PU.1转录因子调控前体脂肪细胞生脂的分子机制研究进展

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摘要 PU.1转录因子是保守的DNA结合蛋白*Ets*家族成员, 因其DNA结合区识别共有序列GAGGAA, 故该区又称为*Ets*结合区或*PU.1* box。*PU.1*主要在造血系统如髓细胞和B淋巴细胞中表达, 调节关键髓系基因的转录从而调控造血系统的分化。*PU.1*周身敲除后, 由于胎儿肝脏中缺乏B淋巴细胞和髓系细胞, 导致小鼠胚胎早期死亡, 表明*PU.1*是调控生命过程的关键转录因子。目前, 在脂肪细胞中*PU.1*对脂肪生成作用及机制的研究报道较少。*PU.1*与脂肪细胞脂肪生成, 与miRNAs、antisense RNA以及C/EBPα/β-PPARγ通路的调控关系将是今后研究的重点。

关键词 *PU.1*; antisense RNA; miRNAs; 前体脂肪细胞; 生脂

动物体脂生成机制的研究, 对改善畜牧业肉用家畜肉质以及治疗人类肥胖症有重要意义。*PU.1*周身敲除后, 由于胎儿肝脏中缺乏B淋巴细胞和髓系细胞, 导致小鼠胚胎早期死亡[1], 提示PU.1是调控生命过程的关键转录因子。尽管哺乳动物多数基因mRNA和蛋白表达呈正相关, 但有部分呈负相关[2]。目前, 对功能基因mRNA和蛋白水平表达相反现象的原因还不清楚。前期研究发现, PU.1转录因子在前体脂肪细胞中mRNA表达较高, 而蛋白表达较低, 在成熟脂肪细胞中则相反: 过表达*PU.1*显著抑制脂肪细胞脂肪生成[3]。推测其机理与*PU.1* antisense RNA或miRNAs的调控有关。此外, 前体脂肪细胞分化为成熟脂肪细胞, 亦即脂肪生成的过程中, *C/EBPβ* (CCAAT enhancer binding protein β)、*C/EBPα* (CCAAT enhancer binding protein α)和*PPARγ* (peroxisome proliferator-activated receptor γ)在其基因网络调控中发挥关键作用。因此, *PU.1*与脂肪细胞脂肪生成, 与miRNAs、antisense RNA以及*C/EBPα/β-PPARγ*通路的调控关系将是今后研究的重点。

1 *PU.1*与脂肪细胞生脂

PU.1 (spleen focus forming virus (SFFV)proviral integration oncogene, *spi-1*或*PU.1*)转录因子是保守的DNA结合蛋白*Ets* (E26 transformation-specific)转录调控因子家族成员, 因其DNA结合区识别共有序列GAGGAA, 故该区又称*Ets*结合区或*PU.1* box。*PU.1*主要在造血系统如髓细胞和B淋巴细胞中表达, 调节关键髓系基因的转录从而调控造血系统的分化[4-6]。目前, 在脂肪细胞中*PU.1*对脂肪生成及其作用机制的研究报道较少。Wang等[3]的研究表明, *PU.1*在白色脂肪细胞中表达并且明显抑制脂肪细胞分化, 但*PU.1*抑制脂肪细胞脂肪生成的机制还需进一步研究。研究中, 发现令人困惑的现象, 即在3T3-L1前体脂肪细胞中mRNA表达非常高, 而蛋白表达较低, 在成熟脂肪细胞中则相反。推测其调控机制有两方面: 其一, 与miRNA调控*PU.1*基因mRNA表达相关; 其二, 与*PU.1*基因反义转录物antisense RNA的调控有关。

脂滴生物学的研究是前沿领域的探索, 脂滴被认为是一类细胞器, 关于脂肪细胞内脂滴的形成、生长、功能和代谢的具体过程仍然不清楚[7], 而这正是脂肪细胞脂肪生成的关键。脂肪细胞脂肪的生成与前体脂肪细胞的分化程度直接相关。前体脂肪细胞分化为成熟的脂肪细胞, 细胞形态发生显著变化, 即由胞内无脂滴到出现脂滴, 再到多脂滴充满细

