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干细胞研究工具与实验方法

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导读版中文序

自1868年德国科物学家Ernst Haeckel提出“干细胞”的概念(德文stammzelle,英文stem cell)以来,经过近一个半世纪的发展历程,这一概念发生了巨大的变化。早期,Haeckel“干细胞”术语主要用于胚胎学,包含两层含义:一层意义是机体所有组织器官的祖先细胞;另一层意思是能够形成所有组织器官的受精卵,与现在“生殖干细胞”的概念相似。中期,自1905年德国科学家Artur Pappenheim提出血细胞生成观点以来,“干细胞”术语主要用于造血系统。到目前为止,造血干细胞不仅是人类认识最清楚的,也是临床应用最为广泛的干细胞。后期,1981年英国科学家Martin Evans建立了小鼠胚胎干细胞系,1998年美国科学家Jumes Thomson首次建立了人类胚胎干细胞系,其后不久,1999年美国科学家Margarel Goodell发现小鼠肌肉组织干细胞可以分化为血液细胞,提出“横向分化”(transdifferentiation)的概念。近来科学界还取得了“治疗性克隆”研究和基因修饰重组成体细胞为胚胎干细胞的巨大进展。这些突破性进展不仅引发了干细胞的重新定义和重新分类,而且引起了社会各界的关注与伦理辩论。尽管如此,随着人类对干细胞本质的认识不断加深,干细胞在未来的应用依然将超越我们的想象。

干细胞从一个术语,逐渐发展为今天的干细胞生物学,这本身得益于细胞生物学、发育生物学、遗传学和生物化学等学科的长足发展。干细胞生物学作为一个独特的研究领域的出现,是后基因组时代最为重要的科学行动之一。在这个过程中,不仅需要理论上的创新,而且需要技术上的创新。只有理论与技术互动,才能使概念上的想法获得证实。本书作为爱思唯尔科学和技术出版社(Elsevier Academic Press)著名的《酶学方法》系列丛书近期推出的三卷关于干细胞专题中的第三卷,分为20章,第一部分(1～12章)搜集了干细胞研究中最有效、最热门的技术,包括基因捕获、基因表达谱分析、RNA干扰、基因转移、人类胚胎干细胞的培养、干细胞的分离纯化和细胞重组等技术;第二部分(13～20章)主要介绍了成体干细胞和胚胎干细胞在组织工程中的应用。

本书由46位来自国际一流干细胞实验室的专家撰写汇集而成。作者不仅简要举例说明最有效、最热门的技术在干细胞各个领域中的应用,而且所介绍的实验操作方案详实,图文并茂,通俗易懂,便于操作,即使是毫无干细胞研究经验的生物学研究人员,也能够在本书的指导下,成功地建立自己的干细胞实验室。对于从事干细胞的研究者而言,这是一本非常实用的参考书,倘若全文翻译,不仅周期长而且较难保证原版的质量和风格;影印版虽然周期短,但完全没有中文的非母语阅读往往使读者费时费力。为了很好地解决原版专著引进中的这些问题,科学出版社进行了大胆尝试,推出了“导读版”原版专著。基于此,应科学出版社的邀请,唐俊明和赵辉召集丁香园“干细胞与组织工程”版块的战友们对本书进行了导读性的翻译介绍,即:对原文中的前言、序、标题、摘要、图例说明、表格抬头、主题索引等进行了翻译。这不仅保证了对原文的忠实,而且可以辅助读者抓住重点,实现快速阅读,取得更好的阅读效果。

本书导读性翻译按如下程序进行:在丁香园的大力协助下,先从全国范围内征集了

20余位从事干细胞相关研究领域的战友们进行初译，随后安排初审、再审，最后终审。初译：周丽（序），黄国良（前言），周庆军（第一章），徐晓雪（第二章），丁玲（第三章），江平（第四章），孟博（第五章），李德冠（第六章），周珏宇（第七章），蔡本志（第八，十一章），徐双年（第九章），龙淼淼（第十章），高莹莹（第十二章），廖文斌（第十三，十六章），张鑫（第十四章），王玮玮（第十五章），翟东旭（第十七章），沈香娣（第十八章），李敬伟（第十九章），李听松（第二十章），张英、霍树辉（主题索引）。初审：周庆军（第一至六章），蔡本志（第七至十二章），余华（第十三至十六章），李听松（第十七到二十章），倪万茂（索引部分等）。再审：徐丹（第一至十二章），杜万良（主题索引）。全书终审：赵辉（第一至十二章），唐俊明（第十三至二十章及索引等）。

