生殖细胞系建立的研究进展与应用

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摘要 细胞系的建立是指特定类型的细胞可以在体外大量增殖和多次传代, 并保持原有细胞主要生物学特征的一项技术。大多数研究发现, 原代细胞在体外培养时易过早分化, 但通过转入外源基因或添加特定营养因子可激活细胞周期运转, 调控信号通路, 实现细胞大量增殖和完成细胞系的建立。生殖细胞系主要是来源于卵巢和睾丸组织内的细胞, 一直是细胞生物学、发育生物学和转基因动物等的研究热点, 也是细胞建系最多的组织。该文将对细胞系建立的相关技术以及与生殖相关细胞系的建立进行综述, 为促进细胞建系和生殖发育方面的研究和应用奠定基础。

关键词 细胞系的建立; 增殖; 细胞周期; 生殖细胞系

Research Progress and Application of Germ Cell Line Establishment

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Abstract Cell line establishment is a technology that specific types of cells can proliferate and passage for many times *in vitro*, and keep the main biological characteristics of the original cells. Most studies have found that primary cells are easy to differentiate prematurely *in vitro*. But cell cycle operation can be activated by transfecting exogenous genes or adding specific nutritional factors, and signal pathways can be regulated, to achieve the cells proliferation and establish cell lines. Germ cell lines are mainly derived from cells in ovarian and testicular tissues. They have always been the research hotspots in cell biology, developmental biology and transgenic animals. They are also the tissues with the most cell lines. In order to lay a foundation for the research and application of cell line establishment and reproductive development, this article will review the related technology of cell line establishment and the establishment of germ cell line related to reproduction.

Keywords cell line establishment; proliferate; cell cycle; germ cell line

细胞系建立是指通过抑制细胞衰老和凋亡[1], 促使细胞在体外大量增殖和传代, 并保持原有细胞的生理特性的一项技术[2]。早期研究显示, 利用自发突变或人工诱导等方法能够完成细胞系的建立[3-4], 但存在耗时长、效率低的问题; 随着分子生物学和细胞生物学技术的发展, 外源基因表达、诱导调控衰老相关基因突变等方法能更容易促使细胞建系[5-6]。生殖活动是生命的起始和后代的延续, 也是生命科学研究的热点。来源于睾丸和卵巢组织内特定细胞所建立的细胞系, 为细胞增殖分化、配子发育和生殖

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激素生成等相关分子机制和转基因技术的应用奠定了基础[6-7]。但在体外培养过程中, 与生殖相关的细胞系建立还存在诸多瓶颈。一方面, 很多具有干性的细胞培养要求苛刻, 生长缓慢, 这制约了该细胞的研究和应用; 另一方面, 建立成功的细胞系因增殖迅速, 导致原有细胞表型及特定生理功能丧失[6]。为解决此问题, 很多学者通过优化培养体系, 实现了体外细胞生长接近于体内细胞生理生殖发育的条件[6], 也有一些学者通过完善细胞建系方法, 为细胞生理功能研究及其体外研究提供了更好的方向。本文主要对细胞系建立的相关技术以及生殖相关细胞系的建立进行综述。

1 细胞系建立的相关技术

细胞系的建立是细胞功能和应用研究的基础, 主要通过理化方法及生物学方法完成(表1)。理化方法是通过放射性因素和致癌物等来完成细胞系建立的[4,8]; 生物学方法则是以猿猴病毒40大T抗原(*Simian* virus 40 large T antigen, *SV40LT*)为代表的病毒基因和端粒酶逆转录酶(telomerase reverse transcriptase, *TERT*)基因所介导的细胞系建立方法[5,9]。最新研究显示, 可逆性细胞建系技术已成为现阶段重要方法之一(表1)。

