Calcif Tissue Int*, 2020, 107: 60-71
- [32] Yoon BH, Jones LC, Chen CH, et al. Etiologic classification criteria of ARCO on femoral head osteonecrosis part 2: alcohol-associated osteonecrosis. *J Arthroplasty*, 2019, 34: 169-74
- [33] 陈亦轩, 朱道宇, 殷俊辉, 等. 酒精性股骨头坏死研究进展. *国际骨科学杂志*, 2018, 39: 28-32
- [34] Cooper KF. Till death do us part: the marriage of autophagy and apoptosis. *Oxid Med Cell Longev*, 2018: 4701275
- [35] Yang Q, Yin W, Chen Y, et al. Betaine alleviates alcohol-induced osteonecrosis of the femoral head via mTOR signaling pathway regulation. *Biomed Pharmacother*, 2019, 120: 109486
- [36] Yu HP, Zhu DY, Liu P, et al. Osthole stimulates bone formation, drives vascularization and retards adipogenesis to alleviate alcohol-induced osteonecrosis of the femoral head. *J Cell Mol Med*, 2020, 24: 4439-51
- [37] Alund AW, Mercer KE, Pulliam CF, et al. Partial protection by dietary antioxidants against ethanol-induced osteopenia and changes in bone morphology in female mice. *Alcohol Clin Exp Res*, 2017, 41: 46-56
- [38] Chao X, Ding WX. Role and mechanisms of autophagy in alcohol-induced liver injury. *Adv Pharmacol*, 2019, 85: 109-31
- [39] Krenn V, Müller S, Krenn VT, et al. Pathophysiologie der aseptischen Hüftkopfnekrose: Pathogenese und histopathologische Differenzialdiagnostik [Pathophysiology of aseptic femoral head necrosis: Pathogenesis and histopathological differential diagnosis]. *Orthopade*, 2018, 47: 710-6
- [40] 魏秋实, 杨帆, 陈晓俊, 等. 激素性与酒精性股骨头坏死患者骨标本坏死区域病理与显微结构特点分析. *中国修复重建外科杂志*, 2018, 32: 866-72
- [41] Guo YJ, Luo SH, Tang MJ, et al. Muscone exerts protective roles on alcohol-induced osteonecrosis of the femoral head. *Biomed Pharmacother*, 2018, 97: 825-32
- [42] 韩杰, 陈跃平, 莫坚, 等. 三七总皂苷干预激素性股骨头缺血坏死模型兔的超微结构评价. *中国组织工程研究*, 2019, 23: 1035-9
- [43] Huang GY, Zhao GL, Xia J, et al. FGF2 and FAM201A affect the development of osteonecrosis of the femoral head after femoral neck fracture. *Gene*, 2018, 652: 39-47
- [44] Shares BH, Smith CO, Sheu TJ, et al. Inhibition of the mitochondrial permeability transition improves bone fracture repair. *Bone*, 2020, 137: 115391
- [45] 朱耀, 孙绍裘, 李益亮, 等. 桃红四物汤对大鼠创伤性股骨头缺血坏死模型外周血中EPCs表达影响. *湖南中医药大学学报*, 2017, 37: 22-5
- [46] 李晨杰, 吕林蔚, 宋阳, 等. 预紧力作用下钛合金人工假体界面骨小梁形态参数测量与统计分析. *中国组织工程研究*, 2021, 25: 516-20
- [47] 章猛奇, 彭笳宸. 股骨颈骨折后股骨头血运评估研究现状及进展. *中国中医骨伤科杂志*, 2019, 27: 82-5
- [48] Xu MX, Sun XX, Li W, et al. LPS at low concentration promotes the fracture healing through regulating the autophagy of osteoblasts via NF- κ B signal pathway. *Eur Rev Med Pharmacol Sci*, 2018, 22: 1569-79
- [49] Tomaru Y, Yoshioka T, Sugaya H, et al. Ten-year results of concentrated autologous bone marrow aspirate transplantation for osteonecrosis of the femoral head: a retrospective study. *BMC Musculoskelet Disord*, 2019, 20: 410
- [50] Sakamoto Y, Yamamoto T, Sugano N, et al. Genome-wide association study of idiopathic osteonecrosis of the femoral head. *Sci Rep*, 2017, 7: 15035
- [51] 王文功, 张德彪, 岳海珠. 非创伤性股骨头坏死与脂质代谢紊乱的机制研究进展. *甘肃医药*, 2019, 38: 109-1+127
- [52] Flores-Romero H, Ros U, García-Sáez AJ. A lipid perspective on regulated cell death. *Int Rev Cell Mol Biol*, 2020, 351: 197-236
