综述

哺乳动物睾丸中*c-kit*基因表达对生精细胞发育的影响

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摘要 c-kit和SCF信号传递系统的特性已被广泛报道。但是哺乳动物*c-kit*基因的结构、功能、时空表达和*c-kit*突变动物模型相关的研究不多。由于研究方法的精度有差别, 造成有些结果不清晰, 有些结论还存在争议。该文对哺乳动物c-kit受体的结构和功能进行了阐述, 并着重介绍了c-kit在模式动物和家畜生精过程中的表达形式及*c-kit*突变个体的相关研究进展。最终深入探讨了c-kit在生精细胞增殖、分化及受精过程中所起的作用。通过系统性介绍, 明确c-kit在生精过程中起到的重要作用, 为生精细胞的增殖、分化及迁移等机理的进一步研究提供了参考。

关键词 c-kit; 哺乳动物; 生精细胞; 表达调控

The Effects of c-kit on Development of Male Germ Cells in the Mammal's Testis

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Abstract The roles and characteristics of c-kit/SCF signaling pathway have been widely reported. But there is not enough research available about mammalian *c-kit* detailed structure, functions, expression and transgenic models. Further the difference of researching pattern caused some unclear results and remaining debates. Here, we described the structure and functions of mammal's *c-kit* gene, then emphasized its expressional pattern and roles in germ cell development of transgenic models and domestic animals. Finally, we discussed in depth about its potential roles in male germ cells proliferation, differentiation and fertilization. By introducing systematically about c-kit, its important functions in spermatogenesis have been defined, which might be a reference for future research about proliferation, differentiation and immigration in male germ cell.

Key words c-kit; mammal; male germ cells; expressional regulation

1 *c-kit*基因结构与功能

从20世纪中期到现在, c-kit(又称kit受体或CD117)和KIT配体(stem cell factor, SCF或KIT ligand, KITL)的一般功能已被广泛报道[1-4]。在研究原癌基因*v-kit*时发现W位点(White-spotting)表达c-kit受体蛋白, 因此认定W位点即为*c-kit*基因[5-6]。在小鼠[7]和人类中*c-kit*基因分别位于4和5号染色体上[8]。作

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为原癌基因, 在人类中*c-kit*基因跨越20 Kb, 包含21个外显子[5,9-10]。在小鼠中同样由21个外显子组成, 转录产生5.5 Kb长的转录物。*c-kit*基因的翻译产物约为145 kDa的c-kit跨膜受体蛋白[5], 属于第三类受体酪氨酸激酶家族。c-kit受体有三个主要功能区域, 分别为: 胞外结构域、跨膜结构域和胞内结构域等。由5个Ig样重复结构组成了与配体结合和二聚体形成相关的胞外结构域[11](图1)。23个氨基酸组成了跨膜的疏水结构域, 具有将受体锚定在细胞膜上的作用。433个氨基酸组成的胞内结构域由三个部分组成, 分别为近膜端ATP结合相关的激酶区域、70-100氨基酸组成的非保守插入结构域(KI区域)和远端的磷酸化激酶区域[12], 其中胞内近膜端酪氨酸残基在信号分子激活过程中有利于信号分子停泊(docking)[13]。完整的c-kit受体蛋白由于其胞外近膜区域的选择性剪切产生GNNK$^-$和GNNK$^+$两种亚型。其区别在于是否具有由四个氨基酸组成的GNNK区域[14-15]。从配体结合和激活能力来看, 两种亚型与KIT配体结合能力相当[16], 只是在骨髓瘤细胞中, KIT配体能够更快速诱导激活GNNK$^-$亚型[17]。除了上述两种剪接变体以外, c-kit受体还有截短型(truncated forms of the KIT protein, Tr-kit)和可溶型(soluble KIT receptor isoform, S-kit)。对小鼠睾丸中单倍体细胞进行Northern blot分析发现3.2 Kb和2.3 Kb的两种不同*c-kit* mRNA剪接体[18]。由3.2 Kb的*c-kit* mRNA转录产生约30 kDa的转录物, 即Tr-kit[19-20], 此转录物含有C端尾部部分非保守插入区域和远端磷酸化激酶区域, 而整个胞外部分和跨膜结构区已失去[21]。Tr-kit不能够与Kit配体相互作用, 因此推测单倍体生精细胞不依赖KIT配体的激活反应。但在小鼠受精实验中利用c-kit抗体封闭精子后发生顶体反应的比例下降, 并伴随精子头对头聚集(head-to-head agglutination)的增加[20,22-23]。说明c-kit不仅在出生前后生精过程中起到作用, 而且在小鼠受精过程中也具有重要作用[20]。已有实验证实, S-kit在造血细胞、肥大细胞和内皮细胞表面及人类血浆中都有表达[24-27]。它由完整的c-kit在胞外结构域和跨膜结构域结合段发生酶裂解而产生。它与KIT配体的结合能力与完整的c-kit受体相同。在体外培养实验中, S-kit能够阻断造血细胞克隆的生长, 表明S-kit具有调节KIT配体生物活性的能力[28-29]。