1.1 传统细胞建系技术

细胞建系技术主要应用于促进细胞周期运转

表1 细胞建系技术

Table 1 Cell line building technology

技术 Technology	方法 Methods	分类 Types	机制 Mechanism	局限性 Limitations	因素 Factors	细胞系 Cell lines	参考文献 References
Traditional cell line building technology	Biological methods	Telomeres method	Activating the activity of *TERT*; prolonging the life cycle	Applying only to specific cells that are the natural host of the virus; changes of cell phenotype	*TERT*	Sheep GCs	[5,28]
		Virus method	Changing the normal gene; bypassing the death period		*SV40*	Porcine GCs	[9]
					HPV	Human embryonic fibroblast line	[29]
					EBV	Human B lymphocyte line	[30]
		Oncogene method	Inhibiting the expression of tumor suppressor genes; activating the activity of *TERT*	The characteristics of the original cells were retained in varying degrees	*C-myc*	Human fibroblast cell line	[31]
					P53	Mouse embryonic fibroblast line	[32]
	Physical and chemical methods	Physical radiation method	Inhibiting the expression of tumor suppressor genes; keeping telomere length	Random damage of genome occurred; losing characteristics of source cells	Radiation	Mouse amniotic fluid cell line	[4,33]
		Chemical carcinogens method	Improving autophagy		4NQO	Human fibroblast cell line	[34]
	Spontaneous mutation		Genetically instable; unclear molecular mechanism	Genotype instability; losing characteristics of source cells		Porcine GCs	[35-36]
Reversible cell line building technology		Cre-LoxP site specific recombination technique	Immortalized genes were inserted between loxP sequences to expand the cells; Cre recombinase recognized loxP site and removed immortalized gene; the characteristics of the cells recovered from the source			Rat pancreatic cell line	[15,19]
		Tet-on system	Dox can combine rTetR with TRE; Dox makes immortalized gene express			CIPGCs	[6]

和抑制细胞衰老。因为理化方法所建立的永生化细胞系会出现细胞基因组损伤和丢失等问题, 所以生物学方法中的病毒法、癌基因法以及端粒法成为了建立细胞系的主要方法。抑制抑癌基因(*p53*和*pRb*)的活性促使细胞进入永生化状态, 或激活/上调*TERT*的活性来维持端粒的长度, 使细胞跨越衰老期[10]。然而, 因细胞类型和种属的差异, 目前还没有一种通用的方法可以使每一种细胞都获得同样的永生[11]。

1.2 可逆性细胞建系技术

可逆性细胞建系技术是指运用永生化基因建立的细胞增殖到一定数量后, 利用特异性整合技术关闭或去除永生化基因使细胞恢复到原始化状态的方法[12], 以解决经*SV40LT*或*TERT*转染的细胞在长期培养过程中生理功能丧失等问题。在可逆性细胞建系技术中, Cre-LoxP特异位点重组技术、四环素(tetracycline, Tet)诱导表达系统(Tet-on)已获得较理想的效果; 运用温度控制技术和光遗传学建立可逆性细胞系将是一个新的研究方向。

Cre-LoxP重组是一种特定位点的重组酶技术, 具有高度保守性和特异性, 包括Cre重组酶和LoxP位点[13]。Cre重组酶是大肠杆菌P1噬菌体中的一个38 kDa的酪氨酸重组酶[14]。LoxP位点是一个包含34个碱基对(base pair, bp)的序列, 由两个13 bp的反向重复序列组成, 并由一个定向的8 bp间隔序列隔开, 其序列为5′-ATAACTTCGTATA-NNNTANNN-TATACGAAGTTAT-3′[15]。Cre重组酶能够有效地识别两个靶位点LoxP以催化位点特异性协同重组, 从而导致两个直接重复的LoxP位点之间的DNA序列被切除[16]。在细胞建系过程中, 永生化基因可以插入到两个同向的LoxP序列之间, 从而构建重组载体; 当原代细胞表达永生化基因并大量扩增后, 通过Cre重组酶识别LoxP位点, 切除永生化基因, 使细胞恢复原代细胞表型和生物学功能(图1)。目前, 运用该技术已成功建立肝细胞系[17]、成纤维细胞系[18]、胰腺细胞系[19]和心肌细胞系[20]等。如在Cre重组酶作用下, 人胰岛β细胞系的永生化基因被切除, 胰岛素的合成量增加2.15倍, 从而为细胞永生化的临床应用和研究策略提供更广阔的应用前景[21]。在生殖细胞方面, 暂时还未发现有通过该技术成功建立的可逆细胞系。

