衰老个体中的组织干细胞

刘清桂　陈佳佳　陈　费　王敏君*　胡以平*

(第二军医大学细胞生物学教研室, 上海 200433)

摘要　　组织干细胞是组织器官稳态维持和损伤修复或再生的结构基础。伴随着年龄的增加, 组织干细胞的数量、增殖和分化等功能及其所处微环境发生改变, 从而使得老年个体再生修复功能降低或异常。此外, 组织干细胞的衰老与老年个体衰老相关疾病的发生有着密切的关系。该文拟介绍几种目前比较明确的组织干细胞在老年个体中发生改变的基本特征及其发生机制, 旨在为正确理解老年组织机能低下提供重要的理论基础, 并希望能够建立有效可行的延缓或逆转组织干细胞衰老的途径以提高老年人群的生活质量。

关键词　　组织干细胞; 衰老; 干细胞巢

Tissue-Specific Stem Cells in the Aged Individual

Liu Qinggui, Chen Jiajia, Chen Fei, Wang Minjun*, Hu Yiping*

(*Department of Cell Biology, Second Military Medical University, Shanghai 200433, China*)

Abstract　　Tissue-specific stem cells play a vital role in tissue homeostasis and regeneration. Aging tissues experiencing a progressive decline in regenerative function is considered as changes in number of tissue-specific stem cells, stem cell niches, proliferative and differentiation capacities. Moreover, the age-dependent diseases may be directly associated with these dysfunctional stem cells and their niches. Here, we explore the effects of aging on several reported tissue-specific stem cells in different tissues and molecular mechanisms underlying stem cells age. With this increased understanding, it is feasible to design and test interventions that delay or reversal tissue-specific stem cell aging and improve both health and lifespan.

Keywords　　tissue-specific stem cells; aging; stem cell niche

组织干细胞是存在于成熟组织器官中的一类未分化的细胞, 具有自我更新(self-renewal)和分化为特定功能性细胞的基本生物学特性, 可直接或间接地参与组织器官的正常生理状态的维持及其组织结构损伤的修复。在个体发育的不同阶段, 组织干细胞的数量、特性及其所存在的微环境有可能发生相应的改变。特别是在衰老的个体中, 组织干细胞发生这些改变的程度非常明显, 而且组织干细胞的这些改变被认为是组织器官乃至整个机体衰老发生的

结构基础。本文将主要介绍在衰老个体中造血干细胞、骨骼肌干细胞、神经干细胞等组织干细胞异常的特征以及其与微环境和之间的相互关系, 希望能够为临床上衰老相关疾病的认识及其防治研究提供一个新的视角。

1　组织干细胞与机体稳态维持的关系

机体稳态的维持是组织干细胞行使正常生命功能的前提。组织干细胞是细胞分化谱系的源头,

分化产生的后代细胞用于补充其特定的分化细胞。各类组织干细胞的体内外生长特性和表型可能不尽相同，但正常情况下大部分处于静息状态，小部分进行不对称分裂。当机体在内在因素和环境因素作用下出现组织损伤性改变，静息的组织干细胞迅速活化，进入细胞周期，增殖产生相应分化细胞的前体细胞。随后，这些前体细胞可进一步增殖分化，产生成熟的分化细胞，从而修复损伤细胞和组织，维护组织和器官结构完整和功能健全(图1)。

　　组织干细胞在不同的器官中参与了组织稳态的维持和损伤的再生修复[1]。例如，成体血液系统中成熟细胞的寿命极短，需要不断更替；而造血干细胞(hematopoietic stem cells, HSCs)则扮演着维持血液系统各个细胞组分平衡的角色。造血干细胞在维持造血系统稳态中，一方面进行自我更新维持自身干细胞数量，一方面分化为淋巴和髓系细胞的前体细胞，进而分化为成熟的淋巴和髓系细胞[2]。与此类似，肠道上皮细胞终生处于不断更新阶段。在正常稳态维持过程中，位于距小肠隐窝(crypts)基部2~7个细胞位置，与潘氏细胞(Paneth cells)混合交叉排列的小肠干细胞(intestinal stem cells, ISCs)起着重要作用[3]。小肠干细胞激活并增殖分化为前体细胞，随后前体细胞分化产生负责消化液分泌的细胞和负责营养吸收的细胞，从而更替小肠上皮细胞的正常消耗。而在损伤刺激条件下，位于"+4"位置的静息干细胞首先被活化，随后分化并补充损伤的细胞[4]。骨骼肌干细胞(muscle stem cells, MuSCs)又称星状细胞(satellite cells)，位于成熟多核肌纤维细胞膜和基底膜之间。正常的肌细胞具有强大的增殖能力，而当

肌肉细胞损伤后，处于静息状态的骨骼肌干细胞则被激活并增殖分化产生肌细胞，从而参与肌肉组织的损伤修复[5-6]。因此，组织干细胞的衰老程度决定了其对组织稳态维持的贡献能力。

2 组织干细胞衰老的特征

　　由于干细胞在生命过程中发挥了组织稳态维持和再生修复的作用，组织干细胞在机体衰老过程中表现特征和功能改变对机体机能的维持有着重要的作用。随着年龄的增加，组织干细胞发生了显著的变化，表现为干细胞数量的变化、静息和活化状态的改变以及增殖和分化能力的降低等[2,7-14]。在老年个体中，骨骼肌干细胞数量仅为年轻个体的50%，从静息状态转变为活化状态的周期延长，自我更新和分化潜能下降以及修复再生损伤肌肉的能力减弱[10-11,15-16]。因此，老年个体容易患肌肉衰减症、肌纤维化、肌肉萎缩、肌营养不良等病症。老年个体神经干细胞的数量明显减少，处于G_1期时间变长而使得神经球的形成率仅为年轻神经干细胞的二分之一[17-18]。神经干细胞分化能力随着年龄的增加而降低，产生神经元的数量不断减少，导致老年个体学习和记忆能力下降，意识退化，易发生郁抑症、精神分裂等老年病[19]。然而，老年个体中造血干细胞的数量却是年轻个体的5~20倍，但大部分的干细胞处于休眠状态，增殖能力低，移植后其再殖修复能力仅为年轻干细胞的50%[7-8]。由于干细胞巢微环境的改变，衰老的造血干细胞分化成淋巴前体细胞数量减少，而分化成髓性前体细胞的数量增加，导致血液中B、T淋巴细胞减少，而髓性细胞大量增多，从
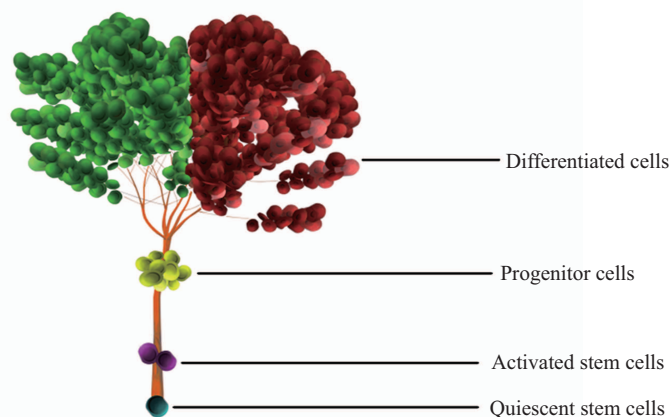

