特约综述

我们实验室主要利用各种细胞模型、转基因小鼠模型和基因敲除小鼠模型, 研究脂肪细胞的发育分化及与肥胖和能量代谢的关系。目前, 主要研究方向有: 在C3H10T1/2细胞模型上研究BMP4对脂肪细胞定向作用的机理; BMP4转基因小鼠和BMP4敲除小鼠脂肪细胞发育分化和能量代谢特点; 脂肪细胞发育分化的转录调控。

诱导性产热脂肪的性质、诱导因素及潜在临床应用

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摘要 当机体能量摄入长期超过能量消耗时, 就会产生肥胖。人们通常把脂肪视为肥胖的“罪魁祸首”, 其实不能一概而论。哺乳动物体内一般存在两种类型脂肪组织, 分别是白色脂肪组织(white adipose tissue, WAT)和棕色脂肪组织(brown adipose tissue, BAT)。两者的解剖位置和形态特征各不相同, 最重要的是在能量代谢方面的作用也相反。白色脂肪组织的主要功能为储存能量, 白色脂肪含量显著增多是肥胖病人的主要特征之一; 棕色脂肪组织主要是消耗能量, 是产热组织。最新研究显示, 有第三种脂肪“诱导性产热脂肪即米色脂肪”的存在。米色脂肪具有有别于白色和棕色脂肪细胞的来源、基因表达谱、诱导因素等, 该文将对此进行综述, 并就诱导性产热脂肪对治疗肥胖的潜在临床意义进行讨论。

关键词 诱导性产热脂肪; 米色脂肪; 肥胖; UCP1; 适应性产热

Characterization of the Inducible Thermogenic Adipose Tissue and Its Potential Application in Treatment of Obesity and Related Complications

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Abstract Obesity results when caloric intake exceeds energy expenditure. The role in energy metabolism of the two types of adipose tissues, white (WAT) and brown (BAT) is well known. Storing energy in the form of triglycerides is the main function of white adipose tissue. Brown adipocytes are specialized to dissipate energy in the form of heat. The brown-like adipocytes that appear in classical WAT depots have been called “brite” (brown-

科技部973计划(批准号: 2011CB910201、2011CBA01103)和国家自然科学基金(批准号: 31271489、81170781)资助的课题

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This work was supported by the National Key Basic Research Project (Grant No.2011CB910201, 2011CBA01103) and the National Natural Science Foundation of China (Grant No.31271489, 81170781)

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网络出版时间: 2013-08-26 14:42 URL: http://www.cnki.net/kcms/detail/31.2035.Q.20130826.1442.003.html

in-white) or “beige” adipocytes and have characteristics similar to brown adipocytes, in particular the capacity for uncoupled respiration. Induction of the beige adipocytes represents a potential strategy for treatment of obesity, diabetes, and other metabolic diseases. This review describes the different determinants that have been linked to inducible thermogenic adipose tissue. Interesting therapeutic perspectives can also be expected from the use of inducible thermogenic adipose tissue.

Key words inducible thermogenic adipose tissue; beige fat; obesity; UCP1; adaptive thermogenesis

1 脂肪组织的分类

人们通常把脂肪视为肥胖的“罪魁祸首”, 其实不能一概而论。哺乳动物体内一般存在两种类型脂肪组织, 分别是白色脂肪组织(white adipose tissue, WAT)和棕色脂肪组织(brown adipose tissue, BAT)。最新研究显示, 有第三种脂肪即米色脂肪存在, 米色脂肪的发现为治疗肥胖和相关代谢疾病提供了新的思路。

1.1 白色脂肪

WAT主要位于腹部皮下、肾周、腹股沟、性腺、腹膜下, 其中的脂肪细胞为白色脂肪细胞。白色脂肪细胞为圆形或卵圆形, 白色脂肪细胞的直径变化比较大, 为25~200 μm。白色脂肪细胞内为单一的大脂滴, 占据细胞90%的体积, 细胞核位于细胞边缘, 线粒体较少、较小、较长。白色脂肪组织在哺乳动物体内分布较为广泛, 其主要功能为储存能量, 能够以甘油三酯的形式在白色脂肪细胞内储存机体摄取的多余能量, 因此, 白色脂肪含量显著增多是肥胖病人的主要特征之一; 同时, 白色脂肪组织还能作为内分泌器官分泌脂肪细胞因子(如leptin、adiponectin等), 作用于效应器官来调节机体的能量代谢。

1.2 经典棕色脂肪

传统观点认为: 在人类, 棕色脂肪只在婴儿期存在, 主要分布在肾周和背部, 成年后基本不再保留; 而多种哺乳动物(如小鼠等)在成年后体内仍保留一定量棕色脂肪组织, 这有利于它们更好地适应低温胁迫[1]。最新研究表明, 在成年人的颈部、锁骨上部和脊柱旁侧有活跃的棕色脂肪存在[2-6]。BAT中的脂肪细胞与骨骼肌细胞有共同的分化来源, 均源自Myf5+成肌细胞[7-8]。棕色脂肪细胞为多边形, 细胞直径大小为15~60 μm, 细胞内含有多个小脂滴, 富含线粒体。棕色脂肪细胞线粒体数目、线粒体大小、线粒体嵴的密度远大于白色脂肪细胞[9]。线粒体不仅使棕色脂肪组织表现为棕色(大鼠、小鼠等, 在人则不明显), 同时也是使棕色脂肪组织具有高度氧化能力的细胞器。贮存能量不是棕色脂肪组织的主要功能。棕色脂肪组织是产热组织, 主要是消耗能量。研究人员估计, 50 g活跃的棕色脂肪组织就可以将人体静息能量消耗提高20%。研究表明, 人体内BAT的量与体重指数(body mass index, BMI)呈负相关, 提示BAT在人体能量平衡调节中起重要作用。棕色脂肪的产热是通过交感神经系统调节的, 脂肪细胞表面密布交感神经纤维, 棕色脂肪细胞上有β3肾上腺素能受体也是棕色脂肪的特异性标志。

