



小鼠胚胎干细胞中2C-like细胞研究进展

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摘要 小鼠胚胎干细胞(embryonic stem cell, ESC)由小鼠囊胚的内细胞团体外培养获得. 小鼠胚胎干细胞可贡献到胚胎的3个胚层, 但缺乏形成胚外组织的能力, 因此, 小鼠胚胎干细胞被认为具有多能性(pluripotent), 而不是全能性(totipotent). 值得注意的是, 小鼠胚胎干细胞中含有少量的亚细胞群(小于1%), 其基因表达模式类似2-细胞期胚胎, 这一类细胞称为2-细胞期胚胎样细胞(2-cell embryo like cell, 2C-like cell). 在适当的体外培养条件下, 小鼠胚胎干细胞和诱导多能性干细胞(induced pluripotent stem cell, iPSC)中均会自发产生少量的2C-like细胞. 2C-like细胞具有全能性和独特的发育特性, 嵌合体实验证明其可同时贡献到胚胎和胚外组织中, 因此2C-like细胞可以作为体外研究全能性干细胞的理想模型. 本文就2C-like细胞的发现、分子调控机制、染色质特征、激活策略以及与体细胞重编程之间的关系等进行综述.

关键词 哺乳动物, 全能性, 合子基因组激活, 胚胎干细胞, 2C-like细胞

全能性(totipotency)是指胚胎发育早期阶段的细胞, 具有分化产生所有细胞谱系的能力^[1,2]; 多能性(pluripotency)是指细胞仅分化成胚胎的3个胚层, 而不能分化形成胚外组织的能力. 然而, 在4-细胞或8-细胞阶段, 胚胎中的卵裂球并不是都具备全能性, 因为在该阶段观察到的单个卵裂球对某些特化谱系的贡献具有偏向性^[3~5]. 因此, 严格意义上的全能性是指单个细胞自身具有发育成完整生物体的能力. 在小鼠中, 只有受精卵和2-细胞期卵裂球具有全能性^[6]. 长期以来, 对全能性的获得和调控机制的研究一直是发育生物学研究领域的重要科学问题. 早期研究认为, 全能性只存在于发育早期阶段的卵裂球中, 而近年来研究发现, 体外培养的小鼠胚胎干细胞处于亚稳态, 在适当的培养条件下可出现类似于全能性的亚细胞群^[7~9], 其基因表达及表观遗传修饰状态均与2-细胞期胚胎较为接近. 有趣的

是, 在含血清的培养条件下, 小鼠胚胎干细胞和诱导多能干细胞中出现部分异质性细胞(小于1%), 可表达一些胚胎基因组激活时期的特异性基因, 如*Mervl*、*Dux*和*Zscan4*等. 因为小鼠的胚胎基因组激活发生在2-细胞期胚胎中, 所以这些细胞也被称为2-细胞期胚胎样细胞(2-cell embryo like cell, 2C-like cell)^[10].

2C-like细胞与2-细胞期胚胎的转录组具有一定的相似性, 不仅可以表达合子基因组激活(zygote genome activation, ZGA)转录本, 而且具有与2-细胞期胚胎相似的表现遗传修饰特征^[11~13]. 通过嵌合体实验发现, 小鼠胚胎干细胞中的2C-like细胞可同时贡献到胚胎和胚外组织中, 说明2C-like细胞具有全能性, 并且与2-细胞期胚胎相比, 2C-like细胞更容易获取^[14](表1). 目前, 2C-like细胞成为常见的体外研究合子基因组激活和全能性的理想模型.