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胞, 最后胞内形成一个或数个大脂滴, 占整个细胞体积的95%以上[8]。因此, 前体脂肪细胞分化的过程也就是细胞脂肪生成的过程, 其实质是一系列标志基因(*C/EBPβ*、*C/EBPα*、*PPARγ*、*LPL* (lipoprotein lipase)、*aP2* (adipose fatty acid binding protein, *A-FABP*或*aP2*)、*HSL* (hormone-sensitive lipase)和*ATGL* (adipose triglyceride lipase)等)时序表达以及网络调控的结果[9]。其中, 以*PPARγ*和*C/EBPα/β*尤为重要, 是启动脂肪细胞脂肪生成的"必经终末通路"[10-11]。研究表明, 在3T3-L1和3T3-F442A前体脂肪细胞中过表达*PU.1*可抑制脂肪生成。因此, PU.1抑制脂肪生成的原因很可能是*C/EBPα/β-PPARγ*通路被抑制。

2 *PU.1*与miRNAs调控

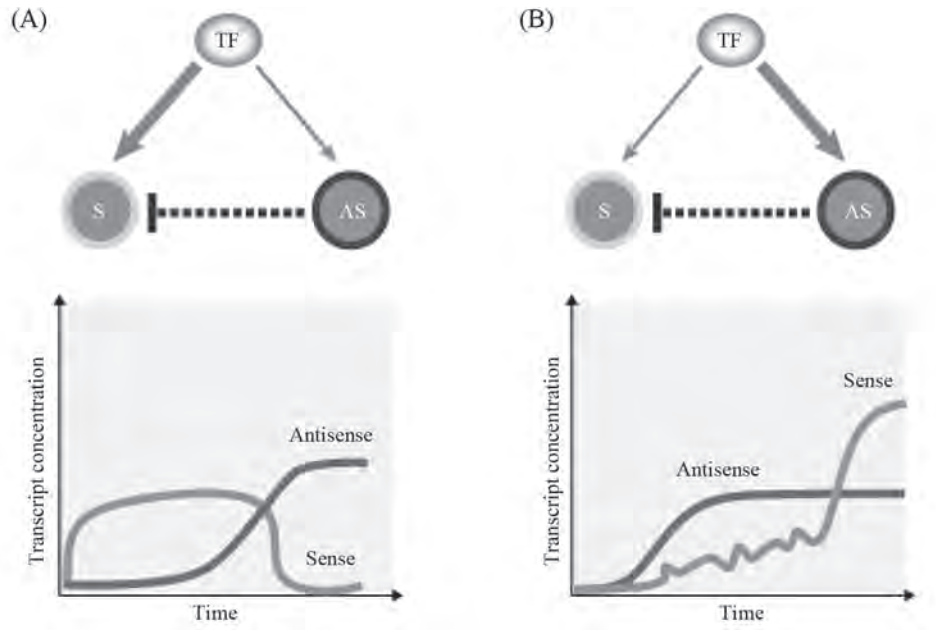
miRNAs属于非编码蛋白的RNAs家族, 调控多个靶基因, 涉及许多基本的生物学过程, 如胚胎发育、细胞增殖、分化和凋亡[12-14]。miRNAs调控靶基因表达的实质, 是与靶基因mRNA 3'和5'非翻译区结合, 降解mRNA, 抑制mRNA翻译[15]。*PU.1*表达与miRNAs调控是双向过程, 即部分miRNAs抑制*PU.1* mRNA的表达, 而*PU.1*则可以调控另一部分miRNAs表达。研究证实, 在髓细胞和B淋巴细胞中, miR-155和miR-124通过直接和间接方式抑制*PU.1* mRNA的表达[16-18], 而*PU.1*则激活miR-23a、miR-146a、miR-223和miR-424的转录[19-22]。在此, 我们关注的是: 抑制*PU.1* mRNA表达的miRNAs对脂肪细胞的脂肪生成是否有此功能呢?

