基因打靶揭示生命奥秘

杨 晓

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生命组学研究所

提　纲

- 基因打靶技术的发展历史及其应用
- 利用基因打靶技术研究组织稳态和疾病发生机制
- 关于科学与人生的几点感悟

遗传与变异是生命的基本特征

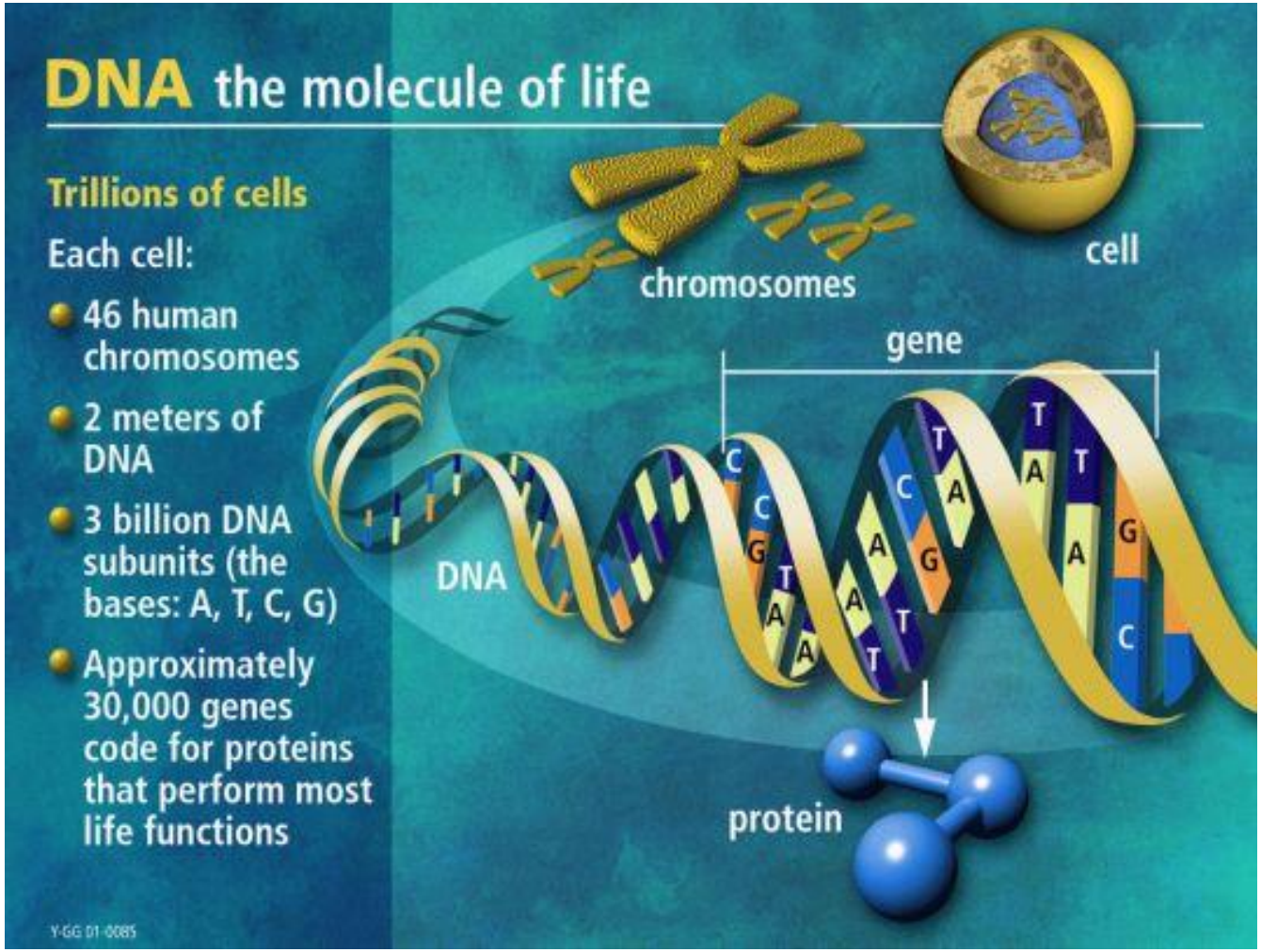








遗传信息储存于DNA中



遗传学的黄金时代