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表 1 小鼠2C期胚胎、囊胚、ESC与2C-like细胞的比较

Table 1 Comparison of mouse 2C embryo, blastocyst, ESC and 2C-like cell

2-细胞期胚胎	囊胚		2C-like细胞	胚胎干细胞
				
E1.5	E3.5	胚胎发育阶段	类似E1.5	类似E3.5
全能性	多能性	发育能力 ^[6]	全能性	多能性
是	是	是否具有分化为胚胎3个胚层的能力 ^[10]	是	是
是	否	是否具有分化为胚外组织的能力 ^[10]	是	否
— ^{a)}	— ^{a)}	是否能够自我更新	— ^{a)}	是
低	高	多能基因(<i>Oct4</i> 、 <i>Sox2</i> 、 <i>Nanog</i>)表达 ^[10]	低	高
高	低	ZGA基因(<i>Zscan4</i> 、 <i>Mervl</i> 、 <i>Dux</i>)表达 ^[10,15-18]	高	低
高	低	染色质开放程度 ^[19,20]	高	低
低	中等	DNA甲基化水平 ^[21]	低	中等
低	高	糖酵解活性 ^[22]	低	高
低	高	耗氧量 ^[10]	低	高

a) “—”表示不存在此项指标

1 胚胎干细胞中2C-like细胞的发现

2007年, Falco等人^[15]利用DNA Microarray数据分析第一次发现*Zscan4*基因, 仅在晚期2-细胞阶段的胚胎和部分胚胎干细胞中表达. 有趣的是, *Zscan4*在胚胎干细胞中呈现一种独特的镶嵌式表达模式, 即在未分化的克隆中仅有少量的胚胎干细胞表达*Zscan4*, 但在囊胚的内细胞团(inner cell mass, ICM)中并未检测到*Zscan4*的表达. 因此, 研究人员推测, *Zscan4*可能在ICM体外衍生胚胎干细胞中发挥重要作用. Morgani等人^[23]观察到2i(MEK和GSK3的抑制剂)培养条件下的胚胎干细胞呈现异质性状态, 在部分细胞中表达胚外内胚层标记基因*Hex*. 而且, 表达单个*Hex*阳性的胚胎干细胞共表达外胚层基因, 嵌合体实验结果也显示, *Hex*阳性细胞可同时贡献胚胎和胚外组织中^[23]. 另外, 在2-细胞期胚胎中, *MuERV-L/MERVL*元件被瞬时去阻遏并

产生3%的mRNA^[24,25]. *MERVL*逆转录元件在2-细胞期胚胎中表达量最高, 随着2-细胞期胚胎的进一步发育, 其表达水平逐渐降低^[26,27]. Macfarlan等人^[10]研究发现, *MERVL*的这种调控模式与100个左右的2C特异性基因的表达模式极其相似. 利用来源于2-细胞期胚胎中表达的逆转录病毒启动子元件来标记胚胎干细胞, 发现体外培养的胚胎干细胞和诱导多能干细胞中都包含少部分具有2C-like转录特征的细胞. 通过流式细胞术将这些2C-like细胞分选后进行嵌合体研究发现, 2C-like细胞具有同时贡献胚胎谱系和胚外组织的独特发育潜能.

2 2C-like细胞的分子调控

2017年, 3个课题组在*Nature Genetics*同时发文报道了小鼠*Dux*和人类*Dux4*转录蛋白可能是激活小鼠ZGA和人胚胎基因组激活(embryonic genome activa-

tion, EGA)的关键因子^[16-18]。这三篇研究论文指出,在体外培养的肌肉细胞或胚胎干细胞中,过表达*Dux/Dux4*会激活一部分ZGA基因,而当胚胎干细胞缺失*Dux/Dux4*便失去转变为2C-like细胞的能力。这一系列的体外实验证明,小鼠*Dux*和人类*Dux4*是在2C-like细胞中激活ZGA基因的必要因子。

