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干细胞的磁性标记研究进展

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摘要 干细胞在组织修复与再生医学中具有广阔的前景, 但是干细胞体内移植后分布、活性、分化方向、作用机制等认知的缺乏成为制约干细胞治疗发展的主要瓶颈, 因此, 干细胞体内示踪技术的发展对于解决上述问题将起到至关重要的作用. 目前利用磁性物质对干细胞进行标记后, 结合磁共振成像技术(magnetic resonance imaging, MRI)可以实现体外无创、安全、持续、动态的示踪观察, 示踪的效果取决于细胞内所携带磁性物质的含量、不同的磁性标记方式、细胞活性的维持. 本文将对干细胞磁性标记的不同方式、磁性物质的胞内代谢及对干细胞的影响等研究进展进行系统综述, 并结合现有的标记技术对如何提高干细胞磁性标记效率进行展望.

关键词 干细胞示踪, 干细胞标记, 磁共振成像, 磁性氧化铁纳米颗粒, 胞内铁代谢

干细胞是一类具有自我更新与分化功能的多能细胞, 体内移植后可实现损伤组织或器官的再生[1,2]. 已有研究报道, 干细胞可用于脊髓、大脑、肝脏等器官损伤的临床治疗, 并且显示良好的治疗效果[3~5]. 移植干细胞在体内的定位、活性与分化等情况是对干细胞治疗安全性与有效性的重要评估, 而归巢和迁移方向的偏差被认为是干细胞治疗中存在的主要问题[6,7], 因此需要长期持续有效且无创的体外跟踪监测手段. 磁共振成像技术(magnetic resonance imaging, MRI)相对于超声(ultrasound, US)、电子计算机断层扫描(computed tomography, CT)、正电子发射型计算机断层显像(positron emission tomography, PET)等其他医学影像方法具有无辐射、信号穿透衰减小、空间分辨率高与组织对比度大等优点[8]. 超顺磁氧化铁纳米粒子(superparamagnetic iron oxide nanoparticles, SPION)在生物医学领域有着广泛应用, 包括药物递送[9]、细胞分选[10]与肿瘤热疗[11]等, 并且由于其具有生物相容性好、对比度强等特点成为目前最常用于细胞标记的MRI示踪剂[12,13]. 尽管如此, SPION应用于干细胞示踪的临床试验依然面临着许多亟待解决的问题, 例如如何在不影响干细胞活性的同时提高SPION对干细胞的标记效率, 如何缓解由于胞内降解与细胞分裂所引起的SPION体内稀释等. 近年来的相关研究正在逐步破解这些难题, 本文将重点针对干细胞的磁性标记方式以及细胞与SPION的相互作用等方面展开综述.

1 干细胞的磁性标记方式

用于示踪的干细胞磁性标记方式主要分为两种: 一种是直接标记, 通过细胞摄入SPION使其携带磁性物质; 另一种标记方式为间接标记, 通过基因转染使细胞表达特定的蛋白质, 增强弛豫率及组织对比度, 提高T1或T2加权成像.

1.1 SPION标记干细胞

1.1.1 细胞对SPION的摄入机制

细胞对SPION的摄入过程分为两个过程: 一是

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Ye D W, Wang Q W, Zhang W G, et al. Recent progress in magnetic labeling for stem cell (in Chinese). Chin Sci Bull, 2017, 62: 2301–2311, doi: 10.1360/N972017-00458

细胞膜与颗粒的接触，二是颗粒由细胞膜向胞内的转运. 其中转运过程主要由网格蛋白和小窝蛋白介导，小窝蛋白尺寸为60~80 nm，不适于大尺寸颗粒的转运，相比之下网格蛋白介导的内吞方式更为广泛，除此之外还有细胞膜穿孔内吞、转运肽介导的直接内化与巨胞饮作用等转运方式[14,15]. 细胞的标记效率不仅取决于细胞的性质(膜的特性以及细胞大小)，还受到颗粒性质(尺寸、形状、分散性、表面电荷)的影响[16]. 比如，颗粒的大小很大程度上影响了颗粒进入细胞的途径，有研究表明[17]，直径大于60 nm的纳米颗粒需要通过与大量受体结合后才可以驱动膜包裹的过程，但同时会限制其他纳米颗粒与膜的结合；直径小于30 nm的纳米颗粒只有与大量受体紧密聚集才能驱动膜包裹过程；而直径30~60 nm的纳米颗粒能够高效地驱动膜包裹过程. 此外，颗粒的形状对吞噬也有一定影响，Zhang等人[18]研究表明细胞对球形颗粒相比圆柱形颗粒有着更强的吞噬效果. 对于一些吞噬性的细胞，如巨噬细胞、中性粒细胞等，采用颗粒与细胞简单的共孵育就能够实现有效标记[19~21]，对于干细胞及其他内吞能力较弱的细胞，采用颗粒表面化学修饰、物理场介导作用等方法可以增强标记效率同时保持细胞原有活性.