1865年，Mendel GJ：分离定律、自由组合定律

1910年，Morgan T：连锁交换定律

1941年，Tatun EL和Beadle GW：一个基因一个酶

1944年，Avery O：遗传物质的化学本质－DNA

1946年，Lederberg J和Tatum EL：原核基因同源重组

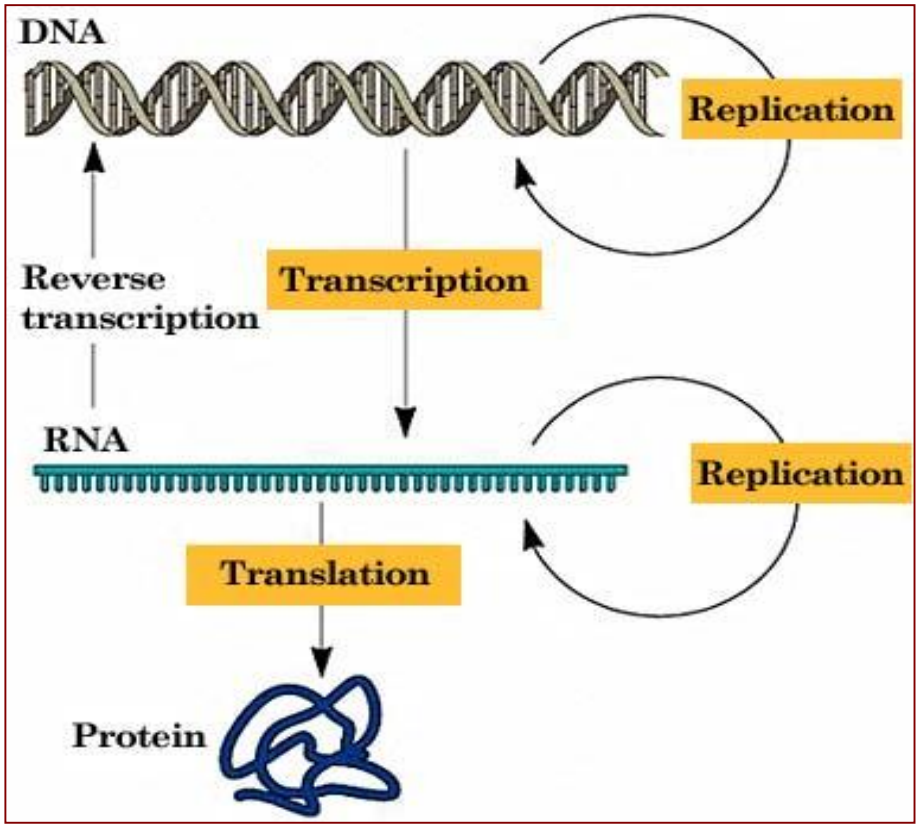
1953年，Waston J和Crick F：DNA双螺旋结构