图1　组织干细胞的分化谱系

Fig.1　The hierarchy of tissue-specific stem cells

而使得老年个体中骨髓增生异常综合征、髓性白血病等各种髓性疾病的发生率明显上升[20-21]。尽管毛囊干细胞数量不会随着年龄的增加而发生改变,但是其出现"静止期停滞(telogen retention)",毛发生长周期增加,增殖速度明显减缓,克隆形成率直线下降,传代10代后,子细胞自我更新能力丧失,无法继续培养[22-23]。

3 组织干细胞衰老的发生机制

正常生理条件下,组织干细胞处于静息状态。在外界刺激或损伤条件下,微环境中的炎性因子、生长因子或胞外基质等发生一系列变化,促进组织干细胞从静息向活化状态转变。同时,在一系列转录信号的调控下,活化的组织干细胞进行增殖和分化,从而实现损伤修复。因此,组织干细胞的衰老不可避免受到内在因素和外在环境的影响。

3.1 组织干细胞衰老的内在因素

个体衰老过程中,组织干细胞由于长期暴露于内源性或外源性毒性物质中,因而DNA损伤不断积累。研究显示,造血干细胞和骨骼肌干细胞中DNA双链断链标志物γ-H2A.X(phosphorylation of Ser-139 of histone H2A.X molecules)的表达水平随着年龄的增加而升高[6,20]。DNA损伤的不断积累会导致细胞应对损伤修复的能力降低,这可能是衰老造血干细胞重构造血系统和克隆形成能力降低的一个主要原因[24-25]。端粒长度缩短是细胞衰老的关键因素。造血干细胞在经过多次移植后端粒长度明显缩短,降低了其自我更新和分化能力[26]。端粒酶缺失的小鼠表现出早衰症状,但是恢复端粒酶活性后,神经干细胞再生髓鞘能力提高,神经元细胞生成增加,嗅球功能恢复正常[27]。线粒体电子传递链中氧化磷酸化会产生活性氧(reactive oxygen species, ROS),而活性氧对线粒体DNA(mtDNA)有毒性,反过来会加剧损害线粒体的功能[28-29]。因此,活性氧水平增高伴随着线粒体功能的异常被认为是细胞衰老的原因之一[28,30]。mtDNA聚合酶核酸外切酶缺失的小鼠模型显示,神经和造血前体细胞功能异常并出现早衰现象[28,31]。mtDNA突变后,造血干细胞的分化异常,导致髓性前体细胞增加,淋巴前体细胞减少,出现贫血症、巴球减少症以及过早衰老现象[32-33]。此外,mtDNA突变降低了神经干细胞的自我更新能力,使得维持静息的神经干细胞数量明显减少[32]。而当过

表达神经干细胞中线粒体抗氧化酶即过氧化物歧化酶2(superoxide dismutase 2, SOD2)后,很大程度上提高了神经干细胞的生存能力[33]。mtDNA突变的小鼠中,小肠干细胞增殖能力降低,凋亡水平升高,体外培养过程中其类器官(organoids)发育明显受到抑制[34]。

表观遗传修饰(包括组蛋白修饰、DNA甲基化、基因沉默等)调控着组织干细胞的命运和衰老进程。大量的研究显示,表观遗传修饰随着年龄的增加而发生改变,从而影响干细胞的功能。在老年个体中,造血干细胞数量的增加和分化能力异常与H3K4me3(trimethylation of histone H3 at lysine 4)水平增加相关[35]。随着年龄增加,骨骼肌干细胞中H3K4me3水平降低,而H3K27me3(trimethylation of histone H3 at lysine 27)水平却增加[36]。敲除或抑制造血干细胞去乙酰化酶家族Sirtuin 1基因后,细胞的增殖能力和DNA损伤水平明显升高,而使得长期造血干细胞(long-term hematopoietic stem cells, LT-HSCs)的数量降低[37]。Sirtuin 3主要参与调控线粒体酶乙酰辅酶A合成酶2(acetyl coenzyme A synthetase 2, AceCS2)和电子传递复合体1活性,在老年造血干细胞移植中起着重要作用[38-39]。过表达Sirtuin 3后可以抵消ROS对造血干细胞的损伤作用,抑制造血干细胞衰老发生[40]。年轻神经干细胞中高表达DNA甲基转移酶1(DNA methyltransferase 1, Dnmt1),一旦Dnmt1缺失,神经干细胞将产生低甲基化的细胞,出现提前分化现象[41]。类似地,毛囊干细胞中特异性敲除Dnmt1基因后,其毛囊干细胞发生耗竭并表现出衰老特征[42]。

除此之外,细胞周期抑制因子、mTOR(mammalian target of rapamycin)信号通路、FoxO家族(Forkhead-box class O)等都与老年个体中干细胞功能异常有关。衰老的造血干细胞、神经干细胞和骨骼肌干细胞中高表达p16[Ink4α]水平,降低了干细胞的增殖能力和分化能力,并促进静息的干细胞进入衰老状态[43-45]。FoxO是干细胞稳态调节的重要蛋白[46-47]。研究显示,FoxO敲除小鼠模型中,神经干细胞巢中干细胞数量耗竭,体外神经球的形成率和分化能力降低。条件性敲除FoxO1、FoxO3、FoxO4后,造血干细胞失去了静息状态,细胞凋亡比例增加,偏向髓系细胞分化等[48-49]。抑制细胞周期抑制因子p57[Kip2]的表达后,神经干细胞可迅速大量扩增,并伴随着神经生成[50]。造血干细胞中mTORC1(mTOR complex 1)表达水平随着年