在非脂肪细胞中, *PU.1*的表达受microRNAs的直接和间接调控。目前, 发现与之直接相关的是miR-155, 现认为miR-155的靶标是*PU.1* mRNA。Vigorito等[16]的研究结果表明, 在miR-155缺失的B细胞中*PU.1* mRNA表达增加, 而且*PU.1* mRNA在3'非翻译区以位点依赖结合方式与预测的miR-155结合位点直接结合, 因此, Vigorito等[16]认为, *PU.1*基因mRNA是miR-155的靶标。然而, miR-155是否是*PU.1*在前体脂肪细胞和成熟脂肪细胞中mRNA和蛋白表达相反的原因, 值得进一步探索。基于此, 在3T3-L1前体脂肪细胞和成熟的脂肪细胞中过表达及shRNA干扰miR-155, 结果却发现miR-155对PU.1蛋白表达没有影响。Ponomarev等[18]推测miR-124与*C/EBPα*的3' UTR (289-295, 345-351, 938-944)有3个结合区域, 并且研究发现, 其靶标是*C/EBPα*, 可直接抑制*C/EBPα*基因mRNA的表达, 进一步分析证实, 在巨噬细胞中过表达miR-124可通过*C/EBPα-PU.1*通路下调*PU.1*的表达。故miR-124对*PU.1*表达抑制是间接的, 推测其在脂肪细胞分化中对*PU.1*表达的抑制作用可能性较小。因此, 目前认为, miRNAs调控*PU.1*促进脂肪细胞脂肪生成的可能性不大。

3 *PU.1* antisense RNA调控

近来基因组研究揭示了真核基因可通过动物基因组双向转录[23], 即DNA正义链(sense strand of DNA)转录信使RNA (sense mRNA或mRNA), DNA反义链(antisense strand of DNA)转录反义RNA (antisense RNA)。以往认为天然的antisense RNA是没有功能的, 现在逐渐认识到其在基因精确调控方面具有重要的作用[24]。可以预测, 探索功能基因antisense RNA与sense mRNA特异性结合降解靶mRNA的调控机制, 将成为今后研究的热点(图1)。然而, 由于缺乏足够的实验证据, 对天然的antisense RNA的功能以及其作用机制了解很少。*PU.1*在前体脂肪细胞和成熟脂肪细胞中的mRNA和蛋白表达模式, 暗示可能存在antisense RNA调控机制。

为此, 我们设计了*PU.1*基因antisense RNA (A区为模板)和sense mRNA (C区为模板)的特异PCR引物(图2), 通过预实验发现, *PU.1*基因antisense RNA和sense mRNA在前体脂肪细胞和成熟脂肪细胞中表达不平衡, 前体脂肪细胞中antisense RNA表达显著高于sense mRNA, 成熟脂肪细胞中antisense RNA和sense mRNA表达下降, 但antisense RNA仍高于sense mRNA。结果提示很可能存在antisense RNA调控, 进一步探索将从sense mRNA和antisense RNA knockdown切入。近年来antisense RNA下调sense mRNA的表达已有报道, 如在斑马鱼、小鼠和人类中, *tie-1* (tyrosine kinase with immuno-globulin-like and EGF-like domains 1) antisense RNA与*tie-1* mRNA选择性结合下调*tie-1*的转录水平, 调控脉管发育[26]: 在人类肺腺癌细胞系中, 通过转染特异*FGF* (fibroblast growth factor) antisense siRNA可以以剂量依赖方式上调互补的转录本及编码蛋白[27-28]: 金黄色葡萄球菌中转染可诱导*mprF* (multiple peptide resistance factor) antisense RNA的质粒载体, 可以抑制*mprF*而重建细菌对达托霉素的易感性[29]。因此, 推测PU.1 mRNA和蛋白表达相反的原因是antisense RNA和