- [53] 杨帆. 不同病因导致的股骨头缺血性坏死的形态学和蛋白质组学分析[D]. 上海: 上海交通大学, 2018: 21
- [54] Narayanan A, Khanchandani P, Borkar RM, et al. Avascular necrosis of femoral head: a metabolomic, biophysical, biochemical, electron microscopic and histopathological characterization. *Sci Rep*, 2017, 7: 10721
- [55] Kim CJ, Shin SH, Kim BJ, et al. The effects of kaempferol-inhibited autophagy on osteoclast formation. *Int J Mol Sci*, 2018, 19: 125
- [56] Li W, Zhang S, Liu J, et al. Vitamin K2 stimulates MC3T3-E1 osteoblast differentiation and mineralization through autophagy induction. *Mol Med Rep*, 2019, 19: 3676-84
- [57] Kale J, Osterlund EJ, Andrews DW. BCL-2 family proteins: changing partners in the dance towards death. *Cell Death Differ*, 2018, 25: 65-80
- [58] Tao SC, Yuan T, Rui BY, et al. Exosomes derived from human platelet-rich plasma prevent apoptosis induced by glucocorticoid-associated endoplasmic reticulum stress in rat osteonecrosis of the femoral head via the Akt/Bad/Bcl-2 signal pathway. *Theranostics*, 2017, 7: 733-50
- [59] Yang F, Qu W, Du M, et al. Stoichiometry and regulation network of Bcl-2 family complexes quantified by live-cell FRET assay. *Cell Mol Life Sci*, 2020, 77: 2387-406
- [60] Ge X, Pan MH, Wang L, et al. Hypoxia-mediated mitochondria apoptosis inhibition induces temozolomide treatment resistance through miR-26a/Bad/Bax axis. *Cell*

- Death Dis, 2018, 9: 1128
- [61] Yao X, Yu S, Jing X, et al. PTEN inhibitor VO-OHpic attenuates GC-associated endothelial progenitor cell dysfunction and osteonecrosis of the femoral head via activating Nrf2 signaling and inhibiting mitochondrial apoptosis pathway. *Stem Cell Res Ther*, 2020, 11: 140
- [62] Yu H, Yue J, Wang W, et al. Icaritin promotes angiogenesis in glucocorticoid-induced osteonecrosis of femoral heads: *In vitro* and *in vivo* studies. *J Cell Mol Med*, 2019, 23: 7320-30
- [63] 朱宁, 许建峰, 马奇, 等. 络灸对激素性股骨头坏死骨细胞凋亡与Bcl-2、Caspase-3表达影响的实验研究. *山西中医学院学报*, 2018, 19: 16-9
- [64] 黎金焕, 陈跃平. 骨细胞凋亡在酒精性股骨头缺血性坏死中的作用. *中国组织工程研究*, 2016, 20: 2241-7
- [65] Ren L, Song ZJ, Cai QW, et al. Adipose mesenchymal stem cell-derived exosomes ameliorate hypoxia/serum deprivation-induced osteocyte apoptosis and osteocyte-mediated osteoclastogenesis *in vitro*. *Biochem Biophys Res Commun*, 2019, 508:138-44
- [66] Nie Z, Chen S, Peng H. Glucocorticoid induces osteonecrosis of the femoral head in rats through GSK3 β -mediated osteoblast apoptosis. *Biochem Biophys Res Commun*, 2019, 511: 693-9
- [67] 龚瑜林, 王玉鑫, 赵振群, 等. 细胞色素C与Caspase-9在激素性股骨头缺血坏死中的作用. *中国组织工程研究*, 2018, 22: 2526-31
- [68] Chen C, Liu TS, Zhao SC, et al. XIAP impairs mitochondrial function during apoptosis by regulating the Bcl-2 family in renal cell carcinoma. *Exp Ther Med*, 2018, 15: 4587-93
- [69] 黎牧帆, 张二洋, 吕雷锋, 等. 维生素E对大鼠早期激素性股骨头缺血性坏死的作用及机制研究. *中国修复重建外科杂志*, 2018, 32: 1421-8
- [70] Chen YH, Peng SY, Cheng MT, et al. Different susceptibilities of osteoclasts and osteoblasts to glucocorticoid-induced oxidative stress and mitochondrial alterations. *Chin J Physiol*, 2019, 62: 70-9
- [71] Wu YX, Wu TY, Xu BB, et al. Protocatechuic acid inhibits osteoclast differentiation and stimulates apoptosis in mature osteoclasts. *Biomed Pharmacother*, 2016, 82: 399-405