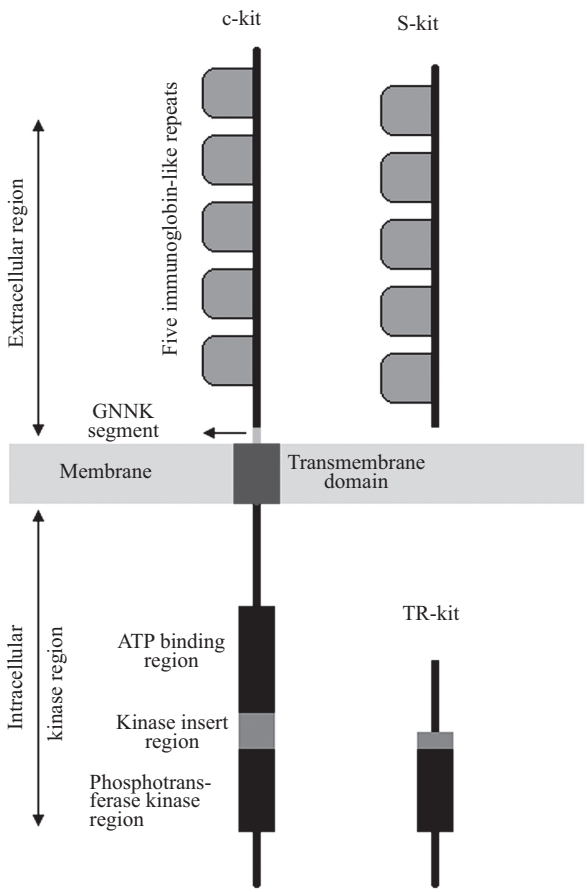


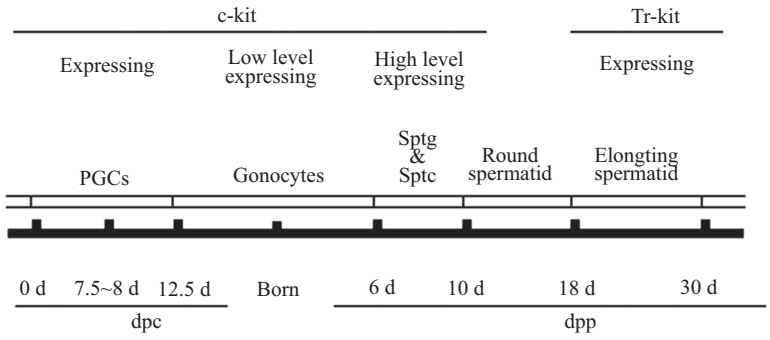
图1 c-kit结构与类型(根据参考文献[29]修改)

Fig.1 The structures and varieties of c-kit(modified from reference [29])