龄的增加而升高, 雷帕霉素抑制mTORC1活性后, 可恢复衰老造血干细胞的自我更新能力[51]。反之, 在年轻小鼠中条件性敲除*Pten*(phosphatase and tensin homolog)可直接激活mTOR, 导致造血干细胞出现早衰和耗竭症状[52-54]。

　　组织干细胞通过不对称分裂产生一个与母细胞相同的干细胞和一个走向分化的前体细胞。因此, 细胞极性是干细胞自我更新的一个重要因素。老年的造血系统中, 造血干细胞高表达Wnt5a而抑制Wnt3a介导的经典Wnt通路, 从而导致下游Cdc42(cell division control protein 42 homolog)活性增强, H4K16Ac(acetylation of histone H4 on lysine 16)乙酰化程度降低, 微管蛋白、细胞极性标记等随机自由分布, 引起细胞极性的丢失, 破坏了造血干细胞的自我更新能力。Wnt5a抑制剂和Cdc42抑制剂处理细胞可达到阻断Wnt5a/Cdc42通路的目的, 从而实现造血干细胞的"返老还童"[55-57]。静息的骨骼肌干细胞接受到损伤修复信号时脱离G_0期, 进入细胞周期, 在FGFR1(fibroblast growth factor receptor 1)诱导下产生极化, 同时在p38和Notch信号调控下, 进行不对称分裂, 实现自我更新和修复或再生肌纤维的功能。但是在衰老过程中, 由于FGF2(fibroblast growth factor 2)的表达水平上升, p38α/β-MAPK信号异常增强, FGFR1受体失活, 无法诱导产生极化, 使得骨骼肌干细胞对称分裂产生两个活化的前体细胞从而降低干细胞巢中的细胞数量[16]。

3.2　组织干细胞衰老的外在因素

　　干细胞存在于特定的微环境(即"干细胞巢")中, 其干性维持和功能都受微环境的调控[58]。随着年龄的增加, 微环境组成细胞的衰老和成分的改变都会影响干细胞的功能。例如在造血系统中, 成骨细胞、网状细胞、间充质细胞、雪旺氏样细胞(Schwann-like cells)等构成了造血干细胞巢的骨内膜和血管区, 而在老年人或小鼠中, 间充质细胞的数量降低并向脂肪细胞分化, 改变了干细胞巢的组成成分, 进而影响了造血干细胞的功能[59-60]。老年小鼠的造血干细胞巢以及血液中高表达Rante/CCL5因子(regulated on activation, normal T cell expressed and secreted/C-C motif chemokine ligand 5), 促进了造血干细胞向髓系前体细胞的分化, 而在*CCL5*敲除的小鼠中则使得淋巴系细胞的数量增加[21]。干细胞巢微环境中TGF-β是调控造血干细

胞自我更新和静息状态的关键因子[60-61], 其表达水平随着年龄的增加而逐渐降低[62]。老年小鼠SGZ(subgranular zone)区高表达Wnt抑制因子*Dkk1*(Dickkopf 1), 导致神经干细胞分化神经元的能力降低。条件性敲除*Dkk1*后, 老年小鼠表现出与年轻小鼠类似的行为水平[62]。神经干细胞巢的内皮细胞中TGF-β的表达水平会随着年龄的增加而增加。通过TGF-β的抗体或者抑制剂抑制TGF-β信号通路后, 老年小鼠可表现出与年轻小鼠一样的神经生成能力[63]。骨骼肌干细胞处于肌纤维细胞和细胞外基质之间, 受肌原细胞分泌因子和细胞外基质的调控。老年肌纤维细胞高表达TGF-β(transforming growth factor-beta), 促进了细胞周期抑制因子的表达, 从而抑制了骨骼肌干细胞的增殖, 而抑制TGF-β-pSmad3信号通路足以促进星状细胞恢复其功能[64]。此外, 老年个体的肌纤维细胞在过表达TGF-β和FGF2的基础之上, 却降低了Notch信号通路的配体Delta1, 抑制了骨骼肌干细胞的增殖和损伤再生能力[65]。在皮肤隆突中, K6阳性细胞、真皮成纤维细胞和皮下脂肪细胞分泌的BMPs(bone morphogenetic proteins)等调控着毛囊干细胞维持静息状态[66]。当受到损伤刺激时, BMP2/4的表达水平下降, 而高表达PDGF-α(platelet-derived growth factor-alpha)、FGF-7、FGF-10、TGF-β2等因子, 这些信号作用于Wnt通路促进毛囊干细胞的活化并增殖[67-68]。个体衰老过程中, 脂肪组织中高表达BMP2/4, 使得BMP/Wnt信号通路的平衡遭到破坏, 而抑制了毛囊干细胞的活化[66,69]。

　　组织干细胞巢存在于血管附近, 因此血液中细胞因子、生长因子等都参与了干细胞功能的调控。例如, 年轻的造血干细胞移植入老年受体后, 其干细胞表现出偏向髓系前体细胞分化等特征[70]。比较年轻和老年血液发现, 老年个体血浆中高表达趋化因子CCL11(C-C motif chemokine ligand 11)不仅影响了学习和记忆能力, 也降低了神经干细胞神经生成的能力[71]。在老年小鼠中, TGF-β表达水平的提高抑制了骨骼肌干细胞的增殖和再生能力[72]。另外, 老年血液中Wnt因子的表达水平升高, 引起Wnt信号的激活, 从而导致活化的骨骼肌干细胞由肌细胞向肌纤维细胞分化而降低了肌肉损伤修复的能力[73]。