1.3 “米色脂肪”的发现

在白色脂肪[10]和肌肉[11]中存在诱导性棕色脂肪(inducible BAT)[12], 也被称为beige[13]或brite[14]。早在30多年前, 就有报道说在小鼠、大鼠和猫的白色脂肪组织内含有解偶联蛋白1(uncoupling protein, UCP1)阳性的多脂滴细胞存在, 类似棕色脂肪细胞, 这些细胞给予长时间冷暴露时, 上述表型会更明显[15-17]。β3肾上腺素能受体激动剂(CL316, 243)作用后也会产生类似现象[18]。后来, 把啮齿类动物的白色脂肪组织在某些诱导因素作用下出现的棕色样脂肪细胞称为brite(brown-in-white)或者米色脂肪细胞(beige adipocytes)[13-14,19]。这些细胞具有许多棕色脂肪的特性, 但不表达肌细胞中富集的基因, 提示这些细胞起源于非Myf5+的细胞系, 因此与经典的棕色脂肪有明显区别[14]。而在成人棕色脂肪主要是指“米色脂肪”。该种脂肪组织如豌豆大小, 散落在成人体内脊柱以及锁骨附近的皮肤下, 而成人棕色脂肪的激活实际指的是白色脂肪的棕色化[13]。正因为米色细胞在接受一定的刺激后可以燃烧脂肪并产热, 在加上成人以米色脂肪为主要产热脂肪, 因此如何激活米色脂肪细胞并作为治疗肥胖症和糖尿病的靶细胞已经受到越来越多的重视。下面我们将米色脂肪的来源、特性、诱导因素、潜在临床应用加以综述。

2 米色脂肪或诱导性产热脂肪的来源

分布在白色脂肪中的米色脂肪细胞来源还不清楚。理论上讲, 在白色脂肪组织中形成的棕色脂肪样细胞可能来源于组织当中已存在的干细胞; 来源于迁移而来的干细胞; 由白色脂肪细胞转分化而来的, 或者几种来源综合作用的结果。Seale等[7]的研究表明, Myf5+前体细胞不能分化为米色脂肪细胞。冷刺激会引起WAT中出现散在的棕色脂肪样细胞, 这些细胞表达UCP1, 却并非由Myf5+的细胞分化而来, 说明米色脂肪细胞与棕色脂肪细胞的来源不同。研究表明, 白色脂肪组织中的棕色脂肪样细胞的前体为PDGFRα3+的细胞[20], 用β3肾上腺素能受体激动剂(CL316, 243)处理后会出现增殖[21], 但由此产生的米色脂肪细胞在附睾周围脂肪中仅占多腔脂肪细胞的25%左右, 而在腹股沟脂肪中所占的比例更少[20-21]。这说明前体细胞的募集并不是肾上腺素能受体激活使白色脂肪棕色化的主要因素。Cinti等[9,19,22]用CL316, 243处理大鼠一周后, 腹膜后的脂肪细胞介于白色脂肪细胞和棕色脂肪细胞之间。并基于以下几点提出米色脂肪更可能由白色脂肪转分化而来的: 冷暴露后, 白色脂肪组织中, 减少的白色脂肪细胞数目与增加的棕色样脂肪细胞的数目相当, 而脂肪细胞的前体细胞的数目并没有增加; 由冷暴露或肾上腺素能受体激活剂作用后新形成的UCP1+棕色脂肪细胞介于白色脂肪细胞和棕色脂肪细胞之间, 且细胞形态和蛋白表达水平与组织的去甲肾上腺素水平相关。目前仍需要更多的研究来阐明米色脂肪究竟是来源于干细胞还是由成熟的白色脂肪细胞转分化而来的, 或者还有其他的来源。

3 米色脂肪的特点

米色脂肪和棕色脂肪关键区别在于, 棕色脂肪细胞表达高水平UCP1, 而米色细胞通常低表达UCP1。米色脂肪可被某些因素诱导, 产生高水平的UCP1, 进而消耗能量, 产热, 其效率会与棕色脂肪细胞几乎一样[13-14]。米色脂肪不表达经典棕色脂肪的特异性分子标志[14]。有研究根据不同部位脂肪对各种刺激的反应, 确定*Zic1*是棕色脂肪特异性的基因, *Hoxc9*是米色脂肪特异的基因, *Hoxc9*和*Hoxc8*是米色和白色脂肪细胞特异的基因, 而*Tcf21*是白色脂肪细胞特异的基因[23]。而Wu等[13,24]也报道了米色脂肪、白色脂肪和棕色脂肪差异表达的基因。米色脂肪特异性表达与发育相关的转录因子(Hoxc9)、脂类代谢通路重要成员(Slc27a1)以及细胞表面蛋白TMEM26和CD137。这种基因表达的差异进一步提示米色脂肪与经典的棕色脂肪的来源不同(表1)。