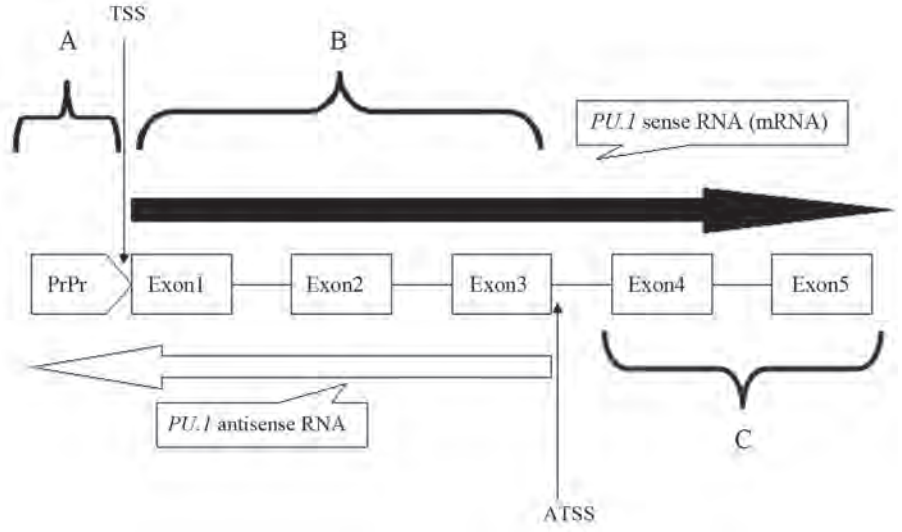


TF: 转录因子; AS: 反义转录本; S: 正义转录本。

TF: transcriptional factor; AS: antisense transcript; S: sense transcript.

图1　正义与反义转录本时序激活的差异[25]

Fig.1　Differences in activation time of the sense compared with the antisense transcript[25]



PrPr: *PU.1*基因临近启动子的区域; A: PrPr区域; B: 反义RNA和正义mRNA外显子1-3重叠序列; C: 正义mRNA外显子4和5; TSS: 正义转录起始位点; ATSS: 反义转录起始位点。

PrPr: *PU.1* gene proximal promoter; A: PrPr; B: antisense RNA and sense mRNA overlap sequence exons 1-3; C: sense mRNA exons 4 and 5; TSS: sense transcriptional start site; ATSS: antisense transcriptional start site.

图2　*PU.1*基因antisense RNA和sense mRNA位置模式图[33]

Fig.2　Model for position of mice *PU.1* gene antisense RNA and sense mRNA[33]

sense mRNA相互结合, sense mRNA降解, 蛋白表达被下调, 从而影响到脂肪细胞中脂肪的生成。

Antisense RNA和sense mRNA结合取决于连续配对碱基的数目。不同物种都存在一定的antisense RNA和sense mRNA对(表1)。生物信息学方法分析, 有15%~25%的哺乳动物基因sense mRNA和antisense RNA重叠(overlap), 产生配对的sense mRNA和antisense RNA对(以配对≥20 bp计)[30]。目前, 认为

表1 几个真核生物全基因组自然反义转录本的数量

Table 1 Genome-wide natural antisense transcripts in several eukaryotic species

物种 Species	重叠的转录本数 Transcripts involved in overlap	转录本总数 Total transcripts	重叠转录本的百分比(%) Percentage (%)	参考文献 References
Human	5 880	26 471	22	[34-35]
Mouse	12 519	43 553	29	[24]
Rat	548	11 332	5	[35]
Chicken	356	7 390	5	[35]
Drosophila	2 054	13 379	15	[35-36]
Rice	1 374	20 477	7	[37]
Arabidopsis	2 680	29 993	9	[38]
Nematode	76	14 406	0.5	[35]
Yeast	610	7 598	8	[39]

antisense RNA的调控机制可能与siRNA的作用模式基本一致, 只不过antisense RNA和sense mRNA形成的dsRNA被RNase H识别而剪切, 而非RNA诱导的沉默复合体(RNA-induced silencing complex, RISC)[31]。近期研究证实, 存在antisense RNA和sense mRNA杂交现象, 且可通过紫外光激发激活RNase H对antisense RNA和sense mRNA对进行剪切[32]。研究发现, 小鼠*PU.1* antisense RNA与sense mRNA在外显子1-3部分重叠互补, 外显子4和5为sense mRNA独有的部分, *PU.1*临近启动子区的PrPr为antisense RNA独有[33](图2)。*PU.1* antisense RNA和sense mRNA这样的结构易产生antisense RNA和sense mRNA对, 也为独立研究*PU.1* antisense RNA和sense mRNA调控基因功能提供了理论依据。