感谢丁香园“干细胞与组织工程版块”战友们的大力支持，感谢科学出版社孙红梅和贾明月两位编辑在本书导读性翻译过程中的编辑和指导。

全书译文以科学出版社出版的《英汉生物学词汇（第三版）》规范所有术语。由于译者水平有限，书中错误和不当之处在所难免。欢迎专家和读者不吝指正，以期再版时加以更正。

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前　言

干细胞能够分化为多种成体组织和细胞，正是这种独一无二的多向分化潜能引起了科学家和临床医生的极大兴趣。除了为移植和再生医学提供细胞来源之外，干细胞还为脊椎动物的发育研究提供了一个很好的模型。近年来，由于不断升温的政治和伦理辩论，对干细胞研究的兴趣不再局限于科学界，而是拓展到了普通公众之中。

干细胞分为两大类：胚胎干细胞（embryonic stem cells）和成体干细胞（adult stem cells）。胚胎干细胞也被称为多能性干细胞（pluripotent stem cell），是从着床前的胚胎分离培养的，在培养时仍保留其多能性（pluripotent），并且能分化为几乎所有成体组织的细胞类型。成体干细胞是在成体器官的大多数组织中发现的；科学家正在开始研究如何将它们分离、培养并且分化成为各种组织特异的细胞。

对干细胞进行培养以及定向诱导分化，除了需要基本的细胞培养技术外，还需要有专门的技术和知识。在本书中，我们试着全面搜集当今国际干细胞研究领域中最有效、最热门的技术（包括各种常规技术和新技术），邀请世界顶尖的科学家对他们专长的技术领域，甚至是他们创立的技术进行具体的方法介绍。在第 418 卷“胚胎干细胞”中提供了一系列涉及胚胎干细胞的来源及其分化等的原理和方法，包括很多备受欢迎的方法，如人类胚胎干细胞的取得和维持、桑椹胚和单分裂球来源的胚胎干细胞、通过单性生殖和核移植技术建立产生的胚胎干细胞等，也提供了分离小鼠、牛、斑马鱼、鸟等来源的胚胎干细胞的方法。在该卷的第二部分中，对三个胚层来源的胚胎干细胞的维持和分化的最新进展作了全面介绍，包括神经细胞、视网膜色素上皮细胞、心肌细胞、造血与血管细胞、卵细胞与雄性生殖细胞、肺细胞与胰岛素分泌细胞等的研究进展。

第 419 卷“成体干细胞”涵盖了三个胚层来源和组织器官来源的干细胞。介绍的方法包括成体干细胞的分离、分析、维持和诱导分化。涉及的成体干细胞有神经、视网膜、上皮、牙齿、骨骼、造血、卵巢、精子、肺、胰腺、肠、滋养层、生殖、脐血、羊水、胎盘等干细胞。

第 420 卷“干细胞研究与组织工程技术”搜集了特定干细胞的应用和一系列技术，包括基因捕获、基因表达谱分析、RNA 干扰、基因转移、用于获取人类胚胎干细胞的胚胎培养、干细胞的鉴定与纯化和细胞重组等技术。该卷第二部分介绍了成体干细胞和胚胎干细胞在组织工程中的应用，包括一些重要的问题，如干细胞的免疫原性和临床应用等。

本书每一章都是以该领域的简要综述开篇，并附以一系列简单实用的操作方案，让即使是毫无经验的研究人员也能在自己的实验室成功使用这些技术。

最后，我们要感谢本系列全三卷书的所有撰稿人，感谢他们无私地奉献自己宝贵的专业技术知识以及易于操作的实验方案。我们也要感谢爱思唯尔公司的 Cindy Minor，感谢她在汇编本系列全部三卷书时所给予的宝贵帮助。

IRINA KLIMANSKAYA

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序

作为干细胞领域的研究者，经常会遇到政治家、病人、记者以及非科学工作者询问以下问题：胚胎干细胞和成体干细胞的研究各有何优劣性，干细胞何时可以作为新的治疗手段应用于治疗人类疾病。以上两个问题长期存在并且一直被关注，表明了干细胞已被人们所熟知，其吸引了大众的注意力并且成为当今科学、社会以及政治事件中的焦点。