- [72] Wu CC, Lee S, Malladi S, et al. The Apaf-1 apoptosome induces formation of caspase-9 Homo- and heterodimers with distinct activities. *Nat Commun*, 2016, 7: 13565
- [73] Fang X, Miao XL, Liu JL, et al. Berberine induces cell apoptosis through cytochrome c/apoptotic protease-activating factor 1/Caspase-3 and apoptosis inducing factor pathway in mouse insulinoma cells. *Chin J Integr Med*, 2019, 25: 853-60
- [74] Chen Y, Dou C, Yi J, et al. Inhibitory effect of vanillin on RANKL-induced osteoclast formation and function through activating mitochondrial-dependent apoptosis signaling pathway. *Life Sci*, 2018, 208: 305-14
- [75] Liu R, Fu C, Sun J, et al. A new perspective for osteosarcoma therapy: proteasome inhibition by MLN9708/2238 successfully induces apoptosis and cell cycle arrest and attenuates the invasion ability of osteosarcoma cells *in vitro*. *Cell Physiol Biochem*, 2017, 41: 451-65
- [76] Fulda S. Therapeutic opportunities based on caspase modulation. *Semin Cell Dev Biol*, 2018, 82: 150-7
- [77] 耿雪松. 基于Cyt-c、AIF、Apaf-1蛋白表达的影响探讨克郁疏神颗粒治疗抑郁发作的凋亡机制的研究[D]. 长春: 长春中医药大学, 2019
- [78] Huber KL, Serrano BP, Hardy JA. Caspase-9 CARD: core domain interactions require a properly formed active site. *Biochem J*, 2018, 475: 1177-96
- [79] He Q, Wang T, Ni H, et al. Endoplasmic reticulum stress promoting caspase signaling pathway-dependent apoptosis contributes to bone cancer pain in the spinal dorsal horn. *Mol Pain*, 2019, 15: 1744806919876150
- [80] Deng S, Nie ZG, Peng PJ, et al. Decrease of GSK3 β Ser-9 phosphorylation induced osteoblast apoptosis in rat osteoarthritis model. *Curr Med Sci*, 2019, 39: 75-80
- [81] 刘庆羊. PINK1/Parkin通路在骨保护素调控破骨细胞线粒体自噬中的作用机制[D]. 扬州: 扬州大学, 2019
- [82] 张明军, 罗俊一, 杨毅宁. PINK1、Parkin与泛素协同调控线粒体自噬的研究进展. *医学综述*, 2020, 26: 1887-92
- [83] Zhang F, Peng W, Zhang J, et al. P53 and Parkin co-regulate mitophagy in bone marrow mesenchymal stem cells to promote the repair of early steroid-induced osteonecrosis of the femoral head. *Cell Death Dis*, 2020, 11: 42
- [84] Yang CN, Kok SH, Wang HW, et al. Simvastatin alleviates bone resorption in apical periodontitis possibly by inhibition of mitophagy-related osteoblast apoptosis. *Int Endod J*, 2019, 52: 676-88
- [85] Ham SJ, Lee D, Yoo H, et al. Decision between mitophagy and apoptosis by Parkin via VDAC1 ubiquitination. *Proc Natl Acad Sci USA*, 2020, 117: 4281-91
- [86] McLelland GL, Goiran T, Yi W, et al. Mfn2 ubiquitination by PINK1/parkin gates the p97-dependent releases of ER from mitochondria to drive mitophagy. *Elife*, 2018, 7: e32866
- [87] Zhen Y, Spangenberg H, Munson MJ, et al. ESCRT-mediated phagophore sealing during mitophagy. *Autophagy*, 2020, 16: 826-41
- [88] Ben HD, Shoshan BV. VDAC1-interacting anion transport inhibitors inhibit VDAC1 oligomerization and apoptosis. *Biochim Biophys Acta*, 2016, 1863: 1612-23
- [89] Pérez MJ, Quintanilla RA. Development or disease: duality of the mitochondrial permeability transition pore. *Dev Biol*, 2017, 426: 1-7
- [90] Shoshan BV, Nahon CE, Shteinifer KA, et al. VDAC1, mitochondrial dysfunction, and Alzheimer's disease. *Pharmacol Res*, 2018, 131: 87-101
