

Stem Cell Technologies: Basics and Applications  
Embryonic Stem Cells

· 导读版 ·

# 干细胞技术基础与应用： 胚胎干细胞

Kaushik D. Deb and Satish M. Totey



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**Embryonic Stem Cells**

**干细胞技术基础与应用：胚胎干细胞**

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## 导 读

《干细胞技术基础与应用》(*Stem Cell Technologies: Basics and Applications*)一书,以《干细胞技术基础与应用:胚胎干细胞》和《干细胞技术基础与应用:成体干细胞》两卷本,在我国以中文导读版的形式出版,从不同的角度看这都是一件让人高兴的事情。我希望我们的研究者、教师和学生,不仅把本书的引进看成一件只与相关学科有关的学术行为。本书不仅在技术层面使我们有了全面、方便的参考书,它还对干细胞研究的历史、现状和未来方向、理论和临床应用等,作了大致的描述,对重要问题进行了讨论。其内容的覆盖面、深度及意义,适合的读者群体等,作者、点评者和原著序都作了简要和清晰的说明。因此,本来中文导读需要做的,比如归纳主要内容、重点、用途、读者对象等,现在看来不免重复了,故在此不再赘述。在接下去的篇幅里我还要回过头来讨论,为什么我坚持认为:对一本题名仅涉及技术的书籍,要认真对待。在此之前,我倒想要先借这个机会,谈点我个人关于干细胞史的经历。

上世纪转折之际,生命科学突飞猛进,而在众多令人眩目的新发现中,我个人以为有两件事,深刻地影响了其后生命科学的发展,并且以我之见,还将长久地影响下去。这两件事,可谓家喻户晓,声名远播:一件就是所谓“Human Genome Project”,即人类基因组计划,它的目标是利用当时已经逐渐成熟的DNA测序技术,完成人类第一个全基因组测序,并为此发展相应的数据处理能力及其在研究和临床中的应用;另一件,就是克隆羊“Dolly”的诞生。

第一件事发生在美国并波及全球,但世界其他地区包括我国的参与,在一开始,无论从思想、技术、资金、规模、时间以及完成的工作量看,多少只是象征性的。而在今天,它的规模和影响力,以及在生物医学中的应用程度,在全世界都已是举足轻重。人类基因组计划是一件真正意义上的伟大工程:实施者除了有宽阔的视野、雄心、勇气,也综合了计算机、生物数据处理及分子生物学等专业技术,以及项目管理能力和巨大的资金。这是一项典型的高科技、商业化、大规模、美国式的科技成就,其对生命科学发展的影响,使得今天的生物学在规模上动辄是十年前的几十乃至几百上千倍。

而这第二件事,即克隆羊“Dolly”1996年于英国Roslin研究所诞生,才与本书的主题干细胞研究及其新近突破密切相关。今天的医学史家们承认,人们对干细胞研究的热情和争论始于两项关键的突破:第一便是Keith Campbell博士及其合作者成功“克隆”Dolly;第二是1998年威斯康辛大学的James Thomson博士成功建立了人的胚胎干细胞株。实际上,不用医学史家们严密的论证,凡亲身经历Dolly诞生并为之震撼和激动的科学家们都会承认这一工作对干细胞研究的意义。

上世纪九十年代,正是现代分子生物学的黄金时代,人们几乎每天都能听到激动人心的新发现。仍然,Dolly的最初报道在同学、同事中引起了极大的震动。此时正值笔者即将离开求学多年的宾夕法尼亚大学,一位来自台湾的同学邀请我到他们夫妇所在的基督教会小组讲解此事的原理和意义。以体细胞核“克隆”生命,对生物学家而言,虽非闻所未闻,但在与我们如此相近的哺乳动物中实现,不可谓不称奇,激动和恐惧兼有。记得被称之为“克隆生命”的这一成就,受到了一位年轻和善的女信徒的强烈抨击,她

认为科学家们在主的面前自恃聪明，亵渎神灵，终将受到严惩。回想起来，仅仅十来年功夫，如今人们对于干细胞、克隆和再生医学的态度，已经完全不同。

与人类基因组计划相比，Dolly 的诞生以完全不同的方式：首先它发生在英国，使我们想起了著名的“下午茶”的传说。该工作的主要核心成员之一 Keith Campbell 博士此前默默无闻，而体细胞核转移技术（所谓“克隆”在这里的实际意思），在当时分子遗传和发育生物学（基因表达和功能）“甚嚣尘上”之际，亦绝非时髦，但有传统。在此，我们遇上了文化、风格、哲学、传统、思辨、好奇和个人的重要性与魅力。这无疑是对每一个默默耕耘的个人及其价值的一种肯定。我们再次看到，人类文明的历史，充满以小博大，以个人的远见和坚持改变或创造世界的传奇。很明显，两种风格，两件大事，相辅相成，互为依托。人类基因组计划的成功，轰轰烈烈，给了我们前所未有的力量和自信；而 Dolly 的克隆和干细胞研究的突破，则更体现思想、坚持和个人，给了我们无穷的想象。而我本人则更欣赏后者：即个人的、安静专注的创造。因为，我坚信世界观的改变是根本的，技术的演进自然也不可忽缺，并且会最终又影响我们的世界观。