干细胞生物学本身作为一个特殊研究领域的出现，很明显是后基因组时代最为重要的科学活动之一。干细胞研究是细胞生物学与发育生物学之间的汇合点。它形成于遗传学和生物化学成熟知识库的每个转折点，并且由于重组 DNA 技术、单克隆抗体技术以及其他生物技术等技术平台的出现而得以加速发展。因为处于未分化状态的干细胞具有增殖和分化的双重能力，所以研究干细胞既有趣又有用。因此人们期待干细胞不仅可以促进我们对于多能性与分化的认识，还能带来细胞周期的调控以及其他方面的知识，从而对从肿瘤到衰老的各个领域都产生影响。

正因如此，研究不同类型的干细胞是很有必要的，这些干细胞包括存在伦理道德争议的胚胎干细胞和不易被发现、培养的成体干细胞。这个问题本身掩盖了干细胞生物学研究的深层次目的，也就是对发育过程中决定细胞命运的本质获得基本了解。对于干细胞为什么能长期维持未分化状态，以及有选择性地沿着特定途径分化，我们知之甚少。只有尽可能获得各种来源的信息，我们才能对干细胞干性及其分化能力获得准确的理解。这个三角测量过程可比喻为全球定位卫星如何找到我们自己：从一个卫星获得的信号告诉我们的信息很少，只有从三个卫星或者更多卫星获得的信号所告诉我们的信息才是准确的。相似的，如果我们要评价相对于正常发育的体外细胞发育的结果，研究多种类型的干细胞以及它们的子代是必要的。

要回答干细胞将何时带来新的临床成果这个问题，需要我们明确干细胞可能的治疗潜能。当然，成体以及新生血液干细胞已用于移植很多年，并且从目前的研究中可能发现其新的来源和用途。然而，其他成体干细胞的研究或者胚胎干细胞分化子代的研究首先应用于移植的可能性并不大。原因在于，当将干细胞植入人体后，需要明确其子代细胞是否长期安全有效。它们作为体外干细胞分化模型的应用，才更有可能推动从干细胞获得的人类特殊类型细胞的临床应用。这些细胞包括培养的神经元、心肌细胞、肾细胞、肺细胞以及其他许多细胞，它们将为新药的发现及药物的检测提供一个新的技术平台。对这些细胞模型的广泛应用，一方面有助于针对人类许多疾病的新药的研发；另一方面在干细胞及其子代细胞移植前，可在体外培养时对其稳定性及功能进行评价。最后，我们不能忽视干细胞及其子代细胞作为发育分化模型在理解人类发育过程中的重要性。虽然我们不能预知其在充分理解人类细胞分化中的作用，然而它潜在的应用将可能超越如干细胞移植等我们所能想象的最显著的应用。

尽管干细胞研究与生物学其他领域以及已建立的相关技术有关联，但是除了基本的细胞生物学和发育生物学技术，在培养体系里要使干细胞有组织地生长和分化还需要特

殊的技术和知识。本系列酶学方法包含了干细胞领域最新的技术，它们由在该领域最领先的科学家们所撰写。每一章节都是有关一些特殊技术方法成果的一个简要综述，并附有简单实用的操作方案，经验不足的研究者依据这些操作方案就能在自己地实验室成功地使用这些方法。总之，这三卷书涵盖了成体及胚胎干细胞，并且为扩展干细胞在组织工程学中的应用提供了工具。希望这些方法的有效性及其广泛普及将使广大科研工作者更容易地进入干细胞研究领域，从而将新的方法引入干细胞研究，促进人们对人类生物学和健康的了解。

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To Richard Latsis, the Teacher
–Irina

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Preface

Stem cells are of great interest to scientists and clinicians due to their unique ability to differentiate into various tissues of the body. In addition to being a promising source of cells for transplantation and regenerative medicine, they also serve as an excellent model of vertebrate development. In the recent years, the interest in stem cell research has spread beyond the scientific community to the public at large as a result of heated political and ethical debate.

There are two broad categories of stem cells – "embryonic" and "adult." Embryonic stem cells – also known as "*pluripotent*" stem cells – are derived from preimplantation-stage embryos and retain the capacity to grow in culture indefinitely, as well as to differentiate into virtually all the tissues of the body. Adult stem cells are found in most tissues of the adult organism; scientists are beginning to learn how to isolate, culture, and differentiate them into a range of tissue-specific types (and are thus considered *multipotent*).