1958年，Meselson M和Stahl F：证实DNA的半保留复制

1961年，Nirenberg MW和Matthaei HJ：破解首个遗传密码

中心法则

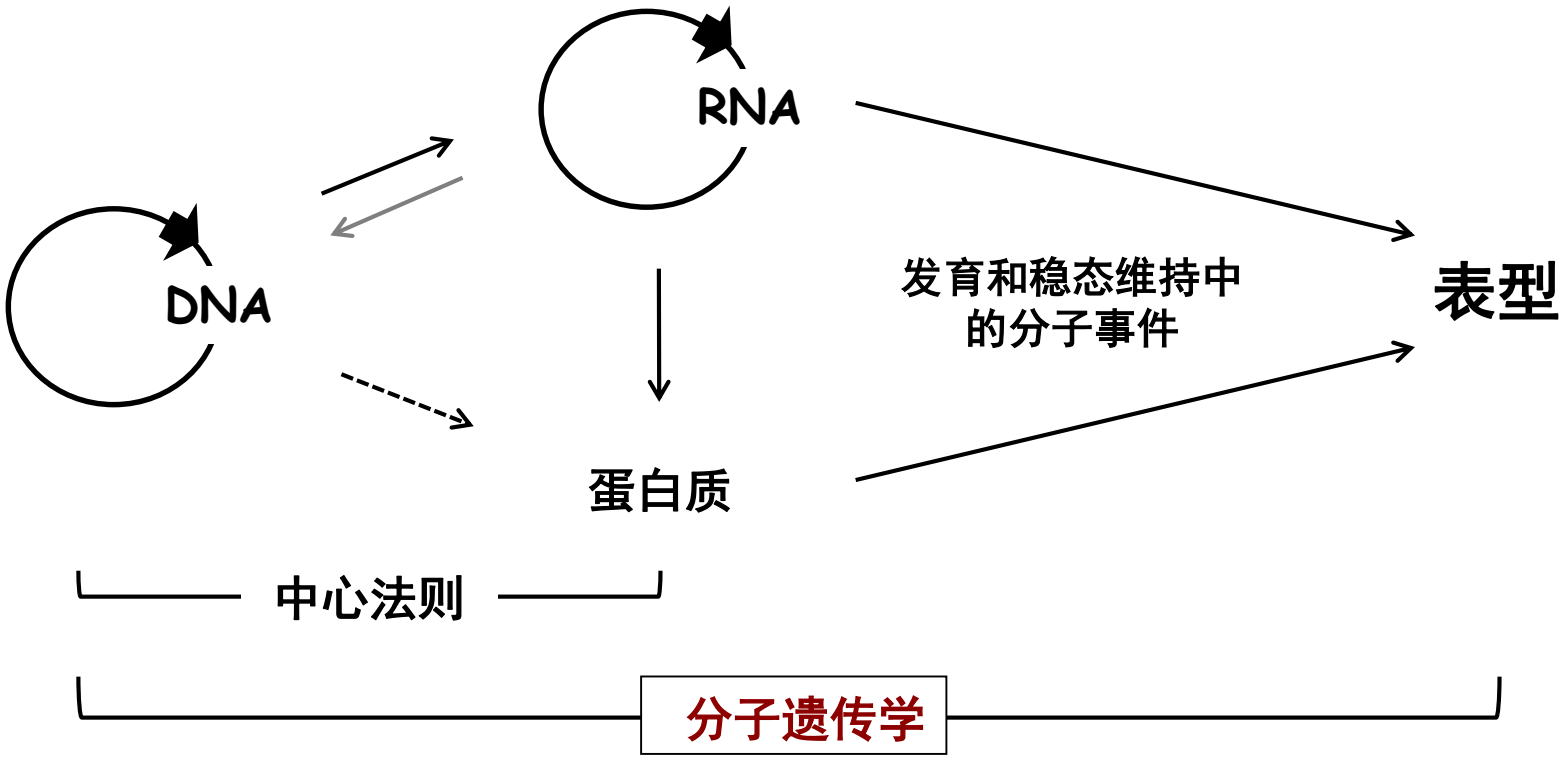
- DNA是遗传信息的储存者，它通过自主复制使遗传密码从亲代转移至子代；
- DNA通过转录生成信使RNA；
- RNA可以逆转录成DNA；
- 信使RNA通过翻译成蛋白质，行使生物学功能。