- [91] Shoshan-BV, Krelin Y, Shteinifer-KA. VDAC1 functions in Ca²⁺ homeostasis and cell life and death in health and disease. *Cell Calcium*, 2018, 69: 81-100
- [92] 刘红娟, 吴德玲, 童小慧, 等. 五子衍宗丸干预线粒体通透性转换孔抑制精子凋亡的机制. *中国实验方剂学杂志*, 2020, 26: 34-39
- [93] Kim J, Gupta R, Blanco LP, et al. VDAC oligomers form mitochondrial pores to release mtDNA fragments and promote lupus-like disease. *Science*, 2019, 366: 1531-6
- [94] Rosenberg N, Rosenberg O, Weizman A, et al. *In vitro*

- effects of the specific mitochondrial TSPO ligand Ro5 4864 in cultured human osteoblasts. *Exp Clin Endocrinol Diabetes*, 2018, 126: 77-84
- [95] 时玉龙, 易成腊. 线粒体融合蛋白2的研究进展. *神经损伤与功能重建*, 2017, 12: 234-7
- [96] Xue R, Zhu X, Jia L, et al. Mitofusin2, a rising star in acute-on-chronic liver failure, triggers macroautophagy via the mTOR signalling pathway. *J Cell Mol Med*, 2019, 23: 7810-8
- [97] 翟启明, 李蓓, 王智伟, 等. 炎症微环境下线粒体融合蛋白2及其介导的内质网-线粒体偶联对牙周膜干细胞成骨分化能力的影响. *中华口腔医学杂志*, 2018, 53: 453-8
- [98] Yao X, Jing X, Guo J, et al. Icariin protects bone marrow mesenchymal stem cells against iron overload induced dysfunction through mitochondrial fusion and fission, PI3K/AKT/mTOR and MAPK pathways. *Front Pharmacol*, 2019, 10: 163
- [99] Jung S, Kwon JO, Kim MK, et al. Mitofusin 2, a mitochondria-ER tethering protein, facilitates osteoclastogenesis by regulating the calcium-calcineurin-NFATc1 axis. *Biochem Biophys Res Commun*, 2019, 516: 202-8
- [100] Gao J, Qin A, Liu D, et al. Endoplasmic reticulum mediates mitochondrial transfer within the osteocyte dendritic network. *Sci Adv*, 2019, 5: eaaw7215
- [101] Park SH, Lee AR, Choi K, et al. TOMM20 as a potential therapeutic target of colorectal cancer. *BMB Rep*, 2019, 52: 712-7
- [102] 李金堂, 高航, 黄楷甫, 等. 异基因造血干细胞移植后股骨头坏死行双髋关节置换术1例报告及文献复习. *吉林大学学报*, 2019, 45: 1168-72
- [103] Ho TT, Warr MR, Adelman ER, et al. Autophagy maintains the metabolism and function of young and old stem cells. *Nature*, 2017, 543: 205-10
- [104] Vannini N, Girotra M, Naveiras O, et al. Specification of haematopoietic stem cell fate via modulation of mitochondrial activity. *Nat Commun*, 2016, 7: 13125
- [105] 白胜超. 一次大负荷离心运动后骨骼肌线粒体分裂的机制及针刺干预研究[D]. 北京: 北京体育大学, 2018
- [106] Wang S, Deng Z, Ma Y, et al. The role of autophagy and mitophagy in bone metabolic disorders. *Int J Biol Sci*, 2020, 16: 2675-91
- [107] Sun K, Jing X, Guo J, et al. Mitophagy in degenerative joint diseases. *Autophagy*, 2020: 1-11
- [108] Jaber FA, Khan NM, Ansari MY, et al. Autophagy plays an essential role in bone homeostasis. *J Cell Physiol*, 2019, 234: 12105-15
- [109] Menk M, Graw JA, Poyraz D, et al. Chronic alcohol consumption inhibits autophagy and promotes apoptosis in the liver. *Int J Med Sci*, 2018, 15: 682-8
- [110] Shen Y, Wu L, Qin D, et al. Carbon black suppresses the osteogenesis of mesenchymal stem cells: the role of mitochondria. *Part Fibre Toxicol*, 2018, 15: 16
- [111] Toscano ECB, Vieira ÉLM, Portela ACDC, et al. Bcl-2/Bax ratio increase does not prevent apoptosis of glia and granular neurons in patients with temporal lobe epilepsy. *Neuropathology*, 2019, 39: 348-57