4 生脂过程中*PU.1*与*C/EBPα/β-PPARγ*通路的关系

既然*PU.1*基因mRNA和蛋白的表达模式很可能受*PU.1* antisense RNA调控, 那么*PU.1*抑制脂肪细胞脂肪生成的分子机制是什么呢？推测与*C/EBPα/β-PPARγ*通路调控相关。*PPARγ*和*C/EBPα/β*被认为是前体脂肪细胞向成熟脂肪细胞分化的“必经的终末通路”, 调控脂肪细胞脂肪生成标志基因的表达, 最终形成成熟的脂肪细胞[40]。研究发现, 在前体脂肪细胞中过表达*PU.1*, 则下调*PPARγ*的表达。在成纤维细胞向巨噬样细胞转化的过程中, *PU.1*抑制*C/EBPα*诱导的*PPARγ*和抵抗素(resistin)基因表达[41]。研究表明, 在非脂肪细胞系中, 与*PU.1*相互作用的转录因子包括*GATA-1* (GATA binding protein 1)[42]、*GATA-2* (GATA binding protein 2)[43]、*C/EBPα*[44]和*C/EBPβ*[41], 提示*PU.1*抑制脂肪细胞脂肪生成与*C/EBPα/β-PPARγ*通路调控有关。

PU.1转录因子可能参与*C/EBPα/β*和*PPARγ*转录与翻译水平的调控。PU.1蛋白包含三个功能域: N端的转录激活区, C端的DNA结合区和中间的PEST (proline, glutamic acid, serine and threonine)蛋白结合区。Feng等[41]认为, *PU.1*通过不同的途径应答细胞内外复杂多样的信号, 发挥对靶基因的调控作用。*C/EBPβ*基因转录产物mRNA中具有4个起始密码子(AUG), 因此有4种选择性转录起始位点(alternative translational initiation, ATI), 通过“核糖体间断扫描机制”可以形成AUG-1、AUG-2、AUG-3和AUG-4 4个*C/EBPβ*同型蛋白产物, 分子量分别为P38 (liver activating protein*, LAP*), P33 (liver activating protein, LAP), P20 (liver inhibitory protein, LIP)和P8.5 (liver inhibitory protein*, LIP*) kDa。其中AUG-1和AUG-2含有N端转录激活域及C端bZIP结构域, 参与基因的表达与调控, 而AUG-3和AUG-4仅含C端bZIP结构域, 因而可与全长型*C/EBPβ*蛋白结合, 发挥竞争性抑制作用, 负调节基因表达[45]。*C/EBPα*只有一种蛋白形态, 其它结构与*C/EBPβ*相似[46]。同样, 由于转录时所用的启动子和拼接方式的不同, PPARγ蛋白有γ1、γ2和γ3三种亚型, 其结构域、DNA结合域及配体结合域等相同, 作用相似。*PPARγ*具有脂肪组织特异性, 能被脂肪酸及外源性过氧化物酶体增殖剂激活, 而调控某些参与脂质代谢的酶的表达。研究发现, *C/EBPα/β*和*PPARγ*的转录活性和表达受到参与脂肪细胞分化过程中其它转录因子(如*GATA-2*)信号传导途径的调节[47], *PU.1*转录因子很可能也参与其中, 对细胞分化脂肪生成起重要作用。

PU.1与C/EBPα/β和PPARγ蛋白之间可能相互作用, 调控脂肪细胞脂肪生成。PU.1蛋白PEST区与其它蛋白相互作用调控PU.1及其靶蛋白的降解[48]。C/EBPβ蛋白可通过亮氨酸拉链结构与自身或者*C/EBPs*家族其他成员形成同源或异源二聚体, 从而改变其对DNA的结合力与亲和力, 进而调节自身及其靶基因的转录活性[49]。尤其是, LAP/LIP的比率常常决定特定基因的表达水平以及是否增强或受到抑制, *C/EBPβ*的这一特性说明了其对基因的转录调节的强弱很大程度上是通过这两类蛋白剪切体的相互拮抗而得以实现的[50]。RNA识别基序(RNA recognition motif, RRM)家族某些成员如*HuB* (*HTLV-I U5* repressive element-binding protein), 在脂肪细胞的分化过程中, 可与*C/EBPβ*直接相互作用, 通过改变LAP/LIP比率来调控细胞的增殖与分化[51]。因此, 推测PU.1蛋白的PEST区域是PU.1与C/EBPα、C/EBPβ和PPARγ蛋白之间相互作用的潜在位点。

5 展望

构建脂肪组织特异性*PU.1*敲缺小鼠(*aP2*-cre-*PU.1* mice)并对其脂肪组织发育的生理学研究, 将有利于从活体水平探索*PU.1*对脂肪形成的调控作用。进一步研究PU.1转录因子及其antisense RNA调控猪和小鼠脂肪细胞的生脂, 将有助于从离体水平揭示*PU.1*调控生脂的分子机制。因此, *PU.1*活体及离体水平的研究结果, 将有助于控制猪体脂沉积, 改善肉质, 同时也为人类肥胖症及相关疾病的治疗提供新靶点。

参考文献 (References)

1 Steidl U, Rosenbauer F, Verhaak RG, Gu X, Ebralidze A, Otu HH, *et al*. Essential role of Jun family transcription factors in PU.1 knockdown-induced leukemic stem cells. Nat Genet 2006; 38(11): 1269-77.