4　组织干细胞衰老特性的可逆性

　　随着对组织干细胞衰老认识的不断加深, 如何

减缓衰老问题或衰老疾病的发生越来越受到关注。研究发现, 通过移植或联体共生实验(parabiosis), 年轻的血液可逆转老年个体中衰老细胞的表型特征和功能。研究显示, 年轻和老年小鼠进行联体共生4~5周后, 老年小鼠肥大的心肌细胞体积明显缩小, 细胞功能明显恢复。置于年轻血循环后, 老年小鼠脑血管中血流量明显增加, 激活侧脑室SVZ区神经干细胞的增殖, 并提高嗅觉、学习和记忆等功能[70-71]。此外, 肌纤维细胞自身的再生能力、骨骼肌干细胞的数量、干细胞损伤激活水平和再生修复损伤肌原纤维的能力以及肌肉功能在年轻的微环境作用下都可以实现"返老还童"[72-73]。相反, 受老年血液环境的影响, 年轻干细胞的功能将受到抑制。通过对年轻和老年血液中细胞因子的表达分析, 发现血液中生长分化因子11(growth and differentiation factor 11, GDF11)、CCL11、TGF-β、Wnt、胰岛素样生长因子-1(insulin-like growth factor-1, IGF-1)等是调控组织干细胞衰老及衰老逆转的关键因子。通过联体共生实验或者体外注射降低老年血液中CCL11浓度, 升高GDF11水平, 可恢复神经干细胞和骨骼肌干细胞的再生功能, 并改善心肌细胞肥大[74,77-80]。加入拮抗剂抑制Wnt通路后可逆转衰老骨骼肌干细胞的功能, 抑制IGF-1信号通路可恢复造血干细胞的正常功能[73,81]。同样, 体外和联体共生模型显示, 通过重新激活Notch通路可逆转衰老骨骼肌干细胞的功能以及维持其干细胞数量[82-84]。在老年皮肤组织中, 抑制NF-κB(nuclear factor-kappa B)活性2周后, 可使一半以上的衰老相关基因的表达水平恢复至年轻状态, 而且衰老皮肤中表现的真皮层变厚、衰老细胞增多以及增殖能力降低等特征都发生了逆转[85]。我们的前期研究显示, 肝细胞随着年龄的增加而表现出衰老表型以及增殖和再殖能力的降低, 而将衰老的肝细胞移植入年轻的受体后, 可逆转其衰老表型使得衰老的肝细胞发生"返老还童"[86]。因此, 年轻的血液微环境对肝干细胞随着年龄的增加而发生的表型变化也会有一定的调控作用。

5　结语与展望

　　组织干细胞主要参与组织稳态的维持和损伤修复或再生功能。为了能够正确地执行其功能, 组织干细胞必须维持其不同细胞状态(静息、活化、分化和自我更新)间的平衡。然而, 随着年龄的增加,

组织干细胞由于在DNA损伤应激、组织微环境和血液环境的交互影响下, 失去了静息、活化、分化和自我更新的平衡, 改变了其增殖和损伤修复或再生的能力, 从而影响了组织稳态维持和再生修复的功能并促进相关疾病的形成。研究显示, 组织干细胞的功能受到许多信号通路的交互调控, 难以选择改变某一条信号通路的调控而逆转衰老干细胞的功能。但是, 通过给予年轻血液微环境、调控血液循环系统中GDF11、TGF-β、Wnt、IGF-1等因子的水平以及将衰老的干细胞通过重编程的方法都可以逆转衰老干细胞的表型特征和功能。随着人口老龄化的增加, 实现老年人群生活质量的提高将会产生巨大的社会和经济效益。尽管目前已经发现造血干细胞(图2)、骨骼肌干细胞(图3)等的衰老和逆转调控机制, 但是包括肝脏干细胞、皮肤干细胞等其他组织干细胞在机体衰老过程中的表型和调控机制以及其对衰老相关疾病的作用是知之甚少。因此, 深入地理解组织干细胞衰老及其衰老逆转的分子机制, 将有助于延缓或逆转老年机体组织的功能, 提高老年人群的生活质量甚至延长生命。

参考文献 (References)

1　Goodell MA, Rando TA. Stem cells and healthy aging. Science 2015; 350(6265): 1199-204.

2　Geiger H, Denkinger M, Schirmbeck R. Hematopoietic stem cell aging. Curr Opin Immunol 2014; 29: 86-92.

3　Clevers H. The intestinal crypt, a prototype stem cell compartment. Cell 2013; 154(2): 274-84.

4　Beumer J, Clevers H. Regulation and plasticity of intestinal stem cells during homeostasis and regeneration. Development 2016; 143(20): 3639-49.

5　Kuang S, Gillespie MA, Rudnicki MA. Niche regulation of muscle satellite cell self-renewal and differentiation. Cell Stem Cell 2008; 2(1): 22-31.

6　Sacco A, Puri PL. Regulation of muscle satellite cell function in tissue homeostasis and aging. Cell Stem Cell 2015; 16(6): 585-7.

7　Wahlestedt M, Pronk CJ, Bryder D. Concise review: Hematopoietic stem cell aging and the prospects for rejuvenation. Stem Cells Transl Med 2015; 4(2): 186-94.

8　Geiger H, de Haan G, Florian MC. The ageing haematopoietic stem cell compartment. Nat Rev Immunol 2013; 13(5): 376-89.

9　Dumont NA, Wang YX, Rudnicki MA. Intrinsic and extrinsic mechanisms regulating satellite cell function. Development 2015; 142(9): 1572-81.