回到本文开初留下的话题，为什么我坚持认为，对一本题名仅涉技术的书籍要认真对待？因为我从本书出版中看到一种我们急需认真面对的东西。须记，我们曾是轻易产生亩产 10 万斤粮食的国家；时至今日，我们还时常见到相似的浮夸。与之相适应，我们不太愿做踏实的“小”事，并使之完美。例如，我们的教科书（就我熟悉的医学教材而言）至今粗糙，观点旧，错误多，缺乏个性——总之，我们需要的是多做实事，少些自欺欺人的热闹！而《干细胞技术基础与应用》一书的编著，就是一件实事，其中有许多值得我们学习之处。

本书正副主编考西柯·德布和萨蒂什·多德均来自印度，是值得我们学习的，首先是其勤奋、敏感，以及做实事、小事的立志，而他们的勇气也使人佩服。两人中虽然萨切斯·多德参与了干细胞早期的一些研究，并且一直坚持下来，是位真正的、勤奋的干细胞专家。但他也并非明星式的“权威”。两人早早在印度创办私企，分任总裁、经理，居然有勇气和号召力动员众多世界干细胞“一线”科学家为本书执笔，可见此计划众望所归。我在此没有用“一流”二字，就是想避免对国家、学校、个人的名气及地位的强调。其实，一线专家就是权威，因为只有实践，才是发现和解决问题的唯一途径。

当然，现在《干细胞技术基础与应用》一书两卷本托科学出版社的努力引进到我国出版，也是一件实事，因此值得高兴和特别推荐，愿我们在技术上参考获益的同时，也享受它。让 Dolly 带来的想象力延续下去，激发更多的思想和科技奇迹。我以一个问题结束本导读——本文力图想说：Dolly 的被“克隆”对干细胞研究的迅速发展起了关键的作用，而这一研究表现了科学以文化传统和个人及其价值为灵魂，那么 Dolly 被“克隆”的生物学本质是什么，它为什么对后来风起云涌的干细胞研究能起这样的作用呢？这是我在通篇啰嗦中并未回答的问题。藉此，留给各位认真的读者了。

高千 博士

2010年6月21日星期一

于空中

## 序

医学史讲述了人类对自己身体、对困扰我们身体的疾病，以及对因病施治的治疗方法，逐渐认知的故事，而医学史引人入胜之处，正在于其将这个演进的过程与一连串不同寻常的事件结合起来：正是这些事件推动了医学的突破和跃进。抗生素的发明正与我们对感染性疾病的深入认识发生重合，心脏外科技术的发展颠覆了心脏病的治疗手段，而功能性影像设备的引入则极显著地改善了诊断技术——凡此种种，不一而足。或许，在数十年之后，当医学史学家们回首世纪之交，也会宣布，培育乃至操控干细胞技术并将之用于成人组织的再生，正是这些颇为重要且不同凡响的医学事件中的一件。

我们已经可以看到再生医学的潜力。依我个人之见，半个世纪以来，我们（在医学上）所取得的巨大进步，例如生物医学工程师们尝试利用人工合成材料替换患病和损伤组织所取得的成就，比之再生医学领域的种种技术将会给我们带来的获益，就只能是相形见绌了。就利用非生命的合成材料来替代人体组织而言，我们所能期待的终将带着明显的局限性。尽管我们在人工关节、人工心脏瓣膜与动脉、可植入性电子装置如起搏器和除颤器，以及人工晶体等方面取得了不少成就，但这些成就仅限于（组织的）机械的或物理的性能方面。如果能够通过受损组织的再生，也来改善其生物学的和生理学的功能，岂不妙哉？而这正是再生医学所要为之——实现细胞治疗、基因治疗与组织工程的完美结合。有一点我们尚需切记，即作为成年人，我们自体组织的再生能力甚为有限，因此，再生医学绝非易事，必须经过包括寻找合适的细胞来源、通过一系列处理使它们改变并使用新的功能组织等过程。