同样, *c-kit*基因在家畜动物中也具有高度保守性。在山羊中, *c-kit*基因的ORF(open reading frame)区域可以翻译产生由987个氨基酸组成的多肽。依照与人源c-kit的高度同源性, 可以推导出山羊c-kit同样存在胞外结构域、跨膜结构域和包内结构域等三个区段。其中山羊c-kit蛋白胞外结构域与牛、人、大鼠和小鼠的序列相似度分别为98.8%、83.5%、77.0%和73.0%。尽管胞外结构中氨基酸序列有一定的变化性, 但在山羊和其他物种中有12个位点是保守的。这些保守的半胱氨酸残基被认为能够支持5个Ig样结构, 从而有利于受体和配体的结合或者受体之间的相互作用[11,30]。c-kit胞内部分氨基酸序列在山羊和其他物种中具有高度保守性, 与山羊序列的相似度分别为99.8%(牛)、96.9%(人)、93.0%(大鼠)和93%(小鼠)[31]。

2 *c-kit*基因在原始生殖细胞(primordial germ cells, PGCs)中的表达

野生型小鼠中, 在受孕后7.5~8.0 d或更早期的



Sptg:精原细胞; Sptc:精母细胞。

Sptg: spermatogonia; Sptc: spermatocytes.

图2 在小鼠第一次生精波期间c-kit受体的表达分布模式图

Fig.2 The model of expressional patter of c-kit during mouse first wave of spermatogenesis

尿囊基底处PGCs内可检测到*c-kit* mRNA的存在[32], 到受孕后第12.5天, 在PGCs到达生殖脊过程中通过原位杂交可以检测到*c-kit* mRNA表达于PGCs, 而KIT配体mRNA则表达于PGCs迁移线路上的体细胞内[33-36]。在上述两个时间段内, c-kit和KIT配体相互作用对PGCs的正常迁移和增殖具有重要意义。在小鼠中, 从胚胎时期第15天(embryonic day 15, E15)到出生后第3天, c-kit蛋白表达明显下调, 并伴随生殖细胞进入静默时期[37]。在出生后第3~6 d时, 生殖母细胞(gonocytes)缓慢从曲细精管索中心迁移至外围, 此时睾丸中c-kit较低水平表达[37]。在此期间gonocytes逐渐转变为c-kit阴性和阳性两种不同状态, 并伴随精原细胞的出现, 标志着第一次生精波的开始[38]。这说明在第一次生精波中c-kit在生精细胞中的表达存在差异, 推导出在此发育阶段不是所有gonocytes均能够形成精原干细胞(图2)。1998年, de Rooij[39]对生精细胞的动态发育进行研究提出gonocytes不仅发育为As型精原干细胞, 而且有些可以直接分化为A2型精原细胞。即gonocytes有一部分亚型发育为As型精原细胞, 而另外一些亚型则发育为更高级的Aal型精原细胞或者直接分化为A2型精原细胞。因为c-kit受体被认为是分化的精原细胞的标记, 因此有人推测c-kit阳性gonocytes是在c-kit阴性的gonocytes建立As细胞群后出现的。此推论也可以解释有些gonocytes直接通过细胞间桥相连形成分化的精原细胞[4,40]。