为了揭示*Dux*是否在体内胚胎发育过程中发挥作用,通过CRISPR-Cas9技术在小鼠早期胚胎中敲除*Dux*基因串联重复序列发现,*Dux*并非小鼠胚胎基因组激活必需的转录因子,虽然在小鼠胚胎发育过程中起重要的作用,但并不起关键作用^[28,29],因此,合子基因组激活和2C-like细胞激活的调控机制及其相互关系有待进一步研究。这说明,尽管2C-like细胞在表观遗传特征和一些ZGA基因的表达与2-细胞期胚胎类似,但是体外实验得到的结果与体内发育胚胎相比,本质上存在一定的差别,今后仍需进一步探讨。因为,*Dux*在2-细胞期胚胎基因组激活中起增强而不是决定性的作用。在该发育阶段,*Dux*瞬时发挥作用之后很快被降解,而*Dux*的持续表达将严重影响胚胎的正常发育。但是,无论内源还是外源*Dux*基因,其编码的mRNA及蛋白在胚胎发育过程中如何被快速降解尚未有定论^[29]。

早期胚胎合子基因组激活是胚胎发育过程中一个关键的发育事件,发生在小鼠的1~2细胞期胚胎和人类的4~8细胞期胚胎中^[13,30-32],与此同时,胚胎细胞获得全能性。由于2C-like细胞中的转录本与合子基因组类似,因此2C-like细胞可以作为体外研究ZGA分子调控机制的理想模型^[11]。

近几年,关于2C-like细胞的研究取得了显著成效,但大多数报道直接或间接与*Dux*基因的调控有关。尤其,Fu等人^[33]通过构建可调控*Dux*基因表达体系,同时结合单细胞转录组测序技术(single cell RNA sequencing, scRNA-seq),揭示了多能干细胞向全能性的2C-like细胞转变过程,发现该转变过程包含两个阶段。第一个阶段是从多能性到中间态的过渡阶段,在该阶段多能性相关基因(如*Sox2*和*Klf4*)的表达受到抑制;第二个阶段是从中间细胞状态向全能性2C-like细胞状态的转变,在该阶段2C-like基因(如*MERV1*和*Zscan4*)开始广泛激活。在小鼠胚胎干细胞向2C-like状态的转变机制研究中,研究人员利用CRISPR-Cas9介导的全基因组筛选技术,筛选出多个调控该过程的关键因子。结果发现,*Myc*阻碍多能性基因的抑制;而*Dnmt1*抑制2C-like基因的激活。

Eckersley-Maslin等人^[11]研究发现,*Dux*的上游调控因子,即发育多能性相关蛋白2/4(developmental pluripotency-associated protein 2/4, DPPA2/4)对2C-like状态、ZGA和生殖细胞增殖等具有重要的调节作用。同时,他们梳理了2C-like细胞的转变过程中不同调控因子之间的上下游关系,明确了*Dppa2/4*与*Dux*基因的启动子结合使*Dux*基因高表达,并随后激活2C-like基因再激活*Zscan4c*等基因的表达。此外,De Iaco等人^[34]研究发现,*Dppa2/4*在ZGA之前就开始表达,并且在小鼠ZGA过程和2C-like细胞中进一步激活*Dux*基因的表达以及长散在重复序列1(long interspersed nuclear elements 1, LINE1),从而成功激活合子基因组,建立2C-like状态。

Yang等人^[35]构建了*Zscan4*-GFP和*MERV1*-tdTomato双报告基因的2C-like细胞检测系统,发现非编码RNA *miR-344*具有诱导双阳性2C-like细胞的作用;同时,他们还验证2C-like细胞的分子调控轴,即*Dux*→*miR-344*-*Zmym2/Lsd1*-*MERV1*。

此外,Hu等人^[22]研究发现,母源性调控基因*Nelfa*促进小鼠胚胎干细胞向2C-like细胞转化,细胞代谢水平的变化可以使胚胎干细胞向2C-like细胞转化,而且还明确了2C-like细胞转变过程中*Nelfa/Dux/Zscan4*轴的调控作用。另外该研究还证明,通过小分子操纵代谢通路可有效促进胚胎干细胞向2C-like细胞转化,为今后研究全能性的分子机制提供了新方法。