4 米色脂肪的诱导因素

4.1 UCP1转录调节

解偶联蛋白1(uncoupling protein, UCP1)是成熟棕色脂肪细胞的特异性标志, 也是白色脂肪棕色化或米色脂肪激活的主要表现。棕色脂肪组织消耗能

表1 棕色脂肪组织与米色脂肪组织的比较(根据参考文献[13-14,23]修改)

Table 1 A comparison between brown and beige fat (modified from references [13-14,23])

特点 Characteristics	棕色脂肪 Brown fat	米色脂肪 Beige fat	参考文献 References
Species	Small rodents (mice, rats) Human infants	Small rodents (mice, rats) Human adults Cats	[13]
Anatomical location	Nterscapular depot Perirenal depot Axial depot	Inguinal subcutaneous depot ?	
Morphology	Multilocular	Unilocular/multilocular	
Basal UCP1	High	Low	
Stimulated UCP1	High	High	
Thermogenic	Yes	Upon stimulation	
Anti-obesity	Yes	Yes	
Development origin	Pax7+/Myf5+	Pax7-/Myf5-	
Marker proteins	Zic1	Hoxc9 Slc27a1 CD137 TMEM26	[14,23]

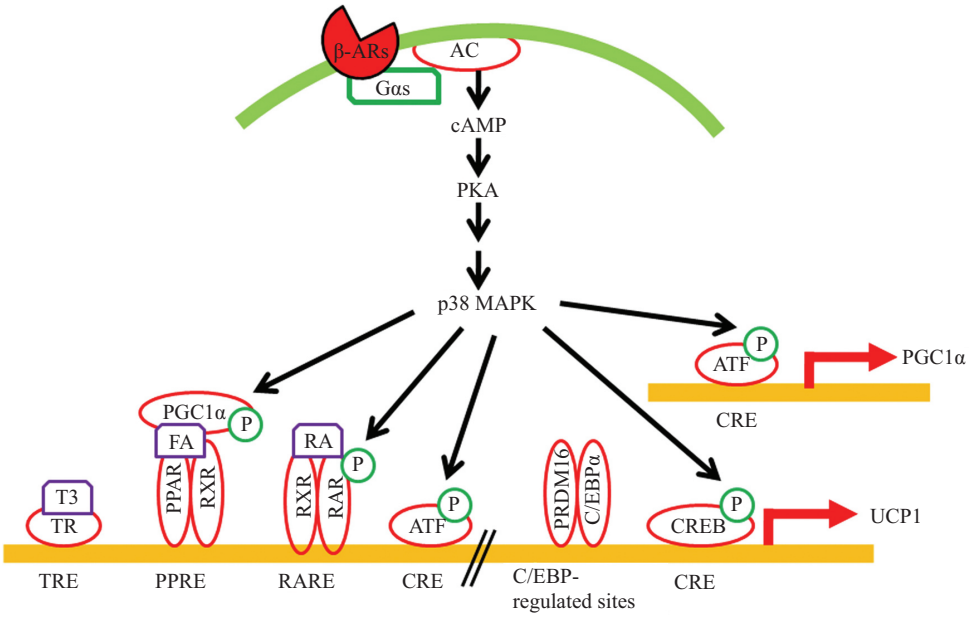

图1 UCP1的激活

Fig.1 Activation of UCP1 expression

量和产热是通过UCP1完成的。电子传递链产生H^+跨线粒体内膜的势能, 在偶联状态下, 势能驱动H^+通过ATP合成酶而重新回到线粒体基质中, 同时势能转化为ATP的化学能。UCP1是棕色脂肪细胞线粒体内膜的一种易化的质子通道, 可以使质子通过该通道回流, 从而破坏呼吸链电子传递过程中所建立的跨内膜的质子电化学梯度, 物质氧化与ATP生成解偶联, 不能驱动ATP合酶合成ATP, ATP合成减少, 从而使ATP的生成受到抑制, 而电化学梯度储存的能量以热能形式释放, 因此UCP1对于维持体温具有重要意义。

UCP1的生物合成主要在转录水平进行调节。通常促进UCP1转录激活的因素也会促进线粒体合成、脂肪酸合成和氧化等其他棕色脂肪细胞募集的一些表现[25-26]。因此, 了解UCP1的表达调控对于更好地理解米色脂肪的激活及诱导剂的作用机制非常重要。*UCP1*基因5′侧翼序列含有近端调节区域(靠近转录起始位点的)和远端增强子区域(220 bp, 鼠和人分别位于转录起始位点−2.5 Kb和−3.9 Kb)。近端调节区域含有C/EBP(CCAAT-enhancer binding protein)调节位点和cAMP调节元件(CRE, cAMP response element)[27-28]。远端的增强子含有另外2个cAMP(cyclic adenosine monophosphate)调节元件和介导UCP1转录激活的复杂的核受体结合位点组合, 如PPAR(peroxisome proliferator-activated receptor)激活剂、维甲酸类及甲状腺激素等的结合位点[29-31](图1)。