10　Cosgrove BD, Gilbert PM, Porpiglia E, Mourkioti F, Lee SP, Corbel SY, et al. Rejuvenation of the muscle stem cell population restores strength to injured aged muscles. Nat Med 2014; 20(3): 255-64.
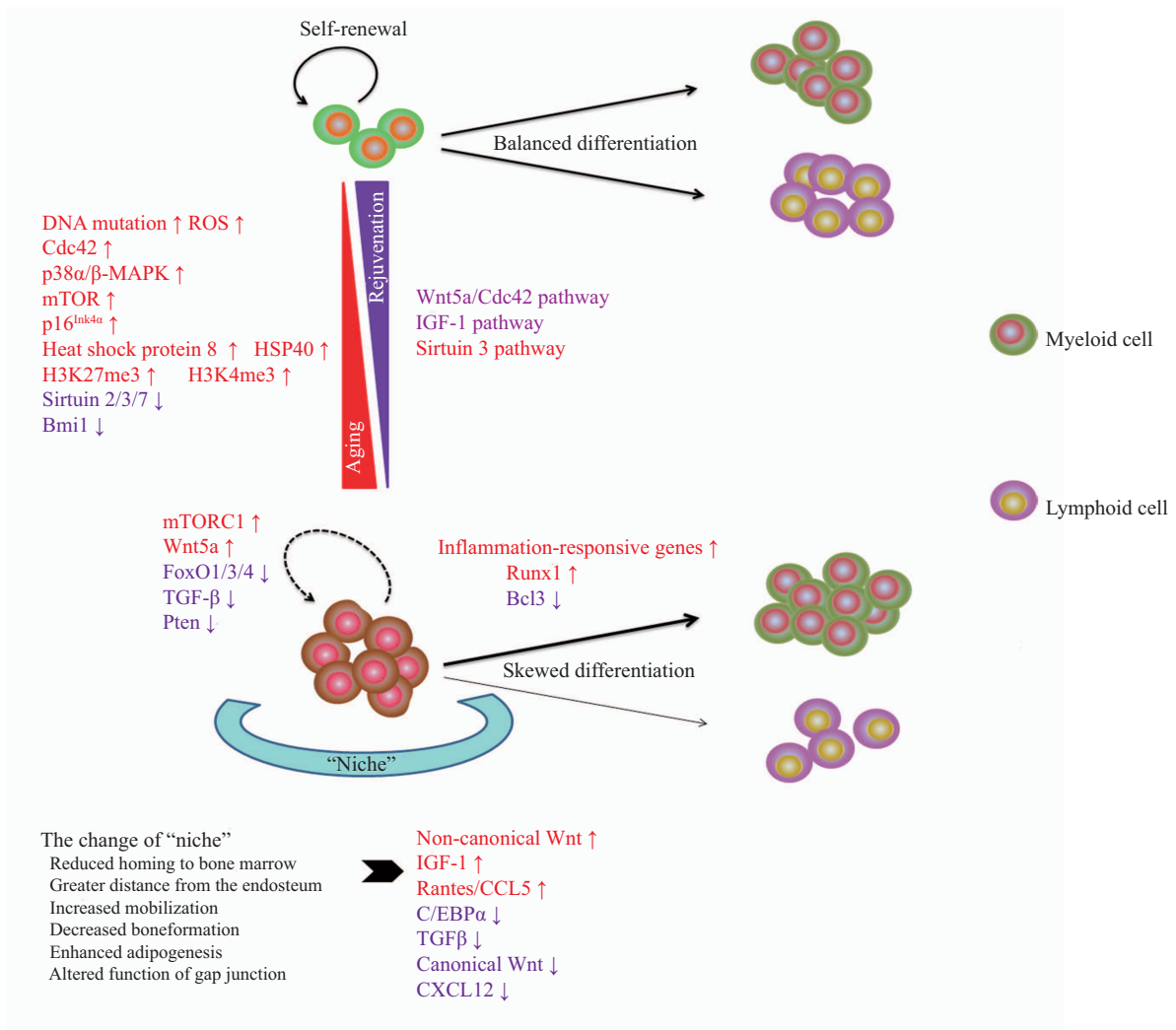
11　Chakkalakal JV, Jones KM, Basson MA, Brack AS. The aged

Self-renewal

Balanced differentiation

DNA mutation ↑ ROS ↑
Cdc42 ↑
p38α/β-MAPK ↑
mTOR ↑
p16^Ink4α ↑
Heat shock protein 8 ↑ HSP40 ↑
H3K27me3 ↑ H3K4me3 ↑
Sirtuin 2/3/7 ↓
Bmi1 ↓

Rejuvenation

Aging

Wnt5a/Cdc42 pathway
IGF-1 pathway
Sirtuin 3 pathway

Myeloid cell

Lymphoid cell

mTORC1 ↑
Wnt5a ↑
FoxO1/3/4 ↓
TGF-β ↓
Pten ↓

Inflammation-responsive genes ↑
Runx1 ↑
Bcl3 ↓

Skewed differentiation

"Niche"

The change of "niche"
　Reduced homing to bone marrow
　Greater distance from the endosteum
　Increased mobilization
　Decreased boneformation
　Enhanced adipogenesis
　Altered function of gap junction

Non-canonical Wnt ↑
IGF-1 ↑
Rantes/CCL5 ↑
C/EBPα ↓
TGFβ ↓
Canonical Wnt ↓
CXCL12 ↓