1.1.2 SPION的表面修饰提高标记效率

SPION标记干细胞时材料本身往往存在两个方面的问题：一是SPION不稳定，颗粒间容易形成聚集；二是SPION与细胞膜间的相互作用弱. 使用一些具有生物相容性的材料对SPION表面进行修饰能够提高颗粒的稳定性、增强颗粒与细胞的接触. 目前广泛使用聚合物(聚乙二醇、多聚糖、聚乳酸)、抗原抗体、细胞穿透肽、无机材料硅[22,23]等对SPION进行修饰结构，作用方式如图1所示.

在SPION表面修饰抗原抗体是一种蛋白之间特异性结合的标记方式[24]，磁性颗粒上携带的配体能够靶向性地与细胞膜上的受体结合，引起局部细胞膜内陷形成小泡，增加细胞对SPION的摄入. RGD三配体、叶酸、转铁蛋白、肝素[25,26]等配体连接在磁性颗粒表面可以靶向表达特定受体的细胞，使得SPION在细胞的含量提高3~4倍，标记量很大程度上取决于细胞表面受体的表达情况[27]. SPION还能够与一些蛋白质结合进而促进细胞吞噬，HIV-1肽蛋白是一种能够自由通过细胞核核膜的物质，可以将颗粒直接转运到细胞内[28,29]. 最早，Weissleder研究组[30]合成了肽链来修饰葡聚糖包被的SPION，紧接着Jackson等人[31]将肽修饰的SPION标记间充质干细胞(mesenchymal stem cells, MSC)，移植到大鼠纹状体中实现了MRI的长时程观察. 然而蛋白质的修饰可能会引起体内的免疫反应，针对这个问题，使用从细胞本身获取的ATP对颗粒进行修饰可以减弱免疫反应带来的影响，由于ATP的一个部分对细胞膜的配体有很强的锚定作用，并且它还具有用于与生物活性分子缀合的偶联部分，在成像示踪上能够起到靶向探针的作用[32].