4.2 交感神经激活剂对米色脂肪的激活作用

棕色脂肪的产热和UCP1转录激活主要是通过去甲肾上腺素控制的。去甲肾上腺素通过作用于棕色脂肪细胞上的β3肾上腺素能受体, 与Gs蛋白偶联活化腺苷酸环化酶(adenylate cyclase, AC), 使细胞内cAMP浓度增加, 从而激活cAMP依赖的蛋白激酶(protein kinase A, PKA), 后者使激素敏感脂酶磷酸化, 最终促进脂滴中贮存的甘油三酯分解为甘油和脂肪酸, 同时也激活UCP1。PKA可通过p38 MAPK(p38 mitogen-activated protein kinases)依赖和非依赖的方式来激活UCP1。PKA使cAMP反应元件结合蛋白(cAMP response element-binding protein, CREB)磷酸化, 成为有活性的形式, 有活性的CREB能结合到启动子近端cAMP调节元件直接诱导UCP1和PGC1α的表达, 这是p38 MAPK非依赖方式。PKA也可通过活化p38 MAPK来发挥作用。p38 MAPK下游两个重要的底物分别是ATF-2(activating transcription factor 2)和PGC1α。一方面, p38 MAPK活化后, 使ATF-2磷酸化, 磷酸化的ATF-2通过各自启动子上的cAMP反应元件驱动UCP1和PGC1α的转录(图1)。另外, p38 MAPK活化后, PGC1α磷酸化并通过共激活因子PPARγ结合到UCP1启动子上, 进而促进UCP1的转录激活[32-34]。

β3肾上腺素能受体除了结合去甲肾上腺素, 介导交感神经系统在棕色脂肪细胞的作用外, 还介导冷暴露小鼠白色脂肪组织棕色化[19]。持续冷暴露或用β肾上腺素能受体激动剂可以使白色脂肪内出现棕色样脂肪细胞, 散在分布在白色脂肪组织内部[14,19]。慢性给与β3肾上腺素能受体激动剂(BRL26830A, CGP-12177, 316243)可以诱导UCP1在白色脂肪组织内的异位表达[10,18,22,35-36], 即出现米色脂肪细胞。β3肾上腺素能受体对白色脂肪组织棕色化的作用要比其对棕色脂肪的激活作用更重要, 因为缺失β3肾上腺素能受体会明显抑制冷暴露引起的白色脂肪组织内多脂滴细胞的形成和UCP1的表达, 但对棕色脂肪组织影响不大, 这种差异可能是因为棕色脂肪组织内其他β肾上腺素能受体代偿的结果[37]。瘦素也可以使白色脂肪组织棕色化[38-43]。选择性抑制瘦素敏感性神经元内的瘦素信号通路的负性调节因子Pten可以增强瘦素在中枢神经系统的作用[44]。瘦素对白色脂肪棕色化的作用主要依赖于其对交感神经系统的调节作用[39,41], 及刺激白色脂肪组织内β3肾上腺素能受体的表达[43]。瘦素也可能直接作用于脂肪细胞, 诱导白色脂肪细胞PGC1α[45]和PPARα[46]的表达。交感神经诱导的白色脂肪棕色化很有可能也是通过cAMP依赖的PKA激活及使p38 MAPK等磷酸化, 导致PGC1α等转录因子或共因子激活。β3肾上腺素能受体激动剂和瘦素也能使PPARα(peroxisome proliferator-activated receptor alpha)表达增加; PPARα缺失小鼠, β3肾上腺素能受体激动剂和瘦素引起的白色脂肪棕色化明显减弱[47-48]。此外, 也有研究显示, microRNA在交感神经诱导的白色脂肪棕色化过程中起作用。冷暴露或β3肾上腺素能受体激动剂能增加白色脂肪的基质血管组分(stromal vascular fraction, SVF)的miR-196a水平, 同时其靶基因*Hoxc8*减少[49-50]。miR-196a转基因小鼠腹股沟白色脂肪和附睾白色脂肪的UCP1及其他棕色脂肪标志明显增加, 但棕色脂肪则没有变化。这些转基因小鼠能量消耗明显增加, 对饮食诱导的肥胖耐受、胰岛素敏感性提高[49]。此外, 也有研究表明, COX-2是肾上腺素信号通路的一个效应分子, COX(cyclooxygenase)是前列腺素生成过程中的限速酶, 有COX-1和COX-2两种同工酶。其中COX-1是组成型表达的, COX-2是诱导表达型的, 其中COX-2对于WAT棕色化也是必需的[51-52]。