3 *c-kit*基因表达对成体生殖细胞发育的影响

在性成熟的小鼠睾丸中通过免疫组化和原位杂交定位发现c-kit在睾丸间质细胞(Leydig cells)、精原细胞(spermatogonia)、初级精母细胞(primary spermatocytes)和精子(spermatids)中表达, 但在未分化的精原细胞和支持细胞(sertoli cells)中不表达[19,40-43]。c-kit在新生和成年个体的睾丸Leydig细胞中均表达, 参与Leydig细胞的类固醇生成并对其存活起到调节作用[38,44-45]。小鼠出生后第6~8 d时, 在支持细胞中SCF蛋白质开始形成[46-48]。对出生后第2~5 d的小鼠生殖细胞和未成熟的支持细胞进行体外培养并用c-kit特异的抗体ACK2阻断处理后, c-kit和KIT配体的相互作用依照剂量依赖形式只对第5 d的小鼠精原细胞的增殖产生抑制作用。表明精原细胞的增殖与其c-kit表达的起始和SCF相互作用直接相关[49]。目前, 常用c-kit蛋白表达与否视为精原细胞的分化标准[50-51], 因为未分化的精原细胞转变为已分化的精原细胞过程与精原细胞内c-kit的表达相对应。基于Real-time PCR和免疫组化结果得知, *c-kit* mRNA可在未分化的精原细胞中检测到, 而c-kit蛋白质只在分化的精原细胞上被检测到[38], 这表明在生精过程中c-kit的转录和翻译存在时空差异。

4 *c-kit*介导的信号通路及其调控

分化的c-kit阳性精原细胞存活及增殖依赖c-kit和KIT配体的相互作用, 但之前的精原细胞分化的起始并不依赖c-kit的作用[52-53]。c-kit信号通路在减数分裂粗线期生精过程的进行和维持中起到关键的作用[54-56]。在精原细胞中c-kit和KIT配体的相互作用可以激活以下四个已知的信号通路。(1)PI3K通路(the phosphoinositide 3-kinase pathway)调节细

胞存活(通过AKT和BAD调节)、黏连(通过c-JUN和c-FOS活化、激活)和增殖(通过AKT和p70S6K调节)。c-kit/SCF信号通路通过PI3K通路募集cyclin D3达到精原细胞的增殖。因此, *c-kit*突变小鼠中PI3K不能正常募集, 导致精原细胞凋亡的增加、增殖的减少, 从而导致不育表型[57-59]; (2)SRC通路中SRC家族蛋白与c-kit胞内结构域近膜端相互作用, 参与小鼠PGCs迁移和AKT的激活[60]; (3)Tr-kit通过PLCG通路激活PLCG, 介导受精卵减数分裂的恢复[61]; (4)RAS通过与c-kit和GRB2结合激活MAPK信号级联途径, 从而介导了PGCs的基因转录和精原细胞的增殖过程[29,58,60]。

除了c-kit受体影响和调控生精过程以外, *c-kit*基因也受到其它调控因子的调节。在人类和小鼠中, *c-kit*启动子转录起始位点位于翻译起始位点上游58 bp处, 具有TATA-less和non-GC-rich启动子结构, 并且其上游2.7~5.0 Kb对其抑制自我转录是必需的[62-64]。在精原细胞分化中, PLZF(promyelocytic leukaemia zinc finger protein, 又称ZBTB16)是目前唯一可知的能够直接作用于*c-kit*基因、并影响其转录的转录因子。敲除*PLZF*基因的小鼠显现出精原干细胞库的不断耗竭, 并伴随生殖细胞的缺乏[65]。PLZF可以直接抑制内源性*c-kit*和由*c-kit*启动子控制的报告基因。从*PLZF*基因敲除小鼠中分离纯化的精原细胞中c-kit水平明显增加[66]。已证实PLZF能够影响*c-kit*的表达, 并帮助造血干细胞从骨髓迁移至外周血中[67], 并在正常的造血前体细胞($CD34^{+}38^{+/-}$ hematopoietic progenitor/stem cells)和急性骨髓白血病(acute myeloid leukemias of M_0/M_1 French-American-British subtypes)中能够介导*c-kit*的负调控[29,68]。