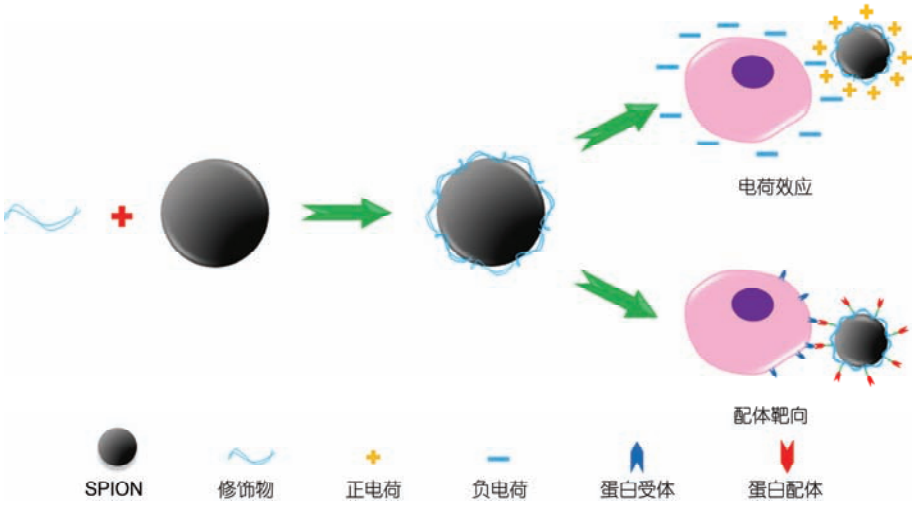

图 1 (网络版彩色)SPION表面修饰原理及作用方式

Figure 1 (Color online) Principle and modes of SPION surface modification

颗粒与细胞表面的电荷作用也是影响细胞吞噬颗粒的重要因素，通过聚阳离子的转染试剂(transfection agents, TA)涂覆表面能够使SPION表面携带正电荷，非特异性接触表面带有负电荷的细胞，从而通过电荷效应增加标记量[33]. Bulte等人[34]提出使用树枝状聚合物修饰SPION能够增加干细胞标记量；随后与Frank等人[35]合作，利用多聚赖氨酸(poly-*L*-Lysine, PLL)、硫酸鱼精蛋白(protamine, PRO)等聚阳离子试剂涂覆SPION，通过颗粒与细胞间静电吸附作用实现了单个细胞中30.1 pg铁含量的标记量. 相比使用单一材料涂覆SPION，两种材料的结合可以起到相辅相成的效果，肝素和鱼精蛋白可以通过静电作用形成体内稳定的复合物[36]，同时证实肝素与鱼精蛋白的不同比例混合修饰SPION对细胞的标记效率具有显著影响[37]. Kim等人[38]在SPION表面修饰了带有强正电荷的2-氨基乙基-三甲基铵(TMA)，对人源间充质干细胞(human derived mesenchymal stem cells, hMSC)进行标记，颗粒吞噬量大大提高. 不同于阳离子转染剂，最近有研究组制备了由柠檬酸盐包被的SPION，柠檬酸盐包被的SPION显示带负电荷，可能是电荷形成的非特异性相互作用使细胞与颗粒之间产生强亲和力，对hMSC实现了69.6±5.1 pg/cell的标记量[39]. 上述的转染试剂能够有效地增加标记浓度，但是潜在的生物毒性不容忽视，无机材料如二氧化硅生物相容性好、更安全，因此也常作为涂覆材料[40,41].

然而基于膜表面受体的内化增强只适用于表达特异性抗原/抗体的细胞，并且存在体内免疫、趋向于定位在细胞核等风险. 而有机聚合物对细胞具有潜在毒性，其毒性大小与使用的转染剂浓度成正比[42]. 另外，共孵育的方式所需时间长，不适于临床应用. 考虑到上述问题，不使用转染剂的标记方式将更具有临床应用价值，因此物理场介导的SPION摄入增强机制以其安全、快速、可调的优势成为目前广受关注的标记方法.

1.1.3 物理场介导的颗粒摄入量增加

物理场介导的颗粒摄入量增加是指在颗粒与细胞共孵育时无需外加转染试剂等材料，取而代之的是对其施加电场、声场、磁场等物理场，可以在增强标记量的同时缩短标记时间. 研究目前主要集中在3种作用：电脉冲穿孔(magnetoelectroporation, MEP)、超声脉冲穿孔(magnetosonoporation, MSP)、磁场作用，原理如图2所示：

MEP是指在颗粒与干细胞共孵育时施加瞬时的电脉冲波刺激干细胞，使干细胞的膜通透性由于电-机械作用发生瞬时改变，使细胞外大分子纳米颗粒等物质内化. 早在20世纪90年代，就出现了利用电脉冲将DNA等大分子物质转运到细胞中用于基因转染. Bulte研究组[43]利用电脉冲(75~400 V, 0.3~30 ms)对鼠来源的神经干细胞(neural stem cell, NSC)进行标记，对细胞作用较低电压(40 mV)、多个脉冲(n=10)后发现标记量增大2倍. 此后，他们对白细胞、淋巴细胞进行了磁标记，这两种细胞由于细胞质空间小难以标记，利用同样方式也观察到了吞噬量的增加，进