Tet诱导表达调控系统是以大肠杆菌Tn10转座子上Tet抗性操纵子为基础而建立的一种用于诱导基因表达的调控系统, 包括抑制型系统Tet-off和激活型系统Tet-on[22]。Tet-on具有更强的严密性和可控性, 是目前应用最广泛的一种操控基因表达的系统[23]。强力霉素(doxycycline, Dox)可以使反义Tet阻遏蛋白(reverse Tet repressor protein, rTetR)与Tet应答元件(Tet-responsive element, TRE)结合, 进而促使目的基因表达; 当Dox不存在时, 则相反[24]。在已建立的可逆性猪颗粒细胞系(conditional immortal porcine granulosa cells, CIPGCs)中, Tet-on系统促使*SV40LT*表达, 添加Dox时, 以红色荧光来监测*SV40LT*表达情况; 不添加Dox, *SV40LT*则不表达, 细胞恢复原有细胞的生理状态; 若再次添加Dox, 细胞可再次进入增殖状态。CIPGCs的建立真正地实现了可逆永生化的调控, 展现出了Tet-on在细胞建系中的强大优势(图2)[6]。

温度控制技术指利用特定的温度控制外源基因使其在特定的时间和空间发挥作用的技术。目前, 通过温度调控基因并建立的细胞系主要集中于温度敏感*SV40LT*(temperature sensitive *SV40LT*, *ts-SV40LT*)。*ts-SV40LT*系呈现出温度依赖性增殖的特点, 在特定温度消失后细胞开始分化或生长停止而出现凋亡[25-26]。光遗传学技术是一种新兴的基因表达调控技术, 融合了光学及遗传学技术[27]。虽然光遗传学系统已广泛运用于基因表达调控方面的研究, 但目前可应用到可逆性细胞建系的相关研究还未见报道。

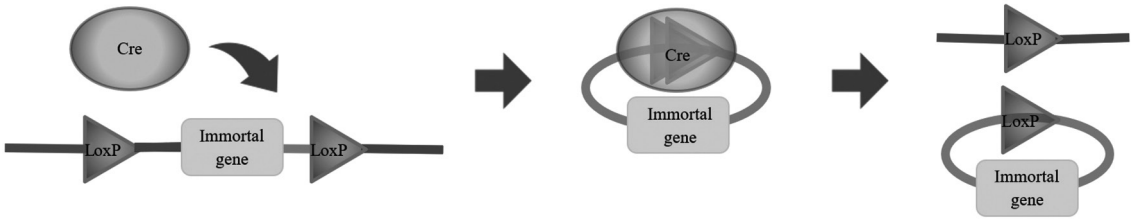
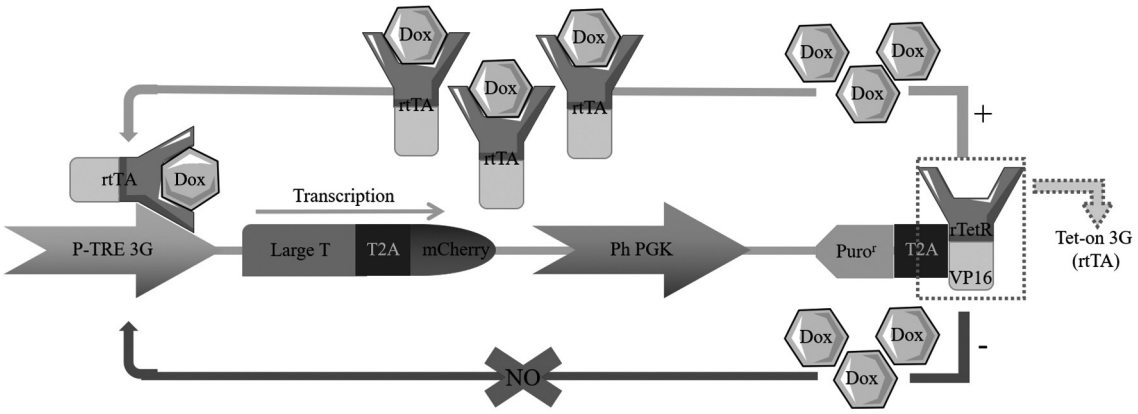