2C-like的激活是短暂的,在小鼠胚胎干细胞培养条件下可以自发地恢复为多能状态^[10]。最近,Fu等人^[36]对2C-like状态向多能性状态的转变过程进行系统性研究,并揭示其精确的调控机制。从2C-like状态退出时,多能性基因表达出现阶段性变化特征,而不是2C-like状态直接向多能性转变。scRNA-seq分析表明,2C-like状态进入和退出胚胎干细胞的多能性状态时遵循不同的转录途径。此外,*Dux*的mRNA降解,特别是通过无义介导的mRNA降解(nonsense-mediated *Dux* mRNA decay, NMD),驱动了2C-like状态的逆转。小鼠胚胎干细胞中可能也存在其他机制,在mRNA水平或蛋白水平上介导*Dux*的降解,以确保小鼠胚胎干细胞培养中2C-like状态的有效逆转。

3 2C-like细胞的染色质特征

通过使用由ZGA转录物启动子驱动的荧光报告系统(例如小鼠内源性逆转录病毒*MERV1*或*Zscan4*)可动

态观察胚胎干细胞中的2C-like细胞^[10,20,37]。2012年, Macfarlan等人^[10]发现, 小鼠胚胎干细胞中多数细胞处于动态循环进出这种2C-like状态, 当胚胎干细胞重新进入2C-like状态时, 它们的染色质状态发生明显改变, 并呈现类似2-细胞期胚胎的染色质特征。

此外, 胚胎干细胞中的2C-like细胞可呈现2-细胞期胚胎的部分特征, 包括2-细胞期胚胎特异性的转录特征、多能性基因的下调^[38,39]、染色质迁移率增加^[40]、染色质去浓缩^[19,20]、染色质可及性的增加以及全基因组DNA低甲基化等^[21,41]。

Eckersley-Maslin等人^[21]通过全基因组DNA甲基化和染色质分析发现, 当胚胎干细胞过渡到 $MERVL^+/Zscan4^+$ 状态时, 伴随染色质可及性增加。由于翻译阻滞导致细胞中DNMT蛋白合成受到抑制, 在全基因组范围内呈现短暂的DNA低甲基化水平。重要的是, DNA甲基化水平随着从 $MERVL^+/Zscan4^+$ 状态退出而恢复, 但是基因组印记状态不能恢复到正常水平。此外, 研究者确定了DNA去甲基化是 $MERVL^+/Zscan4^+$ 转录网络激活的结果而并非 $MERVL^+/Zscan4^+$ 激活的原因。Schüle等人^[42]的研究显示, 在小鼠胚胎干细胞中, GADD45蛋白(生长阻滞和DNA损伤诱导蛋白)与DNA去甲基化酶Tet蛋白相互作用, 通过促进基因座特异性去甲基化和防止从头甲基化来促进2C-like状态的激活。

除了DNA甲基化外, 当胚胎干细胞进入2C-like状态时, 组蛋白修饰也发生明显的变化。在异染色质区域, 活性组蛋白修饰标记H3K4me3、H3K9ac、H3K14ac和H3K27ac的水平显著增加, 尤其是H3K27ac。在2C-like状态激活期间, 小鼠胚胎干细胞的组成型异染色质经历快速去抑制和再抑制, 因此导致了胚胎干细胞循环进出2C-like状态的现象。此外, 抑制性组蛋白修饰标记H3K27me3的表达在早期胚胎发生和2C-like转变过程中表现出较低的水平^[43]。

目前, 关于全能性和2C-like细胞的调控机制尚不清楚。但是, 之前的研究表明, G9a^[44]、miR-34a^[45]、PRC1.6、EP400-TIP60复合物^[46]、PIAS4 SUMO E3连接酶^[47]、KAP1/TRIM28^[48,49]、染色质组装因子1(chromatin assembly factor 1, CAF1)^[20]、组蛋白去甲基化酶LSD1/KDM1A^[10]和LINE1等^[50]均抑制小鼠胚胎干细胞中2C-like状态的激活。因此, 研究人员通过敲除或敲减这些抑制性基因达到激活2C-like状态的目的。此外, Lu等人^[51]通过CRISPR/Cas9技术产生的Tet TKO(triple knockout)的小鼠胚胎干细胞也表现出2C-like细胞群体