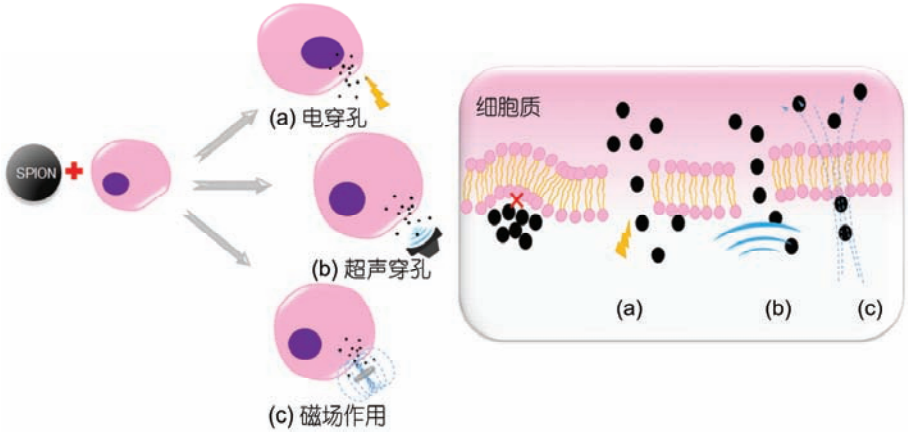

图 2 (网络版彩色)物理场介导的细胞标记. (a) 电穿孔促进细胞标记; (b) 声脉冲刺激促进的细胞标记; (c) 静、脉冲磁场作用的细胞标记

Figure 2 (Color online) Physical field mediated cell labeling. (a) MEP mediated cell labeling; (b) MSP mediated cell labeling; (c) static/pulsed magnetic field mediated cell labeling

一步说明了MEP的有效性[44]. 最近, Kim等人[45]利用电穿孔对脂肪间充质干细胞(adipose derived mesenchymal stem cells, ADMSC)进行标记, 合成了一种氧化硅涂覆的中空氧化锰纳米颗粒, 颗粒与干细胞共孵育时对其施加脉冲电场作用(电压 100 V, 脉冲波个数n=5, 占空比95%), MRI结果显示相对于普通孵育, 电脉冲穿孔具有更短的T1时间. 研究表明施加的电刺激根据细胞、颗粒的特性决定, 电刺激过大会导致细胞的死亡.

MSP是通过超声波作用, 瞬时可逆地改变细胞膜的通透性. 对于声穿孔的机制研究, 普遍认为细胞所处的培养环境(血液、培养液)中都含有微小气泡, 在超声作用时气泡的收缩振荡使细胞膜通透性增大. 最早, Bao等人[46]观察到卵巢细胞在低声作用压下细胞膜对大分子暂时打开, 而后一段时间内自动闭合. 此后, 利用超声空化进行细胞给药的治疗研究广泛开展, Qiu等人[47]首次利用超声对神经干细胞C17.2进行了磁标记, 对细胞作用5 min后标记量达到5.22 pg/cell, MRI成像观察到小鼠体内明显的信号增强. 该研究组[48]之后对超声参数进行了优化, 通过对比不同强度、占空比的超声得到最适参数, 标记量达到8 pg/cell. Lei等人[49]利用“封闭”的超声发射装置对神经干细胞C17.2进行了磁标记, 相比开放的装置, 该设计能够减少细胞污染的损失, 细胞标记量可以达到26.8 pg/cell, 并且较低的占空比更适用于细胞标记, 高的占空比会导致作用间隙过长的SPION从细胞中释放. 气泡作为一种介质是MSP作用的关键, 因此, 在孵育时额外加入适量微气泡可以起到进一步增强标记的效果. Fan等人[50]在气泡表面修饰了具有靶向性的配体, 能够与细胞表面表达的受体特异性结合, 气泡在细胞膜上定位后在超声作用下局部穿孔, 实现单细胞水平上标记量增加的同时能够控制颗粒在细胞内的分布. 最近, 本研究组[51]在微气泡表面修饰磁性颗粒, 超声作用下观察到超过80%的微泡在超声暴露后破裂, 区别于定位在溶酶体, 磁性颗粒大部分定位在细胞质中, 颗粒引起的毒性显著降低.