2 Conrads KA, Yi M, Simpson KA, Lucas DA, Camalier CE, Yu LR, *et al*. A combined proteome and microarray investigation of inorganic phosphate-induced pre-osteoblast cells. Mol Cell Proteomics 2005; 4(9): 1284-96.

3 Wang F, Tong Q. Transcription factor PU.1 is expressed in white adipose and inhibits adipocyte differentiation. Am J Physiol Cell Physiol 2008; 295(1): C213-20.

4 Wei F, Zaprazna K, Wang J, Atchison ML. PU.1 can recruit BCL6 to DNA to repress gene expression in germinal center B cells. Mol Cell Biol 2009; 29(17): 4612-22.

5 Takemoto CM, Brandal S, Jegga AG. PU.1 positively regulates GATA-1 expression in mast cells. J Immunol 2010; 184: 4349-61.

6 Choe KS, Ujhelly O, Wontakal SN, Skoultchi AI. PU.1 directly regulates cdk6 gene expression, linking the cell proliferation and differentiation programs in erythroid cells. J Biol Chem 2010; 285(5): 3044-52.

7 Robert VF, Tobias CW. Lipid droplets finally get a little R-E-S-P-E-C-T. Cell 2009; 139: 855-60.

8 Desruiseaux MS, Nagajyothi, Trujillo ME, Tanowitz HB, Scherer PE. Adipocyte, adipose tissue and infectious disease. Infect Immun 2007; 75(3): 1066-78.

9 Stephane G, Tseng YH, Kahn CR. Developmental origin of fat: Tracking obesity to its source. Cell 2007; 131: 242-56.

10 Otto TC, Lane MD. Adipose development: From stem cell to adipocyte. Crit Rev Biochem Mol Biol 2005; 40: 229-42.

11 Lefterova MI, Lazar MA. New developments in adipogenesis. Trends Endocrinol Metab 2009; 20(3): 107-14.

12 Krichevsky AM, Gabriely G. MiR-21: A small multi-faceted RNA. J Cell Mol Med 2009; 13(1): 39-53.

13 Bi Y, Liu G, Yang R. MicroRNAs: Novel regulators during the immune response. J Cell Physiol 2009; 218(3): 467-72.

14 Gangaraju VK, Lin H. MicroRNAs: Key regulators of stem cells. Nat Rev Mol Cell Biol 2009; 10(2): 116-25.

15 Ambros V. The functions of animal microRNAs. Nature 2004; 431(7006): 350-5.

16 Vigorito E, Perks KL, Abreu-Goodger C, Bunting S, Xiang Z, Kohlhaas S, *et al*. microRNA-155 regulates the generation of immunoglobulin class-switched plasma cells. Immunity 2007; 27(6): 847-59.

17 Thompson RC, Herscovitch M, Zhao I, Ford TJ, Gilmore TD. NF-κB down-regulates expression of the B-lymphoma marker CD10 through a miR-155/PU.1 pathway. J Biol Chem 2011; 286(3): 1675-82.

18 Ponomarew ED, Veremeyko T, Barteneva N, Krichevsky AM, Weiner HL. MicroRNA-124 promotes microglia quiescence and suppresses EAE by deactivating macrophages via the C/EBP-α-PU.1 pathway. Nat Med 2011; 17(1): 64-70.

19 Kong KY, Owens KS, Rogers JH, Mullenix J, Velu CS, Grimes HL, *et al*. MIR-23A microRNA cluster inhibits B-cell development. Exp Hematol 2010; 38(8): 629-40.

20 Jurkin J, Schichl YM, Koeffel R, Bauer T, Richter S, Konradi S, *et al*. miR-146a is differentially expressed by myeloid dendritic cell subsets and desensitizes cells to TLR2-dependent activation. J Immunol 2010; 184(9): 4955-65.