Growing stem cells in culture and differentiating them on demand requires specific skills and knowledge beyond basic cell culture techniques. We have tried to assemble the most robust and current techniques (including both conventional and novel methods) in the stem cell field and invited the world's leading scientists with hands-on expertise to write the chapters on methods they are experts in or even established themselves. Volume 418, "Embryonic Stem Cells," offers a variety of know-how from derivation to differentiation of embryonic stem cells, including such sought-after methods as human embryonic stem cell derivation and maintenance, morula- and single blastomere-derived ES cells, ES cells created via parthenogenesis and nuclear transfer, as well as techniques for derivation of ES cells from other species, including mouse, bovine, zebrafish, and avian. The second section of this volume covers the recent advances in differentiation and maintenance of ES cell derivatives from all three germ layers: cells of neural lineage, retinal pigment epithelium, cardiomyocytes, haematopoietic and vascular cells, oocytes and male germ cells, pulmonary and insulin-producing cells, among others.

Volume 419, "Adult Stem Cells," covers stem cells of all three germ layers and organ systems. The methods include isolation, maintenance, analysis, and differentiation of a wide range of adult stem cell types, including neural, retinal, epithelial cells, dental, skeletal, and haematopoietic cells, as well as ovarian, spermatogonial, lung, pancreatic, intestinal, throphoblast, germ, cord blood, amniotic fluid, and placental stem cells.

Volume 420, "Tools for Stem Cell Research and Tissue Engineering," has collected specific stem cells applications as well as a variety of techniques, including gene trapping, gene expression profiling, RNAi and gene delivery, embryo culture for human ES cell derivation, characterization and purification of stem cells, and cellular reprogramming. The second section of this volume addresses tissue engineering using derivatives of adult and embryonic stem cells, including important issues such as immunogenicity and clinical applications of stem cell derivatives.

Each chapter is written as a short review of the field followed by an easy-to-follow set of protocols that enables even the least experienced researchers to successfully establish the techniques in their laboratories.

We wish to thank the contributors to all three volumes for sharing their invaluable expertise in comprehensive and easy to follow step-by-step protocols. We also would like to acknowledge Cindy Minor at Elsevier for her invaluable assistance assembling this three-volume series.

IRINA KLIMANSKAYA
ROBERT LANZA

Foreword

As stem cell researchers, we are frequently asked by politicians, patients, reporters, and other non-scientists about the relative merits of studying embryonic stem cells *versus* adult stem cells, and when stem cells will provide novel therapies for human diseases. The persistence of these two questions and the passion with which they are asked reveals the extent to which stem cells have penetrated the vernacular, captured public attention, and become an icon for the scientific, social, and political circumstances of our times.

Focusing first on the biological context of stem cells, it is clear that the emergence of stem cells as a distinct research field is one of the most important scientific initiatives of the 'post-genomic' era. Stem cell research is the confluence between cell and developmental biology. It is shaped at every turn by the maturing knowledge base of genetics and biochemistry and is accelerated by the platform technologies of recombinant DNA, monoclonal antibodies, and other biotechnologies. Stem cells are interesting and useful because of their dual capacity to differentiate and to proliferate in an undifferentiated state. Thus, they are expected to yield insights not only into pluripotency and differentiation, but also into cell cycle regulation and other areas, thereby having an impact on fields ranging from cancer to aging.

This directs us to why it is necessary to study different types of stem cells, including those whose origins from early stages of development confers ethical complexity (embryonic stem cells) and those that are difficult to find, grow, or maintain as undifferentiated populations (most types of adult stem cells). The question itself veils a deeper purpose for studying the biology of stem cells, which is to gain a fundamental understanding of the nature of cell fate decisions during development. We still have a relatively shallow understanding of how stem cells maintain their undifferentiated state for prolonged periods and then 'choose' to specialize along the pathways they are competent to pursue. Achieving a precise understanding of such 'stemness' and of differentiation will require information from as wide a variety of sources as possible. This process of triangulation could be compared to how global positioning satellites enable us to locate ourselves: signal from a single satellite tells us relatively little, and precision is achieved only when we acquire signals from three or more. Similarly, it is necessary to study multiple types of stem cells and their progeny if we are to evaluate the outcome of cellular development *in vitro* in comparison with normal development.