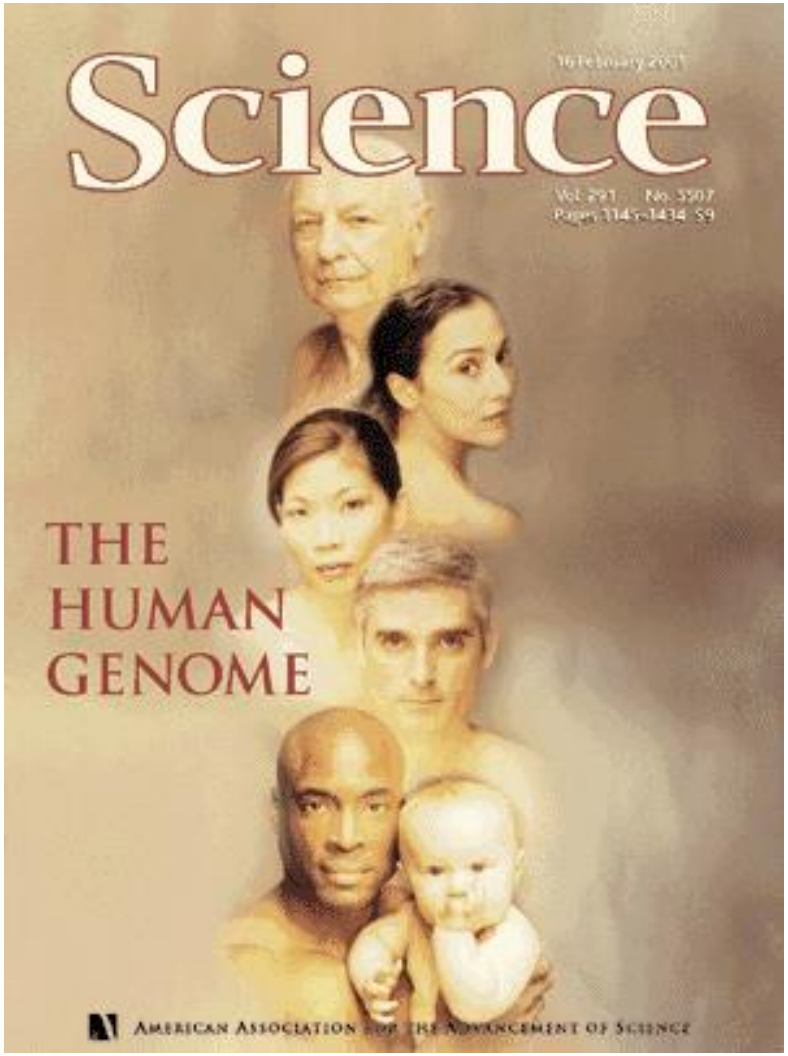
Francis Crick: Symp Soc Exp Biol XII, 1958, 139-163

Nature, 1970, 227:561-563

遗传学的涵义



基因组时代


Science
THE HUMAN GENOME
AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE


15 February 2001
nature
www.nature.com
the human genome
Nuclear fission
Five-dimensional energy landscapes
Seafloor spreading
The view from under the Arctic ice
Career prospects
Sequence creates new opportunities
naturejobs
genomics special

基因打靶技术

基因打靶技术是在胚胎干细胞和同源重组技术基础之上发展起来的、一种定向改变生物个体遗传信息的体内诱变方法。

小鼠基因打靶技术

(1987-至今)

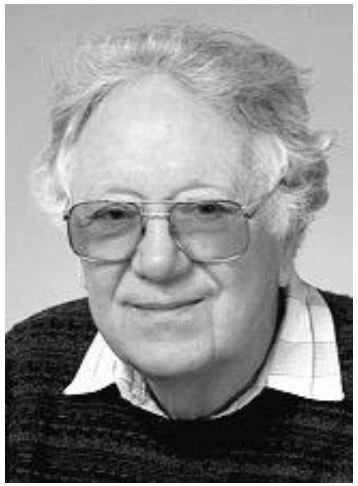
— 开创遗传学新纪元

— 研究基因功能的“金标准”

2007

Mario Capecchi

Martin Evans



Oliver Smithies

Capecchi, Evans and Smithies have revolutionized the study of human health and diseases.