的增加。

4 2C-like状态的激活策略

揭示多能干细胞如何转换成2C-like细胞以及哪些因子对该过程起调节作用是体外研究全能性的基础, 但2C-like细胞在胚胎干细胞中出现的数量稀少, 维持时间较短, 尚未得到系统的研究。因此, 如何有效提高哺乳动物胚胎干细胞中2C-like细胞的比率也成为当今全能性干细胞研究的热点之一。

近年来, 已有不少实验室通过不同的方法将小鼠胚胎干细胞向2C-like细胞转化, 如过量表达*Dux*或敲减*Caf1*的表达等方法驱动胚胎干细胞向2C-like细胞转换^[20,22], 进而解决2C-like细胞较少的瓶颈。

2020年, Hu等人^[22]发现, 2C-like细胞中糖酵解(glycolysis)水平与胚胎干细胞相比显著降低, 进一步使用糖酵解抑制剂2-脱氧-D-葡萄糖(2-deoxy-D-glucose, 2-DG)处理小鼠胚胎干细胞, 发现可显著提高2C-like细胞的比率。通过体内实验验证, 该诱导手段获得的2C-like细胞同时具有贡献ICM和滋养外胚层(trophoblast, TE)的发育能力, 证实抑制糖酵解通路可显著提高小鼠胚胎干细胞向2C-like细胞转化。

Ishiguro等人^[52]在经典的血清/白血病抑制因子(Serum/LIF, S/L)的培养条件下, 分别加入组蛋白乙酰化酶抑制剂丙戊酸(valproic acid, VPA)和视黄酸(retinoic acid, RA)后, 可促进*Zscan4c*介导的2C-like细胞的激活。

2021年, Shen等人^[53]报道了剪接体抑制可以激活小鼠胚胎干细胞从多能到全能状态的转变。通过使用剪接抑制剂普拉二烯内酯B(pladienolide B, PlaB), 实现了全能胚胎干细胞的体外稳定培养, 并称之为全能卵裂球样细胞(totipotent blastomere-like cell, TBLC)。这些细胞在分子水平上具有与2细胞和4细胞卵裂球相似的转录组特征。单细胞RNA-seq分析发现, TBLC中全能性基因的数量比2C-like细胞多, 表达水平也比2C-like细胞高。小鼠嵌合体分析结果显示, TBLC具有强大的胚胎嵌合能力和胚外细胞谱系分化能力^[53]。

但是到目前为止, 关于在化学成分明确的培养条件下获得长期稳定培养2C-like细胞的报道很少。最近我们研究发现, 在成分明确的培养条件下, 视黄酸通过*Nelfa*激活胚胎干细胞中2C-like状态, 与对照组相比2C-like细胞的比率可提高16倍。视黄酸长期处理不影响胚胎干细胞多能性, 即2C-like细胞可同时贡献ICM和TE

中;而且视黄酸通过*Nelfa*激活的2C-like状态,与表观遗传修饰和干细胞代谢调控密切相关^[54]。

5 2C-like细胞与体细胞重编程

2C-like细胞除了在胚胎干细胞培养过程中出现以外,Zhao等人^[55]在利用小分子化合物将体细胞重编程为多潜能干细胞(CiPSCs)的研究中发现,从胚外内胚层样(extraembryonic endoderm, XEN-like)细胞向CiPSCs转变过程中,也存在与2-细胞期胚胎相似的细胞群,并命名为Ci2C-like(chemically induced two-cell embryo-like)细胞,这从表观遗传角度证明了Ci2C-like与2-细胞期胚胎的相似性,而这里的Ci2C-like细胞与胚胎干细胞中2C-like细胞很接近。更重要的是,他们还证明了Ci2C-like相关基因(如*Zscan4*、*Tcstv1*)在CiPSCs重编程过程中发挥关键的作用。在此基础上,通过改进小分子化合物的处理方式,使用组蛋白去乙酰化酶抑制剂VPA来促进2C基因的激活,进而加速了重编程进程,使化学重编程的诱导周期从40 d缩短至16 d^[55]。