图1 Cre-LoxP系统建立可逆性细胞系基本原理

Fig.1 Basic principle of Cre-LoxP system for establishing reversible cell lines



rTetR: 反义Tet阻遏蛋白; VP16: VP16蛋白; Dox: 强力霉素; TRE: Tet应答元件; PhCMV: 人巨细胞病毒早期启动子; rtTA: 反义Tet转录活化因子; P-TRE 3G: 第三代四环素诱导型启动子; rtTA: 第三代强力霉素反应性反式激活蛋白; T2A: 2A自裂解肽; mCherry: 一种红色荧光蛋白; Ph PGK: 人磷酸甘油酸激酶1启动子; Puro^r: 吟霉素抗性基因蛋白。

rTetR: reverse Tet repressor protein; VP16: viral-protein 16; Dox: doxycycline; TRE: Tet-responsive element; PhCMV: human cytomegalovirus promoter; rtTA: reverse tetracycline tranional activator; P-TRE 3G: third-generation Tet-inducible promoter; rtTA: third-generation doxycycline-responsive transactivator; T2A: 2A self-cleaving peptides; mCherry: a red fluoresecent protein; Ph PGK: human phosphoglycerate kinase 1 promoter; Puro^r: puromycin resistance gene protein.

图2 诱导性*SV40LT*抗原表达慢病毒质粒的构建

Fig.2 Construction of lentiviral plasmid expressing inducible *SV40LT* antigen

2 原始生殖细胞系的建立

原始生殖细胞(primordial germ cells, PGCs)是动物个体发育过程中最早出现的生殖干细胞, 经性别分化, 分别转化为雄性和雌性生殖细胞[37-38]。迄今为止, 通过添加生长因子与饲养层细胞, 成功建立了鼠[39]、猪[40]、牛[41]、人[41]、羊[43-44]、兔和鸡[46]等多种动物的PGCs长期培养系统(表2); 激活素、成纤维细胞生长因子2(fibroblast growth factor 2, FGF2)、胰岛素和转铁蛋白的组合能够维持细胞体外长期增殖[47-48]。PGCs作为功能性配子的前体, 在发育过程中表现出独特的多能性、分化潜能和迁移性, 不同于哺乳动物。2006年, VAN DE LAVOIR等[49]将从鸡胚胎脉管系统中抽取含有PGCs细胞的血液置于水牛–大鼠肝脏(buffalo rat liver, BRL)条件培养基, 以维持PGCs的增殖能力并建立其细胞系。WANG等[38]运用同样的方法成功分离并获取了鸡胚血液PGCs细胞, 分别建立了三个稳定的雄性鸡PGCs系, 这为种系嵌合体的遗传改良提供了有效资源。由于鸡的特殊发育不同于其他动物, 致使PGCs较难获得[50]。此外, 因不同物种PGCs还存在很大差异、PGCs的培养需要大量的细胞因子和添加剂等, 使得该研究在生殖发育、功能基因和转基因动物等方面具有一定的局限性。重要的是, 具有多潜能性的PGCs可广泛应用于许多领域, 如发育生物学、基因组编辑和遗传资源保存等, 故研究拟建立条件诱导永生的PGCs系势在必行。

3 雄性动物生殖细胞系建立

睾丸组织中包括各个阶段的精原细胞、支持细胞和间质细胞, 是精子发生和雄激素分泌的场所, 也是维持雄性动物内分泌和繁殖活动的重要器官。

3.1 精原干细胞

精原干细胞(spermatogonial stem cells, SSCs)是睾丸中最原始的精原细胞[51], 既能自我更新又能分化为成熟的精子的成体干细胞[7], 在睾丸中数量较少[52], 体外分离和培养非常困难。1998年, 首次建立了鼠胚胎成纤维饲养层中长期增殖的小鼠SSCs[53]; 随之, 现已建立了多种小鼠SSCs培养系统, 包括STO饲养层[53]、Matrigel无饲养层培养系统[54]和三维软琼脂培养系统[55]等。但目前, 依然只有啮齿动物SSCs在体外可通过添加多种细胞因子和添加剂实现长期培养, 而其他物种仍未获得真正成功[56]。

通过用*SV40LT*抗原转染大鼠SSCs, 首次建立了具有干细胞特征的大鼠SSCs永生细胞系, 这个细胞系极大地促进了SSCs的研究[57]。同样地, 小鼠SSCs系C18-4通过过表达*SV40LT*而被建立, 能够表达胶质细胞源性神经营养因子受体α1(glial cell line derived neurotrophic factor receptor alpha1 gene,