-Lasker Fundation Press Release 2001

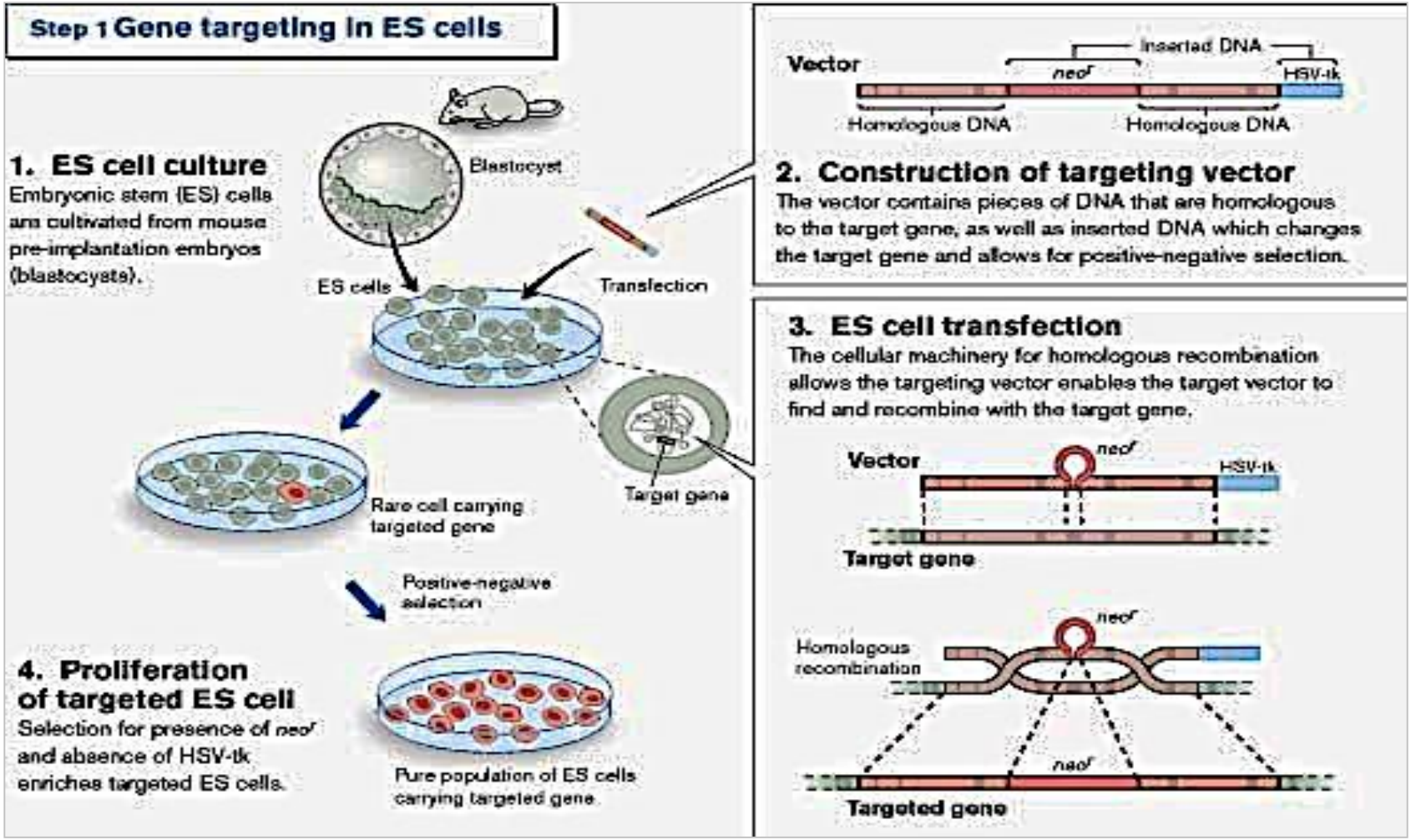
It is now being applied to virtually all areas of biomedicine – from basic research to the development of new therapies.