21 Fukao T, Fukuda Y, Kiga K, Sharif J, Hino K, Enomoto Y, *et al*. An evolutionarily conserved mechanism for microRNA-223 expression revealed by microRNA gene profiling. Cell 2007; 129(3): 617-31.

22 Rosa A, Ballarino M, Sorrentino A, Sthandier O, de Angelis FG, Marchioni M, *et al*. The interplay between the master transcription factor PU.1 and miR-424 regulates human monocyte/mac-

rophage differentiation. Proc Natl Acad Sci USA 2007; 104(50): 19849-54.

23 Werner A, Berdal A. Natural antisense transcripts: Sound or silence? Physiol Genomics 2005; 23(2): 125-31.

24 Katayama S, Tomaru Y, Kasukawa T, Waki K, Nakanishi M, Nakamura M, *et al*. Antisense transcription in the mammalian transcriptome. Science 2005; 309(5740): 1564-6.

25 Lapidot M, Pilpel Y. Genome-wide natural antisense transcription: Coupling its regulation to its different regulatory mechanisms. EMBO Rep 2006; 7(12): 1216-22.

26 Li K, Blum Y, Verma A, Liu Z, Pramanik K, Leigh NR, *et al.* A noncoding antisense RNA in tie-1 locus regulates tie-1 function *in vivo*. Blood 2010; 115(1): 133-9.

27 MacFarlane LA, Murphy PR. Regulation of FGF-2 by an endogenous antisense RNA: Effects on cell adhesion and cell-cycle progression. Mol Carcinog 2010; 49(12): 1031-44.

28 MacFarlane LA, Gu Y, Casson AG, Murphy PR. Regulation of fibroblast growth factor-2 by an endogenous antisense RNA and by argonaute-2. Mol Endocrinol 2010; 24(4): 800-12.

29 Rubio A, Conrad M, Haselbeck RJ, G C K, Brown-Driver V, Finn J, *et al*. Regulation of mprF by antisense RNA restores daptomycin susceptibility to daptomycin-resistant isolates of *Staphylococcus aureus*. Antimicrob Agents Chemother 2011; 55(1): 364-7.

30 Carninci P, Kasukawa T, Katayama S, Gough J, Frith MC, Maeda N, *et al.* The transcriptional landscape of the mammalian genome. Science 2005; 309(5740): 1559-63.

31 Wang JH, Hendry BM, Sharpe CC. Silencing genes in the kidney: Antisense or RNA interference? Nephrol Dial Transplant 2008; 23(7): 2115-8.

32 Tang X, Su M, Yu L, Lü C, Wang J, Li Z. Photomodulating RNA cleavage using photolabile circular antisense oligodeoxynucleotides. Nucleic Acids Res 2010; 38(11): 3848-55.

33 Ebralidze AK, Guibal FC, Steidl U, Zhang P, Lee S, Bartholdy B, *et al*. PU.1 expression is modulated by the balance of functional sense and antisense RNAs regulated by a shared cis-regulatory element. Genes & Dev 2008; 22: 2085-92.

34 Chen J, Sun M, Kent WJ, Huang X, Xie H, Wang W, *et al.* Over 20% of human transcripts might form sense-antisense pairs. Nucleic Acids Res 2004; 32: 4812-20.

35 Sun M, Hurst LD, Carmichael GG, Chen J. Evidence for variation in abundance of antisense transcripts between multicellular animals but no relationship between antisense transcription and organismic complexity. Genome Res 2006; 16: 922-33.

36 Misra S, Crosby MA, Mungall CJ, Matthews BB, Campbell KS, Hradecky P, *et al.* Annotation of the *Drosophila* melanogaster euchromatic genome: A systematic review. Genome Biol 2002; 3: 83.

37 Osato N, Yamada H, Satoh K, Ooka H, Yamamoto M, Suzuki K, *et al.* Antisense transcripts with rice full-length cDNAs. Genome Biol 2003; 5: R5.

38 Wang XJ, Gaasterland T, Chua NH. Genome-wide prediction and identification of cis-natural antisense transcripts in *Arabidopsis thaliana*. Genome Biol 2005; 6: R30.