表2 生殖细胞系的建立

Table 2 Establishment of germline

细胞系 Cell lines	技术 Technology	动物 Animal	特点 Characteristics	参考文献 References
PGCs		Chicken	Both male and female cell lines were present	[49]
			all male; with germline cell characteristics	[38]
SSCs	*SV40LT*	Rats	No morphologic changes; normal DNA content	[57]
		Mice	Expression of GFRα-1 and Ret receptors	[58]
		Human	Abnormal karyotype	[59]
		Porcine	The ability of colonization and proliferation *in vitro*	[7]
SCs	*ts-SV40LT*	Mice	Temperature sensitive type	[65]
		Rats		[26]
	SV40LT	Porcine	The low expression of CD59	[70]
	TERT	Mice	Expression of FSH receptor	[67]
		Human	Normal Y chromosome; no tumor formation	[71]
LCs	*ts-SV40LT*	Mice	Temperature sensitive type; no expression of *LH* receptor mRNA	[25]
	TERT	Goat	Properties, genes and receptors of steroidogenic	[75]
GCs	*SV40LT*	Rats	High progesterone levels	[88]
		Mice	Steroidogenic properties	[89]
		Porcine	Progesterone could not be detected	[9]
	TERT	Goat	Progesterone could be detected	[5]
	Tet-on	Porcine	Reversible	[6]
CLCs	*ts-SV40LT*	Rats	Temperature sensitive type	[93]
		Goat		
	TERT	Goat	Normal karyotype; there was no tumor formation	[95]
		Porcine	Progesterone secretion	[92]

GFRα1), 被广泛应用; 相反, 运用TERT转染的方法建立的小鼠SSCs系S4无此特性[58]。2015年, 首次报道了通过表达*SV40LT*抗原所建立的人SSCs系, 该细胞系具有人类SSCs表型和功能, 可在体外大量增殖; 在受体小鼠体内定植时, 研究发现小鼠体内不存在Y染色体的缺失和肿瘤的形成, 可以为揭示人类SSCs系的分子调控机制提供理想的细胞模型[59]。2020年, 首次报道的SSCs系在没有形态变化的情况下已成功传代35次以上, 不仅可对视黄醇信号通路产生反应, 而且能在移植后不形成肿瘤的情况下定植于受体小鼠睾丸, 成为研究猪SSCs自我更新和分化机制的重要材料[7]。迄今为止, 啮齿类动物SSCs的体外培养、移植技术和建系技术已经趋向成熟, 但对其他动物SSCs的建系仍处于探索阶段[60]。总之, 当建立其他动物SSCs系时, 运用*SV40LT*抗原转染细胞的方法是可行的。

3.2 睾丸支持细胞

睾丸支持细胞(sertoli cells, SCs)对于正常的精子发生必不可少, 是睾丸生精小管中唯一的体细胞类型, 为雄性生殖细胞提供结构和营养支持[61]; 具有较强的可塑性, 在治疗糖尿病[62]、神经系统疾病[63]与激素缺乏症[64]等研究中也显示出很大的应用价值。转染*ts-SV40LT*建立的小鼠和大鼠SCs系在33 °C下能够无限期增殖, 为研究SCs提供模型[26,65]。SCs的基因表达受垂体的促卵泡激素(follicle stimulating hormone, FSH)和睾丸间质细胞的雄激素等调控[66], 但经*SV40LT*永生化的啮齿类SCs系均不表达雄激素和FSH受体[67], 如激素诱导型小鼠睾丸SCs系[68]、大鼠睾丸SCs系(SCIT-C8、ASC-17D和93RS2)[69]。然而, 通过*SV40LT*抗原建立的猪SCs系除CD59表达相对较低外, 均能稳定增殖, 具有与原代细胞相似的表型, 可表达FSH受体与雄激素受体等[70]。由于*TERT*为非癌基因, 利用TERT转染后, 永生的小鼠B6Sc-2和B6Sc-3细胞系形态和基因表达模式保留了亲代细胞原有的特征, 能够表达FSH受体[67]。WEN等[71]首次通过过表达*TERT*获得的稳定的人类SCs系HS1也同样具有无限增殖潜力和高安全性, 为基础研究以及生殖和再生医学提供了依据。由于SCs系有助于