图2 造血干细胞衰老和衰老逆转的调控机制

Fig.2 Mechanisms of HSCs aging and rejuvenation

Young　Quiescence　Activation　Proliferation　Differentiation

γ-H2A.X ↑
p16^Ink4α ↑
p38 ↑
p21^Cip1 ↑
H3K27me3 ↑
Antioxidant activty ↓

Rejuvenation

Aging

Stem cell expansion
Stem cell proliferation
Stem cell commitment

GDF11 pathway
Oxytocin pathway
TGF-β-pSmad3 pathway

Wnt ↑
FGF2 ↑
p38α/β-MAPK ↑
Delta/Notch ↓

Wnt ↑
JAK/STAT ↑
TGF-β-pSmad3 ↑
Notch ↓

p38α/β-MAPK ↑
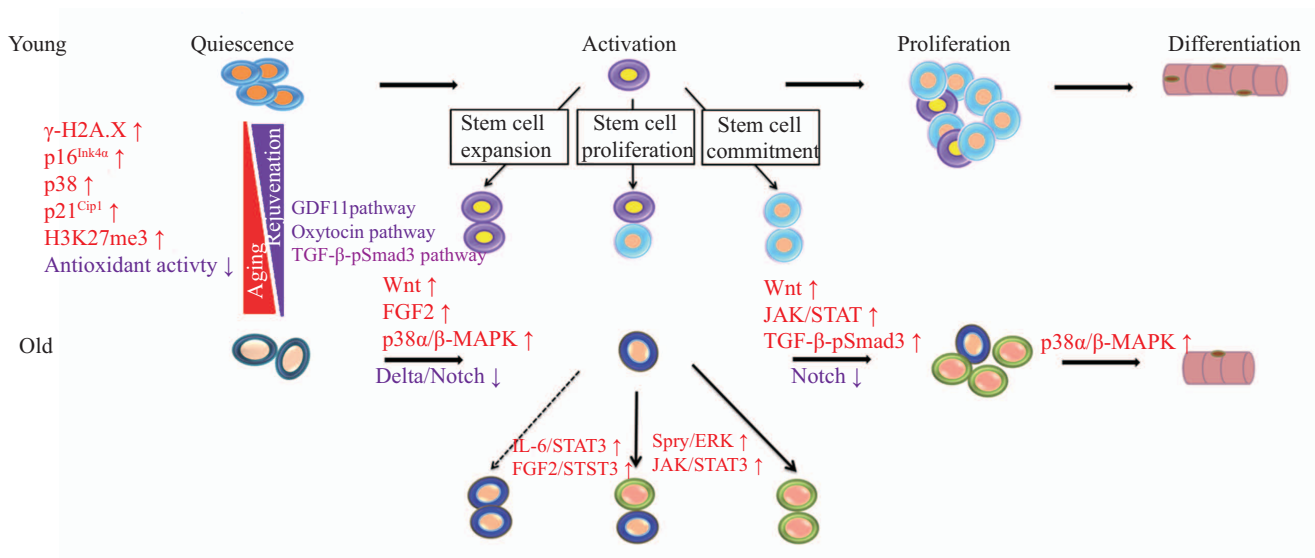
Old

IL-6/STAT3 ↑
FGF2/STST3 ↑

Spry/ERK ↑
JAK/STAT3 ↑

图3 骨骼肌干细胞衰老和衰老逆转的调控机制

Fig.3 Mechanisms of MuSCs aging and rejuvenation

niche disrupts muscle stem cell quiescence. Nature 2012; 490(7420): 355-60.

12 Silva-Vargas V, Crouch EE, Doetsch F. Adult neural stem cells and their niche: A dynamic duo during homeostasis, regeneration, and aging. Curr Opin Neurobiol 2013; 23(6): 935-42.

13 Wang YZ, Plane JM, Jiang P, Zhou CJ, Deng W. Concise review: Quiescent and active states of endogenous adult neural stem cells: Identification and characterization. Stem Cells 2011; 29(6): 907-12.

14 van Wijngaarden P, Franklin RJ. Ageing stem and progenitor cells: Implications for rejuvenation of the central nervous system. Development 2013; 140(12): 2562-75.

15 Shefer G, Van de Mark DP, Richardson JB, Yablonka-Reuveni Z. Satellite-cell pool size does matter: Defining the myogenic potency of aging skeletal muscle. Dev Biol 2006; 294(1): 50-66.

16 Sousa-Victor P, García-Prat L, Serrano AL, Perdiguero E, Muñoz-Cánoves P. Muscle stem cell aging: regulation and rejuvenation. Trends Endocrinol Metab 2015; 26(6): 287-96.

17 Conover JC, Shook BA. Aging of the subventricular zone neural stem cell niche. Aging Dis 2011; 2(1): 49-63.

18 Daynac M, Pineda JR, Chicheportiche A, Gauthier LR, Morizur L, Boussin FD, et al. TGFbeta lengthens the G₁ phase of stem cells in aged mouse brain. Stem Cells 2014; 32(12): 3257-65.

19 Artegiani B, Calegari F. Age-related cognitive decline: Can neural stem cells help us? Aging (Albany NY) 2012; 4(3): 176-86.

20 Cheng CW, Adams GB, Perin L, Wei M, Zhou X, Lam BS, et al. Prolonged fasting reduces IGF-1/PKA to promote hematopoietic-stem-cell-based regeneration and reverse immunosuppression. Cell Stem Cell 2014; 14(6): 810-23.

21 Ergen AV, Boles NC, Goodell MA. Rantes/Ccl5 influences hematopoietic stem cell subtypes and causes myeloid skewing. Blood 2012; 119(11): 2500-9.

22 Keyes BE, Segal JP, Heller E, Lien WH, Chang CY, Guo X, et al. Nfatc1 orchestrates aging in hair follicle stem cells. Proc Natl Acad Sci USA 2013; 110(51): E4950-9.

23 Chen CC, Murray PJ, Jiang TX, Plikus MV, Chang YT, Lee OK, et al. Regenerative hair waves in aging mice and extra-follicular modulators follistatin, dkk1, and sfrp4. J Invest Dermatol 2014; 134(8): 2086-96.

24 Rossi DJ, Bryder D, Seita J, Nussenzweig A, Hoeijmakers J, Weissman IL. Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. Nature 2007; 447(7145): 725-9.

25 Nijnik A, Woodbine L, Marchetti C, Dawson S, Lambe T, Liu C, et al. DNA repair is limiting for haematopoietic stem cells during ageing. Nature 2007; 447(7145): 686-90.

26 Allsopp RC, Cheshier S, Weissman IL. Telomere shortening accompanies increased cell cycle activity during serial transplantation of hematopoietic stem cells. J Exp Med 2001; 193(8): 917-24.

27 Jaskelioff M, Muller FL, Paik JH, Thomas E, Jiang S, Adams AC, et al. Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice. Nature 2011; 469(7328): 102-6.

28 Trifunovic A, Wredenberg A, Falkenberg M, Spelbrink JN, Rovio AT, Bruder CE, et al. Premature ageing in mice expressing defective mitochondrial DNA polymerase. Nature 2004; 429(6990): 417-23.

29 Kujoth GC, Hiona A, Pugh TD, Someya S, Panzer K, Wohlgemuth SE, et al. Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. Science 2005; 309(5733): 481-4.

30 Raule N, Sevini F, Li S, Barbieri A, Tallaro F, Lomartire L, et al. The co-occurrence of mtDNA mutations on different oxidative phosphorylation subunits, not detected by haplogroup analysis, affects human longevity and is population specific. Aging Cell 2014; 13(3): 401-7.

31 Ahlqvist KJ, Hamalainen RH, Yatsuga S, Uutela M, Terzioglu M, Gotz A, et al. Somatic progenitor cell vulnerability to mitochondrial DNA mutagenesis underlies progeroid phenotypes in Polg mutator mice. Cell Metab 2012; 15(1): 100-9.