5 *c-kit*突变动物相关研究

一般认为, c-kit通路对肿瘤生长和多种癌症的发展具有重要作用[69], 而且*c-kit*基因突变会发生不依赖配体的磷酸化, 这被认为对胃肠道间质细胞瘤等的发生起到关键作用。经过几十年的研究, 发现在小鼠中W位点和Sl(steel)位点突变的纯合子个体出现如不孕、发育不全性贫血(先天性贫血)、肥大细胞和黑色素细胞的缺失和白苔斑等多种表型[3,70-72]。在人类、小鼠和大鼠中已报道的W位点的突变包括大片段缺失、基因重排和点突变等类型[72]。这些突变影响到c-kit蛋白的表达量、跨膜结构及激酶活性水平[71,73]。所有报道的*c-kit*基因的点突变均位于蛋白质磷酸化结构域内[74], 这证明此结构域的重要性。受体亚基的二聚化对于其自我磷酸化及信号传导是必需的[15,75-76]。然而在一些含有突变*c-kit*基因的杂合子群体中, 杂合子或纯显性个体常表现出信号传导部分受阻的表型[71,77-78]。*c-kit*和KIT配体基因敲除小鼠可以表现出各不相同的表型, 其中有的缺少睾丸内全部生精细胞, 而有的生精效率低下(表1)。在*Sl17H*突变小鼠中, KIT配体缺少胞质内尾部, 导致不能够与细胞膜正常结合, 从而Aal向A1型精原细胞的转变被抑制[79], 提示了c-kit在体内的功能。在*Sl17H*突变小鼠中其生精细胞在青春期发生增殖, 但发育到成年的小鼠是不育的。这表明, mSCF(mouse SCF)在成年个体的生精过程中对生精细胞自我更新可能起到作用。在steel正常的熊猫或*steel*突变对照小鼠中雌性是不育的, 而雄性是可育的, 但在*steel17H*突变小鼠中, 雄性是不育的, 雌性是可育的[8]。利用c-kit特异抗体ACK2周期性地处理小鼠, 可导致个体中缺少A1-A4型的精原细胞[40], 在体外培养曲细精管体系中加入SCF则能够促进精原细胞的存活[56], 说明c-kit参与调节生精的下游过程。将野生型小鼠混合生精细胞[80]或*Sl*缺陷小鼠混合生精细胞移植入$W^{-/-}$小鼠睾丸后能够启动完整的生精过程产生成熟的精子[81], 证明c-kit在生精过程中发挥着重要的作用。在W/W_v和Sl/Sl_d突变个体中, 均缺少生精细胞, 但其机理却各不相同。在啮齿类睾丸中完整的c-kit主要表达于已分化的A型精原细胞到粗线期精母细胞群体中, 但不表达于As型精原细胞内, 而KIT配体只由支持细胞分泌产生。因此, 在W/W_v突变个体中缺少生精细胞是因为*W*突变中c-kit缺少78个氨基酸, W_v突变中c-kit的络氨酸激酶结构域中发生了一个点突变, 导致生精细胞的前体细胞具有缺陷。而在Sl/Sl_d突变个体中是因为*Sl*突变中缺失整个*Sl*基因, Sl_d突变中缺少SCF的跨膜和胞内结构域, 最终支持细胞无法合成有功能的SCF, 导致缺少生精细胞。在上述两个突变个体中即使出现少数几个个别的生殖细胞也是因为PGCs的增殖和迁移发生了病理性损伤导致[2,52,82]。综上所述, *c-kit*突变小鼠(W/W_v小鼠)可作为良好的受体小鼠用于精原干细胞的移植和研究, 而且对*c-kit*突变模式动物的研究将有助于揭示c-kit在生精过程中起到的作用。