磁场作用是指在细胞与颗粒共孵育时施加磁场提高标记率. 最初研究发现将SPION与DNA结合, 在静磁场的作用下观察到SPION摄入增强, 由此DNA大量进入细胞实现了高效转染, 磁转染技术得到广泛关注和使用. Smith等人[52]在350 mT大小的静磁场下, 对比了无修饰SPION与穿透肽修饰的SPION标记细胞的差异, 结果显示标记了穿透肽的SPION在静磁场作用下标记量显著增多, 对内吞的机制分析表明磁场可能对网格蛋白起到了诱导作用. 对于磁场增加颗粒吞噬量的机制分析, 有研究通过温度的改变发现颗粒进入细胞并非被动扩散和磁力牵拉, 而是一种能量依赖的过程[53]. 此外, 脉冲磁场也被发现能够促进细胞标记, Lee等人[54]合成了一种负载蛋白质的SPION, 外加高强度的脉冲磁场标记贴壁的HELA细胞, 磁场强度为0.6 T, 作用3次, 每次间隔6 s. 结果显示24 h后脉冲磁场作用下标记量由1.9 pg/cell提高到7.6 pg/cell, 作者将这一现象称为“磁轰击”, 认为是由于磁性颗粒在脉冲磁场作用下剧烈而射入细胞质中. 物理场作用时的参数设置尤为重要, 施加的刺激过大会导致细胞死亡, 寻找最合适的标记条件是研究中的关键任务, 外加场刺激对细胞内部能量、机械力、膜性质的改变, 以及标记后细胞与SPION相互作用还有待进一步研究.

1.2 报告基因转染干细胞

SPION标记法一个主要的限制是检测到的信号不能够反映细胞的活性以及生物学特性, 导致检测的信号存在假阳性. 为了克服直接细胞标记的这些问题, 研究者开发了一种对细胞进行转染使其表达某种特定蛋白质用于MRI成像的方法, 所转染的基因称为报告基因.

目前使用的报告基因主要集中在两类蛋白质, 其中一类蛋白质是通过金属蛋白和金属离子转运蛋白的过表达以提高细胞内顺磁含量, 从而增强核弛豫率并在T1或T2加权MRI中产生高对比度[55,56], 铁蛋白报告基因不会被细胞分裂所稀释, 使得它们成为通过MRI跟踪靶细胞的理想方式. Campan等人[57]用人铁蛋白重链作为报告基因在体内跟踪干细胞, 他们用慢病毒载体对猪心脏球体进行转染实现人铁蛋白重链的过表达, 组织学分析表明铁蛋白过表达不影响细胞分化. Vande Velde等人[58]在啮齿动物脑中过表达铁蛋白实现T2*加权MRI的对比度增强. 有研究发现通过融合L和H亚基可以形成一个新的嵌合铁蛋白分子, 铁负载的增多使细胞表现出显著的MRI对比增强[59].

另一类是利用具有大量碱性或酸性氨基酸的蛋白质, 使其和水质子之间产生对比[60]. Gilad等人[61]

开发了一种非金属、可降解、富含赖氨酸的蛋白质(lysine-rich protein, LRP)报告基因，利用射频辐射非侵入性地标记与水交换的质子实现细胞活性状况的区分，并且在多个细胞分裂后也能够产生恒定的内源表达水平. 基于化学交换饱和转移(chemical exchange sturation transfer, CEST)的原理，富含精氨酸(47%精氨酸残基)的人类精蛋白-1也可以由此产生高磁共振对比度，由于这种蛋白质形成源于人类基因，因此具有很好的生物相容性[62]. 最近，Mukherjee等人[63]发现了一种用于MRI成像的新型报告基因：源于人的水通道蛋白的基因，基于增加组织水扩散的原理，当含有10%的细胞表达水通道蛋白就足以增强造影.

报告基因标记效率关键在于过表达蛋白质量的多少，以及这些特殊蛋白质本身性质. 而利用SPION磁性标记细胞时，颗粒在摄入后会经历细胞内的降解代谢，因此标记效率一定程度上会受到细胞内代谢的影响，以下将从SPION在细胞内的代谢活动方面进行展述，进一步对磁性标记的效率进行探讨.

2 SPION的细胞内代谢

SPION的细胞内代谢是指在细胞内经历连续的消化、吸收、外排过程，是标记后细胞内铁浓度不断减少的主要原因之一[64]. 因此明确SPION在细胞内的代谢机制可以在维持细胞活性的同时寻找提高标记效率的方法. 代谢途径会因内吞途径的不同而有所区别，例如，当内吞依赖网格蛋白介导时，网格蛋白内陷形成小泡，最后大部分经过内体进入溶酶体. 当内吞依赖小窝蛋白介导时，小窝蛋白由于体积较小导致颗粒摄取较慢，形成小泡随后进入到溶酶体、内质网、内体等多种细胞器中.