4.3 BMP家族成员对米色脂肪的激活作用

4.3.1 BMPs信号通路 BMPs(bone morphogenetic proteins)是TGF-β(transforming growth factor-β)超家族中的成员, 是一类功能广泛的生长因子。迄今为止已经发现的BMP超过20个。BMPs主要通过两种类型的受体传递信号, 即BMP-I型受体(BMPR-I)和BMP-II型受体(BMPR-II), 两类受体均为跨膜的丝氨酸/苏氨酸受体。I型受体包括BMPR-IA(又称activin receptor-like kinase 3, Alk3)、BMPR-IB(又称activin receptor-like kinase 6, Alk6)和活化素(activin)I型受体; II型受体包括BMPR-II、活化素II型及活化素IIB型受体[53-55]。受体与配体结合后, 它们形成由两对I、II型复合体组成的异四聚体激活受体复合物[[56-57]。在BMPR-I丝氨酸/苏氨酸激酶区域前方, 有一个包含特征性SGSGS标志的区域(GS区域)。GS区域在BMP信号传导中发挥重要作用, BMPR-II可使BMPR-I的GS区域磷酸化, 引起BMPR-I激酶的激活[58-59]。BMP受体下游最重要的信号分子是Smads[60-61]。活化的BMPR-I可募集受体调节Smads (receptor-regulated Smads, R-Smads, Smad1、Smad5和Smad8), 使之磷酸化。R-Smads磷酸化后共同介导Smad(common-mediated Smad, Co-Smad, Smad4)形成复合体并转位至细胞核内与不同的DNA连接蛋白结合, 包括共激活因子和抑制因子, 调节下游相关基因转录。抑制性Smads(inhibitory Smads, I-Smads, Smad6, Smad7)通过与BMPR-I结合或者与Smad4竞争结合磷酸化的Smad1/Smad5/Smad8, 生成无活性复合体两种方式发挥抑制作用。除Smad信号通路可被BMPs激活以外, 参与细胞生长分化的p38 MAPK也可被BMPs激活[57,62-64]。BMPR-I通过桥蛋白XIAP(X-linked inhibitor of apoptosis protein)、TAB1再与TAK1(MAPKKK)间接连接, TAK1激活MEK3和MEK6(MAPKK), 随后磷酸化并激活p38 MAPK[57]。活化的p38 MAPK可进入细胞核内调控相关基因表达。

4.3.2 BMP7对米色脂肪的激活作用 BMPs在多潜能干细胞定向中作用非常复杂, 并且依赖于BMPs的类型和浓度。Tseng等[65]比较多种BMPs发现, 仅BMP7能促进棕色脂肪细胞的分化过程中UCP1的表达。BMP7能够强烈激活p38 MAP激酶和它下游的ATF-2(activating transcription factor-2), 并通过PGC1来激活UCP1的表达及线粒体生成。此外, 如果用

BMP7诱导间充质干细胞向棕色脂肪细胞系定向, 并将这些细胞移植到裸鼠体内, 就可以形成棕色脂肪组织。BMP7敲除后, 小鼠胚胎棕色脂肪缺乏, 并且完全不表达UCP1; 而BMP7过表达能显著增加小鼠体内棕色脂肪, 增加能量消耗、减轻体重。由此可见, BMP7在褐色脂肪细胞分化过程中发挥着重要的作用。那么BMP7对白色脂肪的棕色化有没有作用呢, 研究发现β3-肾上腺素受体激动剂(CL316, 243)和BMP7可以协同作用促进棕色脂肪的特异基因在白色脂肪组织内表达[12]。提示BMP7对米色脂肪的激活也有作用。

4.3.3 BMP8B对米色脂肪的激活作用 最近有研究发现, BMP8B蛋白特异性地调控了棕色脂肪的产热活性。BMP8B在成熟的棕色脂肪细胞中高表达, 高脂饮食和肥胖可诱导BMP8B表达。当小鼠缺乏BMP8B时很难维持正常体温, 小鼠更易肥胖, 这种作用在高脂饮食时更明显。BMP8B能使棕色脂肪细胞对神经系统的激活反应更敏感。此外, 大脑特异区域给予BMP8B, 会提高棕色脂肪组织神经的激活, 棕色脂肪细胞燃烧更多的脂肪, 使小鼠体重减轻[66]。BMP8B对白色脂肪的棕色化作用还没有文献报道。

4.3.4 BMP4对米色脂肪的激活作用 在脂肪组织, BMP2和BMP4能增加间充质干细胞向前细胞定向[67-69], 或促进前脂肪细胞向成熟白色脂肪细胞的分化[70]。Tang等[69]研究表明, BMP-4能够诱导多潜能C3H10T1/2细胞完全向前脂肪细胞定向。BMP-4诱导产生的前脂肪样细胞经皮下注入裸鼠体内, 也可分化为与正常脂肪完全相同的脂肪组织。Bowers等[71]用5-氮杂胞苷处理C3H10T1/2细胞筛选到一株前脂肪细胞系A33细胞, 该细胞系高表达BMP-4, 并且这种内源的BMP-4对于多潜能干细胞获得前脂肪细胞特性是必需的。进一步研究发现, BMP4促进多潜能干细胞定向为前脂肪细胞主要是通过激活Smad信号通路, 而P38/MAPK信号通路也参与部分作用[72]。我们也发现, 赖氨酰氧化酶(lysyl oxidase, LOX)在此过程中起很重要作用[72]。BMP引起的多潜能干细胞向前脂肪细胞定向究竟是定向为前白色脂肪细胞、前棕色脂肪细胞或者米色脂肪细胞还不清楚, 因为相关的特异基因并未被检测。而体内实验显示, 脂肪组织内特异性高表达BMP4后, 皮下白色脂肪组织细胞出现棕色化改变, 皮下白色脂肪内棕色脂肪细胞的特异基因表达明显增加[73]。利用转基因小鼠模型在脂肪组织内特异性过表达BMP4后发现, BMP4促使小鼠白色脂肪细胞脂滴和细胞大小显著减小。进一步实验证实, 白色脂肪细胞内线粒体生成增加, 脂肪酸氧化基因表达增加, 从而导致小鼠整体基础氧耗增加、血脂降低和胰岛素敏感性增加等一系列代谢变化。另外, 体外前脂肪细胞3T3-L1分化过程中加入BMP4处理也可使细胞获得类棕色脂肪细胞的表型。干扰BMP4转基因小鼠白色脂肪内*PGC1α*的表达可以逆转BMP4所引起的白色脂肪棕色化的表型, 说明BMP4所引起的脂肪组织发育的变化是通过*PGC1α*这一关键基因起作用的。同样, 脂肪组织特异性敲除BMP4的小鼠出现脂肪组织体积增加、血脂水平增高、胰岛素敏感性降低等一系列变化, 从反方面证实了BMP4对于诱导白色脂肪棕色化和改变代谢的作用。研究也发现, 人体白色脂肪组织内*BMP4*的mRNA水平与体重指数(BMI)呈负相关, 提示脂肪组织内高表达的BMP4有利于控制体重。BMP4这种新功能的阐明可为临床干预肥胖和改善胰岛素敏感性提供新思路。