表1 W位点突变小鼠及其特性

Table 1 The W locus mutant mice and its characteristics

突变位点 Mutation sites	纯合子(W*/W*) Homozygous				杂合子(W*/+) Heterozygous				突变类型 Type of mutations
	可致死性 Lethality	贫血程度 Degree of anemia	生育能力 Fertility	毛色特性 Color of coat	可致死性 Lethality	贫血程度 Degree of anemia	生育能力 Fertility	毛色特性 Color of coat	
$W^{[96-98]}$	Mostly die	Severe	Sterile	Entirely white	Fully viable	Non-anemic	Fertile	White spot	78 aa deletion mutation
$W^{v[96-100]}$	Mostly die	Severe	Sterile	Entirely white	Fully viable	Mild	Fertile	White spot	Point mutation at 660 aa
$W^{sh[98,100-101]}$	Viable	Non-anemic	Fertile	Almost white	Low viability	—	Fertile	White band across the loins	—
$W^{J[100]}$	Viable	Severe	Sterile	Entirely white	Fully viable	Non-anemic	Fertile	White spot	—
$W^{f[97,100]}$	—	Mild	—	White spot	Viable	Moderate	Fertile	Extensive white spot	—
$W^{a[97]}$	Mostly die	Severe	Sterile	Entirely white	Fully viable	Non-anemic	Fertile	White spot	—
$W^{x[97]}$	Mostly die	Severe	Sterile	Entirely white	Fully viable	Non-anemic	Fertile	White spot	—
$W^{b[97]}$	Mostly die	Severe	Sterile	Entirely white	Fully viable	Non-anemic	Fertile	White spot	—
$W^{e[97]}$	Mostly die	Severe	Sterile	Entirely white	Fully viable	Non-anemic	Fertile	White spot	—
$W^{s[97]}$	Perinatal lethality	Severe	Sterile	Entirely white	Fully viable	Non-anemic	Fertile	White spot	—
$W^{pw[97]}$	Perinatal lethality	Severe	Sterile	Entirely white	Fully viable	Non-anemic	Fertile	Almost white	—
$W^{19H[7,96,102]}$	Pre-implantation die	—	Sterile	—	Mostly die	Non-anemic	Fertile	Minimal white spot	Deletion mutation, no kinase activity
$W^{28[102]}$	Lethal	—	—	—	—	—	—	—	—
$W^{29H[102]}$	Viable	—	—	—	—	—	—	—	—
$W^{34[3]}$	Perinatal lethality	Severe	Sterile	Entirely white	Fully viable	Non-anemic	Fertile	White spot	—
$W^{35[97,100]}$	Perinatal lethality	Severe	Sterile	Entirely white	Fully viable	Non-anemic	Sterile	White spot	—
$W^{37[96-98,100]}$	Perinatal lethality	Severe	Sterile	—	Fully viable	Non-anemic	Fertile	Extensive white spot	Point mutation at 582 aa
$W^{38[97,100]}$	Perinatal lethality	Severe	Sterile	Entirely white	Fully viable	Non-anemic	Sterile	White spot	—
$W^{39[97]}$	Viable	Moderate	Fertile	Entirely white	Fully viable	Mild	Fertile	White spot	—
$W^{40[97,100]}$	Perinatal lethality	Severe	Sterile	Entirely white	Fully viable	Non-anemic	Sterile	White spot	—
$W^{41[96-98]}$	Viable	Moderate	Fertile	Extensive white	Viable	Mild	Fertile	Almost black	Point mutation at 831 aa
$W^{42[3,96-97,100]}$	Death in utero	Severe	Sterile	—	Viable	Severe	Low fertility	Entirely white	Point mutation at 790 aa
$W^{43\ [97,100]}$	Perinatal lethality	Severe	Sterile	Entirely white	Fully viable	Non-anemic	Sterile	White spot	—
$W^{44[98,100]}$	Viable	Non-aneic	Sterile	Almost white	Viable	Non-anemic	Fertile	White spot, mostly black	Rearrangement of the genome
$W^{55[98]}$	Viable	Severe	Sterile	Entirely white	Viable	Mild	Fertile	White spot	Point mutation at 660 aa
$W^{57[98]}$	Viable	Mild	Fertile	White spot	Viable	Non-anemic	Fertile	White spot	Reduced kinase activity
$W_v/W^{19H[102]}$	×	×	×	×	Low viability	—	—	Entirely white	—
$W^e/W^{19H[102]}$	×	×	×	×	Ebroynic death	—	—	—	—
$W/W_v^{[98-99]}$	×	×	×	×	Semi-lethal	Anemic	Sterile	White	No kinase activity

W*: 表示任何一种W位点的突变; —: 表示缺少报道; ×: 表示无此项。

W*: indicated any type of W mutants; —: no available report; ×: no this item.