2.1 SPION的胞内降解

溶酶体是细胞降解SPION的主要细胞器，可以对聚合物及无机氧化物进行降解、胞吐，SPION在细胞内的转运消化是缓慢的过程，目前研究集中在SPION在细胞短时程的代谢分析，而代谢的长时程监测分析还很缺乏. 通常认为溶酶体将SPION消化成铁离子，随后在细胞质内通过Fenton反应催化细胞内的H_2O_2产生·OH，引发活性氧增加，由此产生了细胞毒性进而导致细胞凋亡[65~67]. 另外，SPION的毒性作用与细胞内微环境相关，Chen等人[68]提出SPION在不同pH下表现出不同的类酶活性，如图3所示，有别于溶酶体的酸性环境，细胞质中SPION由于其中性环境而表现出过氧化氢酶活性，催化H_2O_2产生H_2O，不会产生细胞毒性. 之后，普鲁士蓝纳米颗粒(prussian blue nanoparticles, PBNPs)被发现具有更高类酶活性，不同于Fe_3O_4，PBNPs在不同pH条件下均具有类超氧化物歧化酶的作用，可以清除超氧阴离子自由基，产生很小的细胞毒性[69]. SPION在细胞内长时程的代谢跟踪也受到了研究者的关注，持续监测的困难主要在于检测方式的缺乏. 最近有研究通过构建标记有SPION的干细胞球组织模型对SPION在细胞内长时程的代谢进行了定量检测，对溶酶体(200 nm)和模拟溶酶体建立的组织铁代谢模型(0.5 mm)这两种不同尺度的分析发现：SPION的降解几乎完全存在于溶酶体，27天后溶酶体里几乎不存在SPION，降解形成的铁离子分散在细胞质后，转化为转铁蛋白，这种铁元素的回收方式会使铁含量处于平衡稳定的状态[70]. 在标记细胞时，除了颗粒对细胞的影响值得我们关注，细胞内的复杂环境对SPION的结构和性质也会产生影响，例如进入细胞后由于涂层的降解、生物分子的吸附，颗粒间会产生聚集[71]，同时其升温能力、超顺磁性亦会产生不同程度的减弱[72].

这些研究对如何减少SPION内吞造成的细胞毒性具有启发作用. 可能的途径之一是改变颗粒内化途径，本研究组[51]在微气泡表面修饰磁性颗粒，利用MSP对HepG2细胞进行标记，研究表明超声穿孔

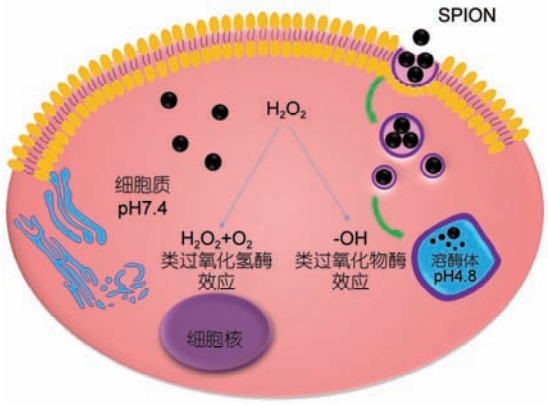

图 3 (网络版彩色)氧化铁颗粒的双类酶效应. Reprinted with permission from Ref. [68], Copyright © 2012 American Chemical Society

Figure 3 (Color online) Dual enzyme-like activities of SPION. Reprinted with permission from Ref. [68], Copyright © 2012 American Chemical Society

后大部分颗粒直接进入细胞质而不是通过内吞摄入, 细胞质内呈中性的酸碱环境使细胞内活性氧水平显著降低. 另一种途径是通过溶酶体逃逸, 使溶酶体中的SPION在被分解前释放到细胞质中, 从而减小由SPION分解引起的氧化应激. 目前研究表明可以通过两种机制实现溶酶体逃逸, 其一是造成溶酶体内外的渗透压差, 外部物质内流引起溶酶体涨裂, 例如聚乙烯亚胺[73]、聚乳酸[74]、聚酰胺[75]. 其二是通过能与膜融合的物质破坏膜结构, 例如流感病毒、黄蜂肽, 物质氯喹最近也被发现能够用于逃逸溶酶体[76].