39 David L, Huber W, Granovskaia M, Toedling J, Palm CJ, Bofkin L, *et al.* A high-resolution map of transcription in the yeast genome. Proc Natl Acad Sci USA 2006; 103: 5320-5.

40 Tang QQ, Zhang JW, Daniel LM. Sequential gene promoter interactions by C/EBPbeta, C/EBPalpha, and PPARgamma during adipogenesis. Biochem Biophys Res Commun 2004; 318(1): 213-4.

41 Feng R, Desbordes SC, Xie H, Tillo ES, Pixley F, Stanley ER, *et al*. PU.1 and C/EBPα/β convert fibroblasts into macrophage-like cells. Proc Natl Acad Sci USA 2008; 105(16): 6057-62.

42 Burda P, Curik N, Kokavec J, Basova P, Mikulenkova D, Skoultchi AI, *et al*. PU.1 activation relieves GATA-1-mediated repression of Cebpa and Cbfb during leukemia differentiation. Mol Cancer Res 2009; 7(10): 1693-703.

43 Zhang P, Behre G, Pan J, Iwama A, Wara-Aswapati N, Radomska HS, *et al.* Negative cross-talk between hematopoietic regulators: GATA proteins repress PU.1. Proc Natl Acad Sci USA 1999; 96(15): 8705-10.

44 Yumi F, Kana K, Ken S, Ryouichi O, Daniel G, Tenen K, *et al.* Role of myeloid transcription factors, C/EBPα and PU.1 in leukemogenesis by MLL-fusion oncogenes. Blood 2010; 116: 1568.

45 Xiong W, Hsieh CC, Kurtz AJ, Rabek JP, Papaconstantinou J. Regulation of CCAAT/enhancer-binding protein-beta isoform synthesis by alternative translational initiation at multiple AUG start sites. Nucleic Acids Res 2001; 29(14): 3087-98.

46 Ramji DP, Foka P. CCAAT/enhancer-binding proteins: Structure, function and regulation. Biochem J 2002; 365(Pt3): 561-75.

47 Takemoto CM, Brandal S, Jegga AG, Lee YN, Shahlaee A, Ying Y, *et al*. PU.1 positively regulates GATA-1 expression in mast cells. J Immunol 2010; 184(8): 4349-61.

48 Aikawa Y, Katsumoto T, Zhang P, Shima H, Shino M, Terui K, *et al.* PU.1-mediated upregulation of CSF1R is crucial for leukemia stem cell potential induced by MOZ-TIF2. Nat Med 2010; 16(5): 580-5.

49 Harmon AW, Patel YM, Harp JB. Genistein inhibits CCAAT/enhancer-binding protein beta (C/EBPbeta) activity and 3T3-L1 adipogenesis by increasing C/ebp homologous protein expression. Biochem J 2002; 367(Pt1): 203-8.

50 Zhu M, Lee GD, Ding L, Hu J, Qiu G, de Cabo R, *et al.* Adipogenic signaling in rat white adipose tissue: Modulation by aging and calorie restriction. Exp Gerontol 2007; 42(8): 733-44.

51 Shao J, Qiao L, Janssen RC, Pagliassotti M, Friedman JE. Chronic hyperglycemia enhances PEPCK gene expression and hepatocellular glucose production via elevated liver activating protein/liver inhibitory protein ratio. Diabetes 2005; 54(4): 976-84.

Progress of PU.1-regulated Adipogenesis in Preadipocytes

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Abstract PU.1 transcription factor is a member of conserved DNA-binding proteins which is called *Ets* family. As the common sequence GAGGAA is recognized in their DNA-binding region, it is named *Ets* binding region or *PU.1* box. *PU.1* mainly expresses in the hematopoietic system, such as myeloid cells and B lymphocytes, regulating the transcription of key myeloid genes to control the differentiation of the hematopoietic system. Global *PU.1* deficiency in mouse leads to early embryonic death due to lack of fetal liver B lymphocytes and myeloid cells, indicating that *PU.1* is a key transcription factor in control of life process. By now, effect of *PU.1* on adipocyte adipogenesis and its mechanism have been rarely reported. The relationship between *PU.1* and regulation of miRNAs, antisense RNA and *C/EBPα/β-PPARγ* pathway during adipocyte adipogenesis will be the focus of future research.

Key words *PU.1*; antisense RNA; miRNAs; preadipocytes; adipogenesis

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