4.4 体育锻炼对米色脂肪的激活作用

运动是改善糖尿病、心血管疾病和其他代谢性疾病的重要手段。传统的观点认为, 作为主要运动器官的骨骼肌, 在人体内分泌、神经的调控下, 可增加葡萄糖消耗, 改善胰岛素敏感性。有研究发现, 肌肉组织中特异性PGC1α转基因小鼠的年龄相关的肥胖和糖尿病发生率较低, 生存期更长[74]。这种现象的发生机制一直困扰着科学家, 有的学者曾猜测, PGC1α可能刺激骨骼肌释放某些特殊因子, 并作用于其他器官。Ostrom等[75]通过比较*PGC1α*转基因小鼠和野生型小鼠的表达谱后发现, PGC1α可刺激肌肉组织中*FNDC5*基因表达。*FNDC5*基因编码一类膜蛋白, 后者经水解后释放入血, 成为一种新的激素Irisin。Irisin是PGC1α依赖的肌因子, 在所有已经测序的哺乳类动物种类中序列高度保守, 小鼠和人类的同源性为100%。由于是多肽类激素, 这也意味着Irisin可能通过某种高度保守的细胞表面受体发挥作用[75]。*PGC1α*基因敲除小鼠的Irisin水平下降了72%。运动可增加Irisin水平, 经3周运动后, 小鼠的血浆Irisin水平显著升高。对健康成人进行10周的耐力性训练后, 其Irisin水平比不运动者增加了2倍。小鼠和人类血浆Irisin水平的增加都与肌肉组织中其mRNA表达增加一致[75]。Irisin的增加对于运动导致

的白色脂肪棕色化是必需的, 可用于解释为什么运动会导致白色脂肪棕色化[75-76]。另有研究表明, 能量限制(calorie restriction)疗法可改善胰岛素抵抗, 降低大鼠脂肪含量, 却不能显著改善Irisin水平, 提示Irisin可能是运动改善代谢的独特机制[77]。无论体外还是体内, Irisin对白色脂肪的棕色化作用都非常明显, 但对棕色脂肪中的棕色脂肪特异性基因的表达影响并不大。Irisin可以有效减少肥胖和胰岛素抵抗, 对高脂饮食小鼠同时导入表达FNDC5的腺病毒后, 小鼠体重轻度下降, 糖耐量明显改善, 空腹胰岛素水平明显降低。Myostatin(Mstn)主要在肌肉中表达, 对调节肌肉的生长、发育、脂肪的沉积起重要作用。肌肉敲除*Mstn*小鼠因肌肉增生和肥大导致肌肉体积增大, 同时因为脂肪体积减少而变瘦。而白色脂肪组织中棕色脂肪的特异性基因明显增加(*UCP1*和*PGC1α*), 出现明显的白色脂肪棕色化, 同时米色脂肪的基因(*TMEM26*和*CD137*)表达也增加, 而这种变化正是由Irisin驱动的。在肌肉内敲除*Mstn*, 增加AMPK的表达和磷酸化, 从而激活PGC1α和FNDC5[78]。目前已证实, 人体内脊柱及锁骨附近皮下也存在米色脂肪细胞, 如果Irisin对人米色脂肪的作用与鼠米色脂肪细胞类似, 将有助于解释通常米色脂肪在人体含量虽相对较低, 而在寒冷刺激后出现PET显像阳性的脂肪沉积明显增加这一现象。因为Irisin的受体还未鉴定成功, 所以Irisin激活米色脂肪细胞的产生的具体机制还不清, 因此Irisin的受体的鉴定将会是今后其分子机制和临床应用的研究热点。