关于用于上述目的的细胞的本质，因科学的、临床的及伦理的种种角度，一直是并且还将继续是多方争论的焦点，争议涉及自（体）源性、同种异（体）源性以及异种源性细胞；完全分化细胞、前体细胞和干细胞；以及成体或胚胎源性细胞各自的品质（等问题）。但是，毋庸置疑，整个争论的重点仍是干细胞在前述各种功能上的适用性。尽管干细胞为我们所知已有一段时间，但也只是在最近十年里，干细胞生物学以及干细胞的临床应用技术才成为人们的研究重点。因此，当前人们迫切需要这样一本书，它囊括了现阶段关于干细胞的知识以及相关应用技术的分析。我要祝贺编辑们为此所作的贡献并相信本书对于确立干细胞科学与技术于二十一世纪医学发展的前沿地位具有相当重要的参考价值。

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《生物材料》杂志主编

（高 千 译）

## 前 言

当前，干细胞研究正在激起极大的兴趣和争论，在专业科学杂志中有关干细胞的科学论文数量较以往激增，干细胞临床试验也越来越多，无疑佐证了这一趋势。政策分析师和科学家们正在达成某种共识，即相信印度在干细胞研究的科学、临床与商业化发展中，或许起着关键性的作用。虽然干细胞的研究与治疗尚处起步阶段，但在印度已是一个迅猛成长的领域，据估计其市场大约在6亿美元，而干细胞治疗的全球市场价值到2010年预期达到200亿美元之谱。

尽管在了解干细胞作用机制并展示其治疗应用前景等方面，我们已经取得了明显的进步，以干细胞为基础的各种治疗方法的广泛使用则尚处萌芽阶段，但日后干细胞治疗一定会造福于广大患者。到目前为止，多种来源的干细胞已被成功的分离和鉴定，而这一名单还在不断扩大。胚胎干细胞，以及骨髓、脂肪组织、脐带、牙髓和一些身体其他部位的干细胞则为将来进行异体或自体移植提供了可能的来源，而新近出现的诱导性多能干细胞技术可能对药物筛选及毒性试验产生革命性的影响。

干细胞的临床转化研究，主要是在成体干细胞的临床转化研究，正以迅捷的步伐向前推进。然而，这些成功在一定程度上，由于可重复性欠佳、治疗效果的不尽人意以及缺乏对机制的理解，而后者又是临床进步之所必需，又显得成就有限。因此，在目前情况下，对所有可能来源的干细胞——胚胎的以及成体的——进行深入研究十分恰当。因为我们尚不清楚，针对不同疾病，哪一种干细胞的治疗途径会取得成功。

在编著本书的过程中，我们有意关注其在世界范围内（包括印度）的教育以及临床研究功能。我们的基本目标是保证本书内容的独特性，这一点使得本书可以成为研究者的首选资料。本书的内容广泛、描述简洁，书中章节清楚地叙述了干细胞生物学领域的各主要分支及其在临床治疗上的潜在应用。很多著名的专家都参与了本书的编写，对于他们为各章节所倾注的宝贵时间与精力我们表示极大的敬意。由于很多作者在写作中描述了一部份自己的研究成果，本书也可作为科研工作者的第一手参考资料。本书涵盖了干细胞生物学的各个领域，对科研工作者、学生和初入此道者都具有重要的参考价值。

本书的每一个章节都包含了干细胞相关领域的基础知识以及最新进展。适用于已掌握基本生物学知识的学生或其他读者。本书分为两个部份：第一部份主涉胚胎干细胞。胚胎干细胞是已知最具潜能干细胞。本部份还专辟一章，阐述以胚胎干细胞为基础的药物筛选平台这一新兴领域。第二部份则探讨源于不同组织类型的多能成体干细胞。这部份内容还包含一些独特的概念，如肿瘤干细胞以及为组织再生设计合适微环境的组织工程技术路径。本书尽量均衡地展示每一领域的临床治疗进展和技术进展。此外，我们还全力以赴，努力确保在本书所涉领域，完整而准确地提供最新的内容资料。毫无疑问，干细胞研究领域进展迅速、涵盖内容非常广泛，因此，本书所涉内容在很多方面还有待进一步改进和提高。

考西柯·德布

萨蒂什·多德

（高 千 译）

## ABOUT THE EDITORS

Dr. Kaushik D. Deb is General Manager, Operations and Research, at Advanced NeuroScience Allies, Pvt. Ltd., a research and development company focusing on different areas of neuroscience and allied research. His current research interests are in the fields of directed cell trafficking technologies and applications of nanotechnology and tissue engineering in regenerative medicine.

Dr. Satish M. Totey is Co-Founder, President, and Chief Executive Officer of Advanced NeuroScience Allies, Pvt. Ltd. He is among a small number of researchers who pioneered the isolation and characterization of pluripotent stem cells from human embryos, which provided an important framework for the development of human embryonic stem cells. Dr. Totey has derived several human embryonic stem cell lines, some of which have been listed at the NIH stem cell registry. He has also developed a method for large-scale clinical scale-up of mesenchymal stem cells and successfully done several pilot studies and clinical trials in various degenerative diseases. Dr. Totey has more than 100 peer-reviewed research papers in international journals and 19 national and international patents to his credit.