6 *c-kit*基因在成体生殖细胞表达状态的争议

长久以来分化的精原细胞被认为不可逆地发生了分化。即认为c-kit阳性细胞不能够转变成c-kit阴性的干细胞。但c-kit在SSCs中的表达目前仍具有争议。在雌性和雄性黑腹果蝇中, 分化的生殖细胞可以转变为具有功能的干细胞来维持生殖细胞系[83-84]。Barroca等[85]在小鼠中的实验表明, 将纯化的c-kit阳性精原细胞移植到γ射线处理的生殖细胞缺乏的成年小鼠睾丸内, 供体来源的精原细胞能够重新形成克隆。作为精原细胞分化的标记, c-kit的功能包括抑制PGCs凋亡, 促进PGCs和精原细胞的增殖, 启动精原细胞减数分裂的开始[8]。在精原细胞中c-kit表示分化的开始, 但在其他多种干细胞中, 如造血干细胞, c-kit是重要的干细胞标记[86]。最近研究表明, c-kit$^-$和c-kit$^+$细胞在移植后均表现出类似的干细胞活性[85,87]。因为依照微环境的不同SSCs可以改变其表型, 因此c-Kit$^+$细胞可能是SSCs自我更新的中间型[88]。 Izadyar等[89]进一步发现POU5F1$^+$/c-kit$^+$的SSCs表达多潜能性ES细胞的标记并且可分化成多种细胞系。但在睾丸移植结果中只有POU5F1$^+$/c-kit$^-$的SSCs可以重新在受体睾丸内启动完整的生精过程。而且利用甲磺酸伊马替尼(Imatinib mesylate, 一种癌症治疗药物)处理精原细胞(包含SSCs和其他类型的精原细胞), 会使其c-kit失活, 最终导致已分化精原细胞数目的减少, 但是不会阻碍SSCs的自我更新[90]。 因此, c-kit看起来并不直接参与调控SSCs的自我更新, 而是对其存活和增殖起到必要的调节作用。引人入胜的是, 尽管c-kit/SCF信号通路的激活对SSCs自我更新并不是必须的, 但是有研究报道表明c-kit可在部分SSCs中表达[88,91-92], 表明了生精调控过程的复杂程度。通过siRNA沉默精原细胞中的c-kit表达, 可使细胞周期停滞, 证实了c-kit对减数分裂的起始起到重要的作用[93]。c-kit在单倍体生殖细胞中是否存在一直存有争议。Muciaccia等[94]报道了c-kit和其mRNA在精子中无法检测到, 但Feng等[95]则报道成熟人精子表达c-kit, 并且其可能参与精子获能中的顶体反应[22]。综上所述, c-kit在精原干细胞中是否表达, 其生物学作用及其在分化的生精细胞中起到的作用是目前关注的重点问题。而且c-kit在生精过程的不同阶段时空表达差异及c-kit相关的信号通路之间的相互作用的阐明是解释生精细胞的增殖及分化的关键。

7 展望

c-kit作为生精过程中重要的信号传递受体, 对PGCs的存活、迁移和精原细胞的维持及分化中起到了关键的作用。目前, c-kit在生精细胞中的生物学作用备受关注。研究热点主要集中在精原干细胞是否表达c-kit、c-kit对精原细胞的分化和增殖的影响及S-kit和Tr-kit在生精细胞中的分布和作用。解决上述问题的关键在于明确界定精原干细胞的定义, 划定精原干细胞分化的标准及建立有效的研究工具(如抗体、模型动物和序列等)和手段(纯化, 移植和分子标记)。对c-kit的深入研究将有助于阐明生精细胞增殖、分化、迁移、凋亡和受精机理, 从而为解决雄性不育和优良育种提供理论基础。

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