An answer to the question of when stem cells will yield novel clinical outcomes requires us to define the likely therapeutic achievements. Of course, adult and neonatal blood stem cells have been used in transplantation for many years, and it is likely that new sources and applications for them will emerge from current studies. It is less likely, however, that transplantation will be the first application of research on other adult stem cell types or of research on the differentiated progeny of embryonic stem cells. This reflects in part the degree of characterization of such progeny that will be needed to ensure their long-term safety and efficacy when transplanted to humans. It is their use as *in vitro* cellular models that is more likely to pioneer novel clinical applications of the specialized human cell types that can be derived from stem cells. These cells, including cultured neurons, cardiomyocytes, kidney cells, lung cells, and numerous others, will imminently provide a novel platform technology for drug discovery and testing. The applications of such cellular models are likely to be extensive, leading to development of new medicines for a myriad of human health problems. The wide availability of these specialized human cells will also provide an opportunity to evaluate the stability and function of stem cell progeny in the Petri dish well before they are used in transplantation. Finally, we should not overlook the importance of stem cells and their progeny as models for understanding human developmental processes. While we cannot foresee the impact of a profound understanding of human cellular differentiation, it has the potential of transcending even the most remarkable applications that we can imagine involving transplantation.

Despite the links of stem cell research to other fields of biology and to established technologies, growing and differentiating stem cells systematically in culture requires specific skills and knowledge beyond basic cell and developmental biology techniques. These *Methods in Enzymology* volumes include the most current techniques in the stem cell field, written by leading scientists with hands-on expertise in methods they have developed or in which they are recognized as experts. Each chapter is written as a short review of outcomes from the particular method, with an easy-to-follow set of protocols that should enable less experienced researchers to successfully establish the method in their laboratories. Together, the three volumes cover the spectrum of both embryonic and adult stem cells and provide tools for extending the uses of stem cells to tissue engineering. It is hoped that the availability and wide dissemination of these methods will provide wider access to the stem cell field, thereby accelerating acquisition of the knowledge needed to apply stem cell research in novel ways to improve our understanding of human biology and health.

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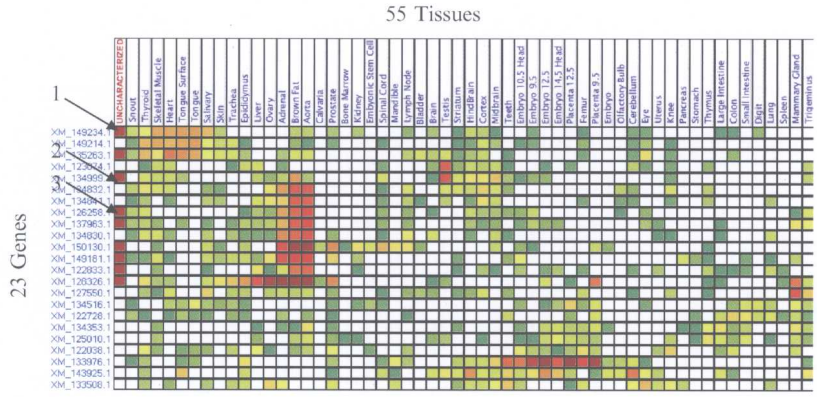

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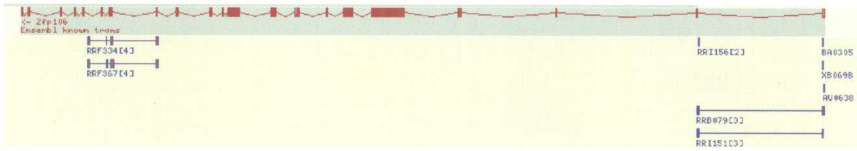
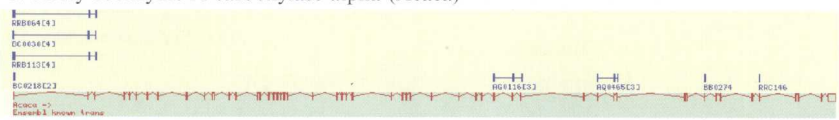


2. Expressed sequence AV340375

3. Acety-coenzyme A carboxylase alpha (Acaca)



STANFORD *ET AL.*, CHAPTER 8, FIG. 4. Expression patterns of 23 genes known or predicted to be involved in insulin receptor signaling (GO:0008286). Predictions were made at a precision of 25% or greater as described (Zhang, 2004). Of the nine predicted genes (indicated by colored boxes in the left column), three have been trapped by members of the IGTC (arrows). The genomic structure of these three genes, together with the position of gene trap sequence tags are illustrated in the bottom panel.