4.5 PPAR激动剂对米色脂肪的激活作用

*UCP1*基因的远端启动子含有PPAR反应元件, 既可以结合PPARγ又可以结合PPARα, PPARγ和PPARα均与RXR(retinoid X receptor)形成二聚体(图1)[79]。一般认为PPARγ控制与脂肪细胞分化相关的*UCP1*基因的表达, 而PPARα控制成熟脂肪细胞*UCP1*基因的表达[80]。在棕色脂肪细胞, PPARγ和PPARα激动剂不仅可以诱导*UCP1*基因的表达[26,79], 也可诱导*PGC1α*基因的表达[81-82]。PPARγ是白色脂肪细胞和棕色脂肪细胞分化的核心转录调节因子, 对棕色脂肪的发育是必需的[83]。*PPARα*则是棕色脂肪特异的标志基因, 许多PPARα的靶基因所编码的蛋白质参与脂肪酸氧化, 因此与棕色脂肪的脂质氧化能力有关[84]。PPARγ和PPARα内源性激活的配体可能为某些脂肪酸或脂肪酸衍生物。在产热因素刺激下, PPARα可被脂解产生的内源性脂类配体激活, 协同调节成熟棕色脂肪细胞脂解代谢相关基因和产热相关基因的表达[79-80,82]。也有研究显示, PPARδ也参与*UCP1*基因的表达调控[85-86]。除了在棕色脂肪中的作用, 在啮齿类动物PPARγ激动剂对白色脂肪棕色化也起作用[87-91]。在人类, PPARγ激动剂对白色脂肪棕色化也起作用[92]。PPARγ激动剂也可使白色脂肪组织来源的原代前脂肪细胞棕色化[14,93]。PPARγ激动剂诱导的白色脂肪棕色化涉及线粒体的生物合成、形成丰富的嵴、诱导*UCP1*及其他线粒体基因及在WAT中形成多腔脂肪细胞等[87,90-92]。也有证据表明, PRDM16蛋白的稳定性在PPARγ激动剂促进白色脂肪棕色化过程中发挥作用[94]。此外, 有研究显示, PPARγ激动剂也可能通过对PPARγ的转录后修饰发挥调节作用, 可以使PPARγ去乙酰化, 这个过程需要NAD依赖的去乙酰化酶Sirt1[95]的参与。而PPARα则通过诱导PGC1α和PRDM16促进白色脂肪棕色化[47,82,96]。PPARα激动剂(bezafibrate, GW7647, WY-14,643)可诱导人和小鼠白色脂肪细胞线粒体生物合成和脂肪酸氧化[94,97-98], 最终促进白色脂肪棕色化。

4.6 甲状腺激素对米色脂肪的激活作用

甲状腺素(thyroid hormone, TH)在能量代谢中具有重要作用[99]。TH包括T3和T4两种形式, T3是甲状腺素的活性形式, 由T4经Dio2(type II iodothyronine deiodinase)转化而来, 能够和TR结合从而激活TR的转录因子功能。Dio2可被交感神经系统激活, 而其底物T4则会强烈抑制Dio2的活性。棕色脂肪组织中UCP1的水平随着T3的水平改变而变化。TR由*TRα*和*TRβ*两个基因编码, 分别会产生TRβ1、TRβ2、TRβ3和TRα1、TRα2几种异构体, 其中TRβ的三种异构体和TRα1能够和T3结合[100], 而T3对UCP1的诱导依赖于TRβ[101]。在*UCP1*基因的远端增强子区域含有结合甲状腺素受体的反应元件(TRE)(图1)。甲状腺素与交感神经系统之间存在串话, 二者协同参与棕色脂肪的产热[102-103]。在棕色脂肪细胞内, 一方面, 肾上腺素能刺激通过激活DIO2, 以产生足够量的T3最大限度的产热。此外, 肾上腺素能系统也被T3调节, 放大肾上腺素能信号传导[104-105]。目前, 对于T3在白色脂肪组织棕色化过程中的作用研究还很少。

4.7 维甲酸对米色脂肪的激活作用

维甲酸(retinoid acid, RA)是一类重要的维生

素A代谢中间物, 包括9-顺式维甲酸(9-cis retinoid acid)和全反式维甲酸(all-trans retinoid acid)两种异构体。维甲酸通过RAR(retinoic acid receptors)和RXR行使功能, 具有广泛的生物学作用。9-顺式维甲酸能激活RAR和RXR, 而全反式维甲酸只能激活RAR[110]。维甲酸也是*UCP1*基因的转录激活因子[106-108]。研究表明, 维甲酸对于β肾上腺素能激动剂和PPARγ激动剂诱导人*UCP1*基因是必需的[109]。在*UCP1*启动子5′端的增强子上存在非经典的RA反应元件(RA response element)[111-112]和PPAR反应元件(PPAR response element)[31]。前者可以和RAR/RXR异二聚体结合, 激活相关基因的表达。后者可以和PPAR/RXR异二聚体结合(图1), 在RA与PPAR配体(可能是一些脂肪酸及其衍生物)同时存在的条件下达到最强的激活作用[113]。在小鼠胚胎成纤维细胞(mouse embryonic fibroblasts, MEF)来源的脂肪细胞加入视黄酸, 会以p38依赖的方式诱导UCP1的表达[114]。维甲酸处理的小鼠, 白色脂肪会出现棕色化, 表现为多脂滴细胞增加, 线粒体基因、产热基因、脂肪酸氧化相关基因增加[115]。非常有趣的是, 视黄醛有类似作用。视黄醛脱氢酶1(retinaldehyde dehydrogenase1), 又称为Aldh1a1, 是一种将视黄醛(retinaldehyde)转化为维甲酸的限速酶。研究发现, 在白色脂肪细胞中, 视黄醛通过选择性激活RAR、招募PGC1α并诱导*UCP1*启动子活性来诱导UCP1的mRNA和蛋白水平的表达。当白色脂肪细胞的Aldh1a1被抑制时, 这些细胞开始变得像棕色脂肪细胞。缺乏Aldh1a1或被抑制了Aldh1a1活性的小鼠变得更能耐受低温。更令人兴奋的是, 研究人员发现通过注射反义链分子来降低其*Aldh1a1*基因的表达, 可抑制饮食所致的小鼠肥胖: 小鼠的内脏脂肪减少、体重减轻, 同时葡萄糖水平降低并更耐受低温。这提示Aldh1a1可能成为肥胖症治疗的靶点。