32 Norddahl GL, Pronk CJ, Wahlestedt M, Sten G, Nygren JM, Ugale A, et al. Accumulating mitochondrial DNA mutations drive premature hematopoietic aging phenotypes distinct from physiological stem cell aging. Cell Stem Cell 2011; 8(5): 499-510.

33 Sakata H, Narasimhan P, Niizuma K, Maier CM, Wakai T, Chan PH. Interleukin 6-preconditioned neural stem cells reduce ischaemic injury in stroke mice. Brain 2012; 135(Pt 11): 3298-310.

34 Fox RG, Magness S, Kujoth GC, Prolla TA, Maeda N. Mitochondrial DNA polymerase editing mutation, PolgD257A, disturbs stem-progenitor cell cycling in the small intestine and restricts excess fat absorption. Am J Physiol Gastrointest Liver Physiol 2012; 302(9): G914-24.

35 Sun D, Luo M, Jeong M, Rodriguez B, Xia Z, Hannah R, et al. Epigenomic profiling of young and aged HSCs reveals concerted changes during aging that reinforce self-renewal. Cell Stem Cell 2014; 14(5): 673-88.

36 Liu L, Cheung TH, Charville GW, Hurgo BM, Leavitt T, Shih J, et al. Chromatin modifications as determinants of muscle stem cell quiescence and chronological aging. Cell Rep 2013; 4(1): 189-204.

37 Singh SK, Williams CA, Klarmann K, Burkett SS, Keller JR, Oberdoerffer P. Sirt1 ablation promotes stress-induced loss of epigenetic and genomic hematopoietic stem and progenitor cell maintenance. J Exp Med 2013; 210(5): 987-1001.

38 Brown K, Xie S, Qiu X, Mohrin M, Shin J, Liu Y, et al. SIRT3 reverses aging-associated degeneration. Cell Rep 2013; 3(2): 319-27.

39 Finkel T, Deng CX, Mostoslavsky R. Recent progress in the biology and physiology of sirtuins. Nature 2009; 460(7255): 587-91.

40 Bratic A, Larsson NG. The role of mitochondria in aging. J Clin Invest 2013; 123(3): 951-7.

41 Fan G, Martinowich K, Chin MH, He F, Fouse SD, Hutnick L, et al. DNA methylation controls the timing of astrogliogenesis through regulation of JAK-STAT signaling. Development 2005; 132(15): 3345-56.

42 Montgomery RL, Hsieh J, Barbosa AC, Richardson JA, Olson EN. Histone deacetylases 1 and 2 control the progression of neural precursors to neurons during brain development. Proc Natl Acad Sci USA 2009; 106(19): 7876-81.

43 Molofsky AV, Slutsky SG, Joseph NM, He S, Pardal R, Krishnamurthy J, et al. Increasing p16INK4a expression

decreases forebrain progenitors and neurogenesis during ageing. Nature 2006; 443(7110): 448-52.

44 Sousa-Victor P, Gutarra S, Garcia-Prat L, Rodriguez-Ubreva J, Ortet L, Ruiz-Bonilla V, et al. Geriatric muscle stem cells switch reversible quiescence into senescence. Nature 2014; 506(7488): 316-21.

45 Janzen V, Forkert R, Fleming HE, Saito Y, Waring MT, Dombkowski DM, et al. Stem-cell ageing modified by the cyclin-dependent kinase inhibitor p16INK4a. Nature 2006; 443(7110): 421-6.

46 Paik JH, Ding Z, Narurkar R, Ramkissoon S, Muller F, Kamoun WS, et al. FoxOs cooperatively regulate diverse pathways governing neural stem cell homeostasis. Cell Stem Cell 2009; 5(5): 540-53.

47 Renault VM, Rafalski VA, Morgan AA, Salih DA, Brett JO, Webb AE, et al. FoxO3 regulates neural stem cell homeostasis. Cell Stem Cell 2009; 5(5): 527-39.

48 Tothova Z, Kollipara R, Huntly BJ, Lee BH, Castrillon DH, Cullen DE, et al. FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. Cell 2007; 128(2): 325-39.

49 Miyamoto K, Araki KY, Naka K, Arai F, Takubo K, Yamazaki S, et al. Foxo3a is essential for maintenance of the hematopoietic stem cell pool. Cell Stem Cell 2007; 1(1): 101-12.

50 Furutachi S, Matsumoto A, Nakayama KI, Gotoh Y. p57 controls adult neural stem cell quiescence and modulates the pace of lifelong neurogenesis. EMBO J 2013; 32(7): 970-81.

51 Chen C, Liu Y, Liu Y, Zheng P. mTOR regulation and therapeutic rejuvenation of aging hematopoietic stem cells. Sci Signal 2009; 2(98): ra75.

52 Kalaitzidis D, Sykes SM, Wang Z, Punt N, Tang Y, Ragu C, et al. mTOR complex 1 plays critical roles in hematopoiesis and Pten-loss-evoked leukemogenesis. Cell Stem Cell 2012; 11(3): 429-39.

53 Lee JY, Nakada D, Yilmaz OH, Tothova Z, Joseph NM, Lim MS, et al. mTOR activation induces tumor suppressors that inhibit leukemogenesis and deplete hematopoietic stem cells after Pten deletion. Cell Stem Cell 2010; 7(5): 593-605.

54 Magee JA, Ikenoue T, Nakada D, Lee JY, Guan KL, Morrison SJ. Temporal changes in PTEN and mTORC2 regulation of hematopoietic stem cell self-renewal and leukemia suppression. Cell Stem Cell 2012; 11(3): 415-28.

55 Florian MC, Dorr K, Niebel A, Daria D, Schrezenmeier H, Rojewski M, et al. Cdc42 activity regulates hematopoietic stem cell aging and rejuvenation. Cell Stem Cell 2012; 10(5): 520-30.

56 Qian D, Jones C, Rzadzinska A, Mark S, Zhang X, Steel KP, et al. Wnt5a functions in planar cell polarity regulation in mice. Dev Biol 2007; 306(1): 121-33.

57 Schlessinger K, McManus EJ, Hall A. Cdc42 and noncanonical Wnt signal transduction pathways cooperate to promote cell polarity. J Cell Biol 2007; 178(3): 355-61.

58 Morrison SJ, Spradling AC. Stem cells and niches: Mechanisms that promote stem cell maintenance throughout life. Cell 2008; 132(4): 598-611.