# PRAISE FOR *STEM CELL TECHNOLOGIES*

*Stem Cell Technologies: Basics and Applications* will be of significant interest to researchers, faculty members teaching biology, medicine or embryology as well as graduate and post-graduate students. The practical aspects of stem cell research will also be of interest to those involved in stem cell banks and therapies. The area of my particular interest covered in this book is isolation and characterisation of germ and spermatogonial stem cells. I believe that experts in the area of human and animal reproduction will find discussions of derivation of gametes from embryonic stem cells highly relevant and revealing. I hope that the book finds a wide audience and fosters interest in stem cell biology and applications.

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*Stem Cell Technologies: Basics and Applications* will be of great interest to undergraduate and post-graduate students as well as scientists who use stem cell technologies for different purposes.

The book covers the basic concepts on isolation, characterisation, culture and differentiation of stem cells, as well as their possible future application in improving human health. The book has brought together scientists from different countries and with different backgrounds, allowing different opinions and perspectives on a relatively new area of biology. Interestingly, the book also has a historical perspective on medicine, covering major milestones from the discovery of stem cells to the evolution of regenerative medicine. It will increase our current knowledge about stem cells derived from different organisms, such as mammal, zebra fish and avian.

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*Stem Cell Technologies: Basics and Applications* presents a comprehensive overview of the 2009-2010 state-of-the-art in the use of stem cells in regenerative medicine, drug discovery, genetics of human development, and the development of cancers. With a virtual explosion in the number and range of papers published on this subject, this book gives its readership an in-depth view of the nature of stem cells from embryonic to umbilical cord to amniotic to adult. My own research with adult-derived stem cells counters the view that adult stem cells have very little plasticity in their ability to differentiate among all tissues of the body. However, I do agree with Prof. David Williams that all avenues of stem cell research, be it embryonic, umbilical cord, amniotic, hematopoietic, spermatogonial, dental pulp, and adult-derived stem cells, should be investigated to their fullest extent to gain insights into all possibilities of harnessing regenerative medicine. We are currently engaged in research on repairing damaged tissues, curing both somatic and genetic diseases, understanding the role of stem cells in the development of cancers, and developing are ethnic-based pharmaceuticals, utilising autologous, allogeneic, and xenogeneic stem cells.

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## FOREWORD

Interestingly the history of medicine combines a story of the evolution of knowledge about the human body, the diseases which affect it and the therapies intended to deal with these diseases, with a number of remarkable events that have themselves moved the subject forward with quantum leap. The discovery of antibiotics superimposed on the evolution of knowledge of infectious diseases, the development of open heart surgical techniques that revolutionised the treatment of heart disease and the introduction of functional imaging devices to markedly improve diagnostic techniques—all come to mind here. Perhaps, after a few decades medical historians, will look back to the turn of this century, and pronounce that the ability to harvest and manipulate stem cells for the purposes of regeneration of tissue in adult humans was one of those major, remarkable events.

We can already see the potential of regenerative medicine. From my own perspective, as that of a biomedical engineer attempting to treat diseased and traumatised tissue through replacement by synthetic structures, the great strides that we have made over half a century are likely to be overshadowed by the gains that will be made by various technologies of regenerative medicine. There will always be significant limitations to what we can expect to achieve through the replacement of tissues by non-living synthetic materials. Although we have had success with joint replacements, prosthetic heart valves and arteries, implantable electronic devices such as pacemakers and defibrillators, and intraocular lenses, such success will be limited to mechanical and physical functions. How much better would it be to improve biological and physiological functions, through the regeneration of the affected tissues? This is what regenerative medicine is about—a combination of cell therapy, gene therapy and tissue engineering. Bearing in mind that as adults we have very limited ability to regenerate our tissues, this is by no means, a trivial task, which essentially has to involve the sourcing of appropriate cells and persuading them, through various manipulations, to using new functional tissues.

The nature of the cells for these purposes has been, and will continue to be, a controversial issue from a variety of scientific, clinical and ethical positions, with debates about the respective qualities of autologous, allogeneic and xenogeneic sources; fully differentiated cells, progenitor and stem cells; and adult or embryonic sources of cells. Without doubt, however, a major factor in this whole debate is the suitability of stem cells for these functions. Although stem cells have been known for some time, the biology of stem cells and their manipulation for therapeutic purposes have become the subject of intense research only in the last decade. It is highly appropriate at this time, therefore,