在克隆胚胎研究中发现,与体外受精胚胎相比,克隆胚胎存在部分胚胎基因表达异常的现象,尤其合子基因组激活相关基因未被完全激活^[56]。最早研究人员通过组蛋白去乙酰化酶抑制剂曲古抑菌素A(Trichostatin A, TSA)^[57-59]或使DNA去甲基化^[60]等处理方法提高核移植效率。最近,研究人员开发了多种方法消除表观遗传屏障来提高体细胞核移植(somatic cell nuclear transfer, SCNT)效率,包括将H3K9me3特异性去甲基酶KDM4D或H3K27me3特异性去甲基酶KDM6A的过表达^[61,62]等。Yang等人^[63]发现,H3K9ac的重新修饰对SCNT效率的优化非常重要,异常的H3K9ac信号会导致2C基因组激活缺陷。而以前的研究中使用TSA处理也正是纠正了SCNT胚胎中异常的乙酰化修饰,从而提高了SCNT的效率。但是TSA在H3K9ac修复中的功能受到供体细胞染色质状态的限制,说明其拯救作用仍然不足。研究还发现,*Dux*的过表达可纠正其靶位点上的异常H3K9ac修饰,从而确保2C基因组正常激活来提高SCNT效率^[63]。因此,2C基因组的激活对体细胞核移植效率的提高起重要的调控作用。

此外,研究表明,在成纤维细胞中强制表达*Zscan4*可提高诱导多能干细胞的重编程效率^[64]。Hernandez等人^[65]在小鼠胎儿成纤维细胞(mouse embryonic fibroblast, MEF)重编程为诱导多能干细胞的过程中,将

*Dppa2*和*Dppa4*过表达,由*Dppa2/4*通过DNA损伤修复等途径调控整体染色质开放程度,从而加速成纤维细胞重编程时间,可在2~4 d内将MEF重编程为诱导多能干细胞^[65]。所以,通过将一个或几个2C相关基因的过表达,或对其他2C基因阻遏物的抑制等方法,可提高体细胞核移植或成纤维细胞重编程效率。

由于2C-like细胞具有与2-细胞期胚胎相似的基因表达特征,2C-like细胞的这些特征将来可能应用到体细胞重编程过程中。例如,将2C-like细胞作为核移植供体细胞进行体细胞克隆研究等。

6 讨论与展望

以上研究证实,2C-like细胞和胚胎干细胞之间存在一定的动态平衡状态,在一定条件下两者之间可互相转换。进入2C-like状态对胚胎干细胞扩展多能性的维持具有一定的帮助作用^[10]。胚胎干细胞中2C-like状态的激活可能是多方面共同调节作用的结果,不仅需要激活上游调控基因,还需要一些表观遗传修饰,包括染色质开放程度以及组蛋白修饰等均与2C-like状态的激活有关。2C-like状态一旦被激活,诸如*MERV1*和*Zscan4c*等基因得到表达,并参与维持胚胎干细胞中的2C-like状态,而*LINE1*^[50]、核小体重塑与去乙酰化酶(nucleosome remodeling and histone deacetylase, NuRD)和CAF1^[20,66]等对2C-like状态的激活起到抑制作用。

虽然,目前为止有很多关于2C-like细胞的报道,但是关于2C-like细胞的精确调控机制仍需要我们进一步探讨,如Eckersley-Maslin等人^[11]的研究显示,*Dppa2*和*Dppa4*并不只在2C-like细胞中表达,而是在所有胚胎干细胞中均匀表达,但只有一少部分ZGA相关基因在2C-like细胞中表达;胚胎干细胞中的2C-like细胞作为合子基因组激活和全能性调控的分子机制研究的理想模型,其目前的培养条件大多数含有血清,尚未建立成分明确的2C-like细胞体外培养体系,而且仍需明确2C-like细胞能否在体外持续稳定培养。此外,关于胚胎干细胞向2C-like状态转换的精确调控机制仍需进一步研究。