2.2 SPION外排

在溶酶体中降解的部分SPION以铁离子的形式排到细胞质中, 而过剩的SPION将被包裹在囊泡中通过胞吐的方式排出细胞, SPION外排的过程通常与降解同步[77]. 研究发现纳米颗粒的尺寸越小越容易被外排, 14, 50和74 nm的金纳米颗粒, 胞吐的比例依次为35%, 10%和5%. 相同的研究还报道, 细胞对转铁蛋白包被的棒状纳米颗粒的胞吞作用较弱而胞吐作用较强, 因此细胞颗粒排泄可以通过大小和形状调制[78].

除了通过溶酶体的胞吐作用, 细胞在应急状态下形成的囊泡也能够将细胞内的SPION运送到细胞外, 紧接着释放出的囊泡会被周围的正常细胞再吞噬, 形成对其他细胞的再标记[79]. SPION的排出与外泌体也存在一定联系, 外泌体是多泡体与细胞膜融合后产生的胞外小囊泡, 直径40~100 nm. Froehlich[64]提到, 纳米颗粒的增加会引起氧化应激反应, 外泌体会随着细胞内的压力和氧化应激增多而增多, 从而纳米颗粒的增多会导致外泌体数量增加, 由此假设可以通过这种通路来促进纳米颗粒自身外排. 最近, Busato等人[80]利用细胞与SPION共孵育的方式获取被标记的外泌体, 研究结果表明普通孵育的方式就能够使排出的外泌体标记上SPION, 也证实了外泌体确实是一种外排方式.

3 SPION对干细胞生理功能的影响

SPION作为干细胞示踪的造影剂, 对于干细胞来说是一种“外来物”, 进入细胞内部后参与细胞的生长代谢, 在这个过程中, 细胞的活性、分化、迁移等性质的改变影响着细胞移植后在体内的治疗、安全性等问题[81~83], 因此标记后的细胞所具有的性质是否和正常干细胞一致值得我们关注.

Pongrac等人[84]为揭示SPION对于NSC的活性影响, 发现用SPION标记的NSCs中细胞内谷胱甘肽水平降低, 超氧化物歧化酶活性降低, 谷胱甘肽过氧化物酶上调, 线粒体膜电位超极化以及DNA损伤增加. 虽然表面涂层理应能够缓解SPION对于细胞的毒性作用, 但结果显示实验中所有SPION类型(未涂覆、涂覆有D-甘露糖或涂覆有聚L-赖氨酸)都会影响NSC, 这表明它们的主要细胞靶标是线粒体稳态, 同时, 最近有研究发现在一些间充质干细胞中检测到类似于凋亡小体的结构[85]. 针对迁移和分化方面的影响, Magnitsky等人[86]利用NSC和C17.2进行了研究, 结果显示用SPION标记后的原代NSC迁移能力受到一定程度限制, 而对C17.2细胞迁移能力影响不大. 约翰霍普金斯大学Walczak研究组[87]也发现标记后神经干细胞运动性的降低. 干细胞的分化影响目前主要集中在成骨分化的变化, 之前的一些研究表明细胞在磁性标记后会抑制软骨形成[88,89]. 本研究组[90]发现SPION可以激活经典的有丝分裂原活化蛋白激酶信号通路, 从而调节下游信号促进人类骨源性间充质干细胞在体外的成骨分化.

目前, 对于干细胞行为和性质的影响认知还不完全, 原因之一在于对其干性改变的分析没有系统的方法和手段, 另一方面, 有关细胞示踪方面的研究, 研究者通常将重点放在示踪效果, 而往往对细胞性质的影响有所忽视. 然而, 对干细胞性质的研究能够起到反馈的作用, 通过检测细胞的变化可以反向优化示踪剂以及标记手段.

4 总结与展望

干细胞治疗是当前最有潜力的组织修复方式, 现有的研究状况表明目前主要还停留在实验阶段, 临床研究比较缺乏, 大部分临床研究主要针对脊髓损伤和脑损伤两类模型, 标记的干细胞移植后成像信号显示大约可以维持3周, 7周之后成像信号基本消失[91], 持续数周的MRI监测结果同时表明移植的干细胞能够自主向损伤部位迁移[92]. Janowski等人[93]针对婴幼儿脑损伤模型将SPION标记的神经细胞经过脑室注射如脑内, 通过外部施加静磁场, 观察到细胞顺着磁场方向发生定向迁移.