59 Moerman EJ, Teng K, Lipschitz DA, Lecka-Czernik B. Aging activates adipogenic and suppresses osteogenic programs in mesenchymal marrow stroma/stem cells: the role of PPAR-gamma2 transcription factor and TGF-beta/BMP signaling pathways. Aging Cell 2004; 3(6): 379-89.

60 Yamazaki S, Iwama A, Takayanagi S, Eto K, Ema H, Nakauchi H. TGF-beta as a candidate bone marrow niche signal to induce hematopoietic stem cell hibernation. Blood 2009; 113(6): 1250-6.

61 Yamazaki S, Ema H, Karlsson G, Yamaguchi T, Miyoshi H, Shioda S, et al. Nonmyelinating Schwann cells maintain hematopoietic stem cell hibernation in the bone marrow niche. Cell 2011; 147(5): 1146-58.

62 Seib DR, Corsini NS, Ellwanger K, Plaas C, Mateos A, Pitzer C, et al. Loss of Dickkopf-1 restores neurogenesis in old age and counteracts cognitive decline. Cell Stem Cell 2013; 12(2): 204-14.

63 Pineda JR, Daynac M, Chicheportiche A, Cebrian-Silla A, Sii Felice K, Garcia-Verdugo JM, et al. Vascular-derived TGF-beta increases in the stem cell niche and perturbs neurogenesis during aging and following irradiation in the adult mouse brain. EMBO Mol Med 2013; 5(4): 548-62.

64 Carlson ME, Hsu M, Conboy IM. Imbalance between pSmad3 and Notch induces CDK inhibitors in old muscle stem cells. Nature 2008; 454(7203): 528-32.

65 Conboy IM, Conboy MJ, Smythe GM, Rando TA. Notch-mediated restoration of regenerative potential to aged muscle. Science 2003; 302(5650): 1575-7.

66 Plikus MV, Mayer JA, de la Cruz D, Baker RE, Maini PK, Maxson R, et al. Cyclic dermal BMP signalling regulates stem cell activation during hair regeneration. Nature 2008; 451(7176): 340-4.

67 Chi W, Wu E, Morgan BA. Dermal papilla cell number specifies hair size, shape and cycling and its reduction causes follicular decline. Development 2013; 140(8): 1676-83.

68 Morgan BA. The dermal papilla: An instructive niche for epithelial stem and progenitor cells in development and regeneration of the hair follicle. Cold Spring Harb Perspect Med 2014; 4(7): a015180.

69 Hsu YC, Li L, Fuchs E. Emerging interactions between skin stem cells and their niches. Nat Med 2014; 20(8): 847-56.

70 Vas V, Wandhoff C, Dorr K, Niebel A, Geiger H. Contribution of an aged microenvironment to aging-associated myeloproliferative disease. PLoS One 2012; 7(2): e31523.

71 Villeda SA, Luo J, Mosher KI, Zou B, Britschgi M, Bieri G, et al. The ageing systemic milieu negatively regulates neurogenesis and cognitive function. Nature 2011; 477(7362): 90-4.

72 Carlson ME, Conboy MJ, Hsu M, Barchas L, Jeong J, Agrawal A, et al. Relative roles of TGF-beta1 and Wnt in the systemic regulation and aging of satellite cell responses. Aging Cell 2009; 8(6): 676-89.

73 Brack AS, Conboy MJ, Roy S, Lee M, Kuo CJ, Keller C, et al. Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis. Science 2007; 317(5839): 807-10.

74 Katsimpardi L, Litterman NK, Schein PA, Miller CM, Loffredo FS, Wojtkiewicz GR, et al. Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors. Science 2014; 344(6184): 630-4.

75 Ruckh JM, Zhao JW, Shadrach JL, van Wijngaarden P, Rao TN, Wagers AJ, et al. Rejuvenation of regeneration in the aging

central nervous system. Cell Stem Cell 2012; 10(1): 96-103.

76 Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL, Rando TA. Rejuvenation of aged progenitor cells by exposure to a young systemic environment. Nature 2005; 433(7027): 760-4.

77 Sinha M, Jang YC, Oh J, Khong D, Wu EY, Manohar R, *et al*. Restoring systemic GDF11 levels reverses age-related dysfunction in mouse skeletal muscle. Science 2014; 344(6184): 649-52.

78 Loffredo FS, Steinhauser ML, Jay SM, Gannon J, Pancoast JR, Yalamanchi P, *et al*. Growth differentiation factor 11 is a circulating factor that reverses age-related cardiac hypertrophy. Cell 2013; 153(4): 828-39.

79 Mendelsohn AR, Larrick JW. Systemic factors mediate reversible age-associated brain dysfunction. Rejuvenation Res 2014; 17(6): 525-8.

80 Poggioli T, Vujic A, Yang P, Macias-Trevino C, Uygur A, Loffredo FS, *et al*. Circulating growth differentiation factor 11/8 levels decline with age. Circ Res 2016; 118(1): 29-37.

81 Mayack SR, Shadrach JL, Kim FS, Wagers AJ. Systemic signals regulate ageing and rejuvenation of blood stem cell niches. Nature 2010; 463(7280): 495-500.

82 Fry CS, Lee JD, Mula J, Kirby TJ, Jackson JR, Liu F, *et al*. Inducible depletion of satellite cells in adult, sedentary mice impairs muscle regenerative capacity without affecting sarcopenia. Nat Med 2015; 21(1): 76-80.

83 Kuang S, Kuroda K, Le Grand F, Rudnicki MA. Asymmetric self-renewal and commitment of satellite stem cells in muscle. Cell 2007; 129(5): 999-1010.

84 Wagers AJ, Conboy IM. Cellular and molecular signatures of muscle regeneration: Current concepts and controversies in adult myogenesis. Cell 2005; 122(5): 659-67.

85 Adler AS, Kawahara TL, Segal E, Chang HY. Reversal of aging by NFkappaB blockade. Cell Cycle 2008; 7(5): 556-9.

86 Wang MJ, Chen F, JX Li, Liu CC, Zhang HB, Xia Y, *et al*. Reversal of hepatocyte senescence after continuous *in vivo* cell proliferation. Hepatology 2014; 60(1): 349-61.