总之,2C-like细胞在一定水平上可能替代2-细胞期胚胎,是体外研究全能性和合子基因组激活的理想模型。相信在不久的将来,我们对2C-like细胞的了解会更加透彻,对其调控机制的理解更加明确,2C-like细胞模型在实际应用中必然占据一席之地。

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Summary for “小鼠胚胎干细胞中2C-like细胞研究进展”

Review on 2C-like state of mouse embryonic stem cells

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Mouse embryonic stem cells (ESCs) are derived from the inner cell mass of mouse blastocysts. Mouse ESCs are pluripotent and contribute widely to the formation of the three embryonic germ layers in chimeric mice but not to that of the extra-embryonic trophoctoderm. A small population of transient cells (less than 1%) has been identified occasionally in the culture of mouse ESCs, which has been shown to exhibit a gene expression profile similar to that of cells in 2-cell stage embryos (2C-like cells). 2C-like cells were found to be generated spontaneously from mouse ESCs and induced pluripotent stem cells (iPSCs) when they were cultured in the serum-supplemented with leukemia inhibitory factor (LIF) on feeder cells *in vitro*. 2C-like cells have been considered to be totipotent. There is evidence suggesting that 2C-like cells can effectively generate both embryonic and extra-embryonic tissues, and therefore, 2C-like cells can be potentially used as a model for *in vitro* totipotent stem cell research. However, only a small population of ESCs could transit to 2C-like state in the serum-containing culture medium. There are several 2C specific genes that express when ESCs enter 2C-like state, such as *Zscan4*, *Mervl*, *Dux*, *Nelfa*, *Tdpz1/2/4* and *Tctst1/3*. Notably, 2C-like cells exhibit highly relaxed chromatin architecture, which is similar to that observed in 2-cell stage embryos. Genome-wide demethylation occurs upon transition of mouse ESCs into 2C-like state, including gene bodies, promoters, and repeat elements. The dynamics of the reprogramming process from ESCs to 2C-like cells are still unclear, which have certainly uncovered an intricate regulatory mechanism mediated by specific genes, proteins, as well as some metabolites and non-coding RNAs.

Additionally, one of the important features of totipotency is that a single cell can form an entire organism. Therefore, totipotent stem cells form a valuable tool to study the early developmental processes *in vitro* and prove to be beneficial for regenerative medicine applications. For instance, the embryonic stem cells have been used to generate embryo-like structures. The artificial embryo-like structures not only facilitate the studies on the key events in early embryonic development, but also serve as a model for investigating the early developmental defects occurring in humans. Several previous studies revealed that ESCs at different totipotent states can arise in *in vitro* cultures, and they focused on identifying culture conditions that induce 2C-like states. For example, knockout serum replacement (KOSR) media promotes the induction of 2C-like cells from ESCs in *in vitro* culture, as indicated by the increased levels of *ZSCAN4*-positive cells. Additionally, the suppression of glycolysis mediated by 2-deoxy-d-glucose (2-DG) in serum plus LIF condition induces the 2C transcriptional programming in ESCs. Interestingly, the most recent research indicates that the spliceosomal repression in mouse ESCs drives the pluripotent-to-totipotent state transition. Using the splicing inhibitor pladienolide B, they achieved stable *in vitro* culture of totipotent ESCs comparable at the molecular levels with 2- and 4-cell blastomeres. In our recent study, we also demonstrated that retinoic acid induces *NELFA*-mediated 2C-like state of mouse embryonic stem cells, which is associated with epigenetic modifications and metabolic processes in chemically defined media. Here, we review the discovery, molecular regulatory mechanism, chromatin characteristics, activation strategies, and the relationship of 2C-like cells with somatic reprogramming.

mammal, totipotency, zygote genome activation, embryonic stem cells, 2C-like cells

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