通过一系列体内外实验验证, MRI成像是现在公认的比较合适的一种体外示踪方式, 因此本文对现有的干细胞磁性标记方式, 以及细胞内代谢、增殖分

化影响进行了描述，并且对不同标记类型的优缺点进行了分析. 应用于临床的标记物一定要保证具有很小的毒性、良好的生物相容性，在体内维持长循环以及高质量的成像效果. 研究证明基于颗粒表面修饰方式具有高标记量的特点，而物理场作用特点在于快速标记，结合两者的优势将是以后标记方式的发展趋势.

结合本研究组即将开展的工作，对于细胞的磁性标记提出了以下思考: 对于提高细胞内标记量，一方面要增强细胞对颗粒的吞噬，另一方面要减少细胞对颗粒的降解、外排. 标记的增加有赖于新型转染剂、报告基因的开发以及与物理场结合的协同作用，通过内吞途经摄入的SPION大部分会进入溶酶体分解，因此加入能使颗粒从溶酶体中逃逸的材料或许可以一定程度上减少颗粒的分解，实现细胞内更长时间的滞留. 同时，细胞外标记的方式也引起了关注，现有的细胞外标记磁珠方式多用于细胞分选[94]，细胞外标记优势在于不涉及胞内的降解，但是用于体内的问题在于细胞上负载了磁性颗粒在体内很容易被内皮系统识别，引起巨噬细胞吞噬，但我们猜想如果在磁性颗粒外部修饰细胞自身来源的物质是否可以避免巨噬细胞的清除，这有待更进一步的实验考证.

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Summary for “干细胞的磁性标记研究进展”

Recent progress in magnetic labeling for stem cell

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Stem cells are cells with self-renewal and multiple differentiation direction characters. Variety of studies have shown that stem cells have broad prospects in tissue repair and regenerative medicine. However, some aspects of stem cells *in vivo* transplantation developed slowly, for instance, distribution, activity, differentiation direction and mechanism, which have restricted the development of stem cell therapy. Thus, exploring novel technologies for tracing stem cell *in vivo* will play a vital role in solving the above scientific problems. When stem cell are labeled magnetically, we can obtain a noninvasive, safe, sustained and dynamic tracing *in vivo* by combining with magnetic resonance imaging (MRI). In addition, major factors influencing the efficiency of *in vivo* trace contain cellular content of magnetic material, the different methods for magnetic labeling and the maintenance of cell activity.

Here, we review the recent progresses on different ways for magnetic labeling stem cell, the metabolism of magnetic substances and the impact on cell systematically. For magnetic labeling, one of the major aspects focuses on labelling stem cells with magnetic materials like superparamagnetic iron oxide nanoparticles (SPION). Surface modification of SPION and exerting additional physical field when stem cell incubated with SPION both have enhanced label efficiency, however, it could not reflect the actual cellular activity *in vivo* which might bring out false-positive results by using this approach. Therefore, the use of reporter gene is developed, which could provide strong tissue contrast after stable transfection. However, whether the transfection alters stem cell properties remains to be proved. In addition, after SPION is uptaken by cells, the labeling efficiency will be decreased by a series intracellular degradation and metabolism, most particles is degraded by lysosomes into iron ions which could not provide effective MRI imaging. On the other hand, the cellular reactive oxygen species (ROS) level will increase substantially through Fenton reaction which has toxic effect on cell activity. Thus, SPION degradation in lysosome shows a huge negative influence on tracing, which could not be ignored. Recent research reveals that cell properties such as migration and differentiation will be altered after labeling with SPION.

By analyzing the working principles of existing label technology, we cover a detailed outlook on improving magnetic stem cell labeling. We prospect that two momentous issues should be considered to achieve efficient cell labeling and long-term tracing: Phagocytosis and metabolic pathway. More advanced methods ought to be developed to realize secure, rapid, massive cell label. Meanwhile, regulation of intracellular metabolism pathway is also critical for long-term cell tracing. To some extent, coating materials of SPION that allows particles to escape from lysosomes degradation will allow SPION longtime retention in cell. Moreover, labeling SPION extracellular also attracts our attention and its advantage is that SPION labelled on external of plasma membrane could not only be imaged, but also be escaped from cellular metabolism.

stem cell tracing, stem cell labeling, magnetic resonance imaging, magnetic iron oxide nanoparticle, intracellular iron metabolism

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