基于不同激素、细胞因子和微生物对妊娠期类固醇生成细胞活性和功能的机制影响的研究, 所以建立各种动物SCs系是极为重要的。

3.3 睾丸间质细胞

睾丸间质干细胞(stem leydig cells, SLCs)是一类位于生精小管外侧壁的干细胞, 能够维持自我更新, 逐步分化为睾丸间质祖细胞(progenitor leydig cells, PLCs)、未成熟睾丸间质细胞(immature leydig cells, ILCs)和成熟间质细胞(adult leydig cells, ALCs)[72]。2020年, 有报道称已成功建立能产生11-酮睾酮的SLCs系, 该细胞系在培育77代后仍能保持细胞的稳定增殖和正常核型; 经特定培养基诱导后, SLCs能分化成睾丸间质细胞(leydig cells, LCs)并产生11-酮睾酮, 为研究SLCs的体外自我更新和类固醇生成提供了理论依据[73]。SLCs对LCs的形成和维持发挥着重要作用。然而, 到目前为止, 研究者们对SLCs的研究主要集中于增殖与分化等调控机制方面。因此, 在今后的研究中可以加强对SLCs建系的研究, 从而为研究相关的内分泌干扰物提供宝贵的体外模型。

LCs可以合成和分泌睾酮, 促进精子成熟与雄性动物第二性征维持[74], 包括胚胎时期间质细胞(fetal leydig cells, FLCs)和ALCs[72]。起初, 虽然已使用原代LCs培养, 但其有限的寿命阻碍了长期效果的评估。有研究报道, 通过转染*ts-SV40LT*, 促使转基因小鼠LCs系TTE1表现出明显的温度敏感型生长表型, 但不表达促黄体生成素(luteinizing hormone, *LH*)受体的mRNA[25]。山羊LCs系转染*hTERT*不仅保留了典型的类固醇特性, 还延长了复制寿命, 成为了研究LCs的有用模型[75]。然而, 目前关于LCs建系的研究报道还太少, 这也在一定程度上阻滞了对精子发生和男性生殖功能机制的研究。因此, 建立稳定的LCs系将成为研究正常LCs发育、功能和调节的极好工具。

4 雌性动物生殖细胞系的建立

卵巢具有雌性生殖干细胞、卵泡颗粒细胞与黄体细胞, 促进卵母细胞生产并分泌性类固醇激素, 如雌激素和孕激素。多种细胞已在体外建立细胞系, 如可逆永生系CIPGCs等。

4.1 雌性生殖干细胞

雌性生殖干细胞(female germline stem cells, FGSCs)是一类既能进行自我更新又能分化为卵母细胞的干细胞, 存在于卵巢皮质[75]。通过生殖生物学分析, 发现雌性哺乳动物在出生后会失去新细胞产生的能力[77]。然而, JOHNSON等[78]提出FGSCs的概念之后, 越来越多的研究证明哺乳类动物卵巢内仍存在FGSCs; ZOU等[79]通过小鼠血管同源基因(mouse vascular homologous gene, *MVH*)进行磁珠分选, 首次建立了具有正常核型和高*TERT*活性的新生小鼠FGSCs品系, 且将其移植到不育小鼠卵巢会促使卵子发生; 随后, ZOU等[80]经进一步优化方法, 通过种系特异蛋白Fragilis膜受体分选获得较多FGSCs; WIHTE等[81]利用较为灵敏的流式分选技术分离出了人FGSCs, 首次证实了育龄妇女卵巢内拥有FGSCs且能产生再生卵子。BAI等[82]从4月龄猪的卵巢皮质中分离出的FGSCs, 可以在无血清和无饲养层的培养基中形成多达8代的细胞集落; 2014年, 通过刮取绵羊卵巢表面上皮(ovarian surface epithelium, OSE)进行MACS、干细胞富集、HE染色, 可见散布少量FGSCs[83]; 同年, 经流式分选技术, 研究者从牛OSE中获得了具有种系干细胞(卵母细胞)特征的细胞, 即FGSCs[84]。目前, 由于FGSCs在卵巢中数量较少, 且存在耗时长、操作繁琐等一系列弊端, 还未通过建系技术进行FGSCs建系。但研究开发各种动物FGSCs细胞系十分必要, 且有助于研究卵巢功能并推动动物优良品种的开发及濒危动物的保种等[76,85]。因此, 优化FGSCs分离方法、建立健全FGSCs培养体系、探索FGSCs建系可能是目前亟待解决的问题。