4.8 饮食因素对米色脂肪的激活作用

饮食因素在白色脂肪棕色化中也起重要作用。饮食中限制某些必需氨基酸的摄入, 如甲硫氨酸会抑制腹部脂肪沉积, 减少脂肪, 增加能量消耗。在甲硫氨酸摄入限制的大鼠[116]和小鼠[117], 白色脂肪中UCP1被诱导。甲硫氨酸限制摄入增加能量消耗部分是通过增加β肾上腺素能受体信号通路来实现的[117]。在啮齿类, 辣椒素(capsaicin, 反式-8-甲基-N-香草基-6-壬烯酰胺, 8-methyl-N-vanillyl-6-nonenamide, 辣椒的主要成分)和一些非辛辣的辣椒素类似物(辣椒素酯类物质)也具有抗肥胖的作用。其作用机理主要包括以下几个方面: 激活棕色脂肪产热和全身的能量消耗[118]; 刺激白色脂肪细胞模型的脂类分解代谢[119]; 诱导白色脂肪的棕色化等[120]。辣椒素的生物学活性是通过激活SNS, 增强肾上腺髓质儿茶酚胺的分泌[121]以及结合并激活在胃肠道和内脏脂肪组织上的瞬时受体电位通道[transient receptor potential(TRP) channel][122-123]来发挥作用的。其中, TRPV1(TRP vanilloid type-1 channel)对于辣椒素预防饮食诱导的肥胖尤其重要[122]。白藜芦醇(Resveratrol, 3,5,4′-trihydroxystilbene)是多酚类化合物, 在葡萄和许多食用蔬菜中含有, 对于改善能量代谢具有重要作用。研究表明, 服用白藜芦醇30天后, 线粒体氧化磷酸化的相关基因上调, 而与炎症有关的细胞因子基因下调[124]。白藜芦醇能增加MEF来源的脂肪细胞的线粒体DNA含量、脂肪酸氧化和UCP1表达水平, 提示具有棕色化作用[125]。到目前还没有体内实验证实白藜芦醇促进白色脂肪棕色化。藻褐素是一种类胡萝卜素, 存在于可食用的褐藻的叶绿体中, 有抗氧化特性。藻褐素或者藻褐素丰富的海藻提取物单独或与其他选择性试剂结合可以抵抗食物诱导的肥胖, 减少腹部白色脂肪, 这种作用是通过刺激腹部白色脂肪棕色化而完成的, 包括诱导腹部脂肪UCP1 mRNA和蛋白质的表达、诱导脂代谢相关基因的表达[126-129], 而藻褐素促进白色脂肪棕色化, 但不影响棕色脂肪UCP1表达, 提示具有作用部位的特异性。

5 米色脂肪与肥胖的治疗及展望

当机体能量摄入长期超过能量消耗时, 就会产生肥胖。因此减少能量摄入和增加能量消耗是抵抗肥胖的重要方式。现有的减少能量摄入的药物并没有起到很好的作用, 因此通过提高能量消耗来减轻体重, 则越来越受到重视。而经典的棕色脂肪和最近发现的米色脂肪均具有产热或能量消耗作用。因此, 激活棕色脂肪或米色脂肪将为治疗肥胖提供新的思路。自从得知成年人体内也有棕色脂肪, 研究人员就希望能够通过激活BAT的产热功能来治疗肥胖及其相关疾病。但是人体内的BAT具有很大的年龄与个体差异, 随着年龄的增长, BAT逐渐减少。这一现象既可解释中老年肥胖发生的原因, 也为BAT

激活来治疗肥胖带来难题[130]。而最新研究显示, 成人棕色脂肪主要是指“米色脂肪”。成人棕色脂肪的激活实际指的是白色脂肪的棕色化[13]。正因为米色细胞在接受一定的刺激后可以燃烧脂肪并产热, 在加上米色脂肪是成人的主要产热脂肪, 因此如何激活米色脂肪, 使米色脂肪细胞作为治疗肥胖和糖尿病的靶细胞已经受到越来越多的重视。诱导白色脂肪棕色化或米色脂肪的活化有很多种方式, 如前所述运动、饮食、小分子药物等。运动和饮食控制是可行而又安全的方式。但鉴于运动要求持之以恒, 而很多肥胖患者不能坚持运动, 因此饮食和小分子药物, 如β3-AR激动剂、PPAR激动剂、维甲酸等, 将有可能成为更行之有效的减肥方式。但值得注意的是, 有些化合物在促进白色脂肪棕色化时还有其他作用, 因此这些试剂安全性有待进一步验证。目前, 白色脂肪棕色化或米色脂肪的实验大部分是在动物体内进行, 尤其是啮齿类动物, 啮齿类动物的脂肪与人类还有很大差别, 因此这些试剂真正用到人身上还有一段距离。然而米色脂肪细胞对全身能量消耗和系统代谢还有很多疑问, 仍需要进一步研究, 尤其在人类这种转变上还存在很大挑战。

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