4.2 卵泡颗粒细胞

颗粒细胞(granulosa cells, GCs)具有维持卵泡膜基质细胞类固醇生成、抑制皮质基质细胞凋亡和提高基质与卵泡膜细胞抗凋亡能力的一类细胞[86]。由于原代GCs会在体外快速黄体化, 导致GCs的体外研究非常困难[87], 建立不同物种的GCs系变得十分重要。研究显示, 当细胞色素P450胆固醇侧链裂解酶(cytochrome P450 cholestero side chain lyase, *P450scc*)表达时, 通过己烯雌酚处理的未成熟GCs经逆转录病毒建立的大鼠GCs细胞系Rao-gel-29, 在含环腺苷酸类似物的培养环境中, 能够分泌孕酮[88]; 当缺乏*P450scc*基因时, 尽管受到FSH刺激, 但无法在猪GCs细胞系PGV中检测到类固醇[9], 即所有P450的表达均受转录因子肾上腺-4结合蛋白(adrenal-4 binding protein, Ad4BP)的影响。相关报道显示, 转

染*Ad4BP*基因和*SV40LT*抗原所建立的小鼠GCs细胞系4B2的类固醇生成能力增强, 从而抑制细胞生长, 与原代GCs的分化过程相似[89]。同样地, 运用TERT转染法建立的山羊GCs系保留了与原代细胞相同的表型以及相似的类固醇生成特性[5], 但由于在细胞永久化过程中, 其许多关键的生理功能丢失。因此, 先前已建立的GCs系并不是研究GCs在正常卵泡发生中功能的理想模型。

为解决通过转染*SV40LT*或*TERT*造成的组成型表达, 并改善长期培养细胞的生理功能丧失的问题[90-91], 研究并建立一种可逆细胞系方法日益重要。目前, 通过使用Tet-on的诱导系统关闭*SV40LT*或*TERT*的表达, 可以恢复永生化细胞系更多的功能表型。CIPGCs不仅保留了维持生理功能的能力, 还能够在促性腺激素的作用下产生雌二醇, 为研究这些重要细胞在卵巢卵泡发育过程中的功能调节构建了模型[6]。因此, 可逆细胞建系技术能够为生殖细胞系的成功建立提供新的见解。

4.3 黄体细胞

黄体细胞(corpus luteal cells, CLCs)在妊娠过程中起着至关重要的作用, 其主要功能是分泌黄体酮维持妊娠和支持胎儿发育。原代CLCs的细胞产量低和体外传代能力弱限制了人们对CLCs的研究[92]。建立CLCs系是研究CLCs在不同疾病中活性和功能的必要条件。在先前的研究中, 通过转染*ts A209*(*ts-SV40LT*突变体)可建立温度依赖性大鼠与山羊CLCs系[93-94]。有研究表明, *TERT*基因能够在第50代山羊CLCs系中稳定表达, 并保持细胞的核型和特性[95]。同样地, 高表达*TERT*的猪CLCs系传代50次以上, 具有与原代猪CLCs相似的核型和表型特征, 这有助于在细胞和分子水平上研究黄体功能[92]。由此可见, TERT转染技术在建立CLCs系上具有重要意义, 可为CLCs系的建立提供参考。

5 小结与展望

随着细胞建系技术的不断发展, 越来越多的生殖细胞系被建立, 为进一步研究生殖、病毒机理、临床试验提供了大量的体外模型。虽然细胞系建立的机制有相似之处, 但同样的建系方法并非适用于所有细胞。目前, 仍有部分生殖细胞以现有的方法不能实现细胞建系, 且可逆性生殖细胞系建立较少。因此, 加强细胞建系技术的研究非常重要, 尤其是在可逆诱导性细胞建系技术方面。

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