-Nobel Assembly Press Release 2007

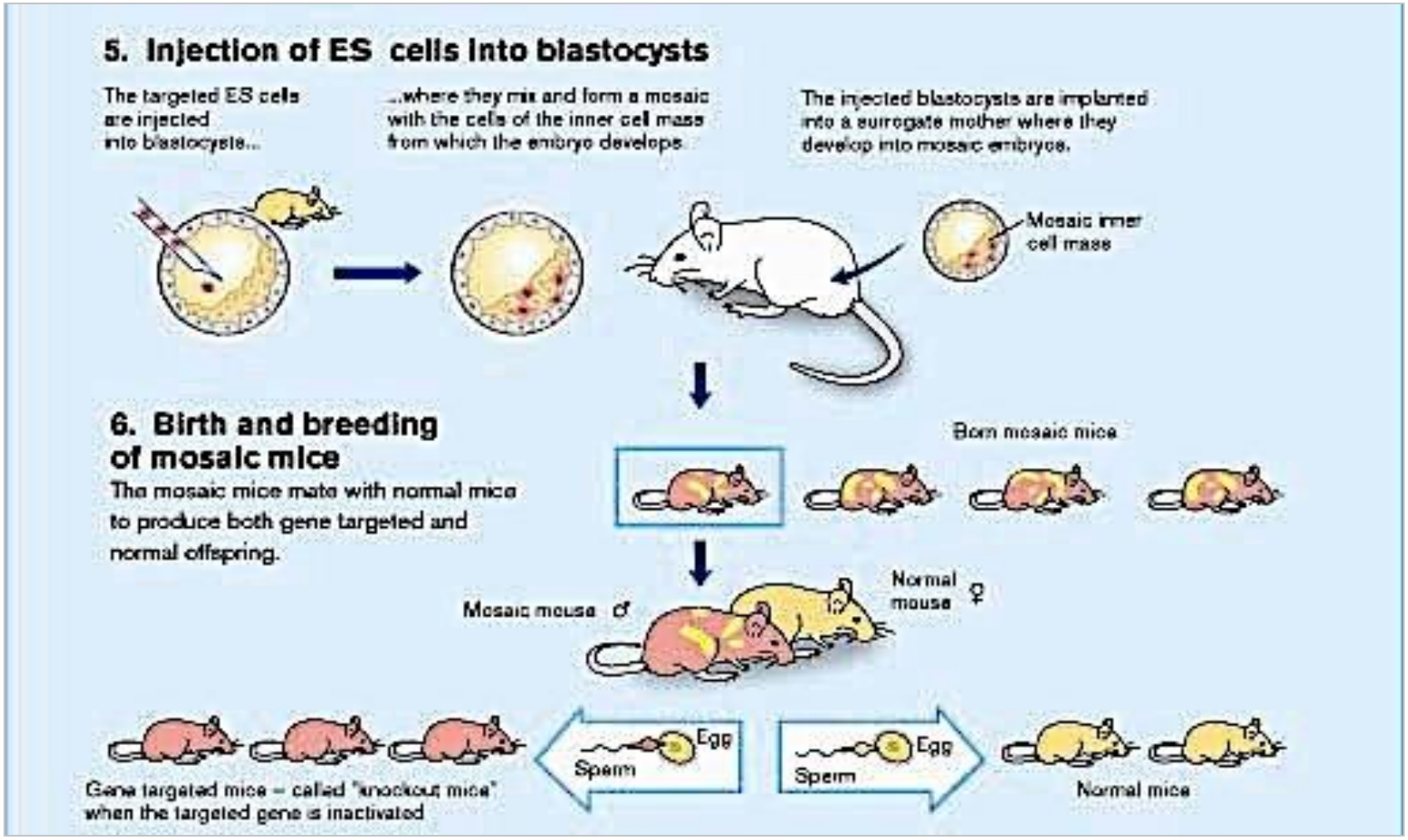
It is not an exaggeration to say that there is no mammalian biologist today who does not use these tools in one way or another.

—Science 2007

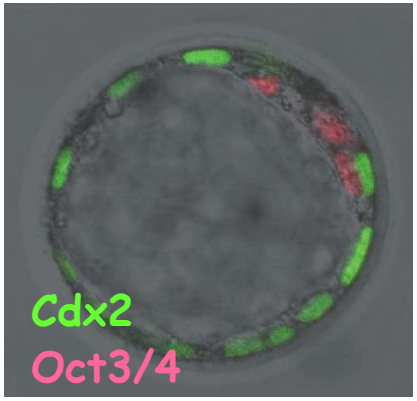
基因打靶的策略—获得中靶ES细胞



基因打靶的策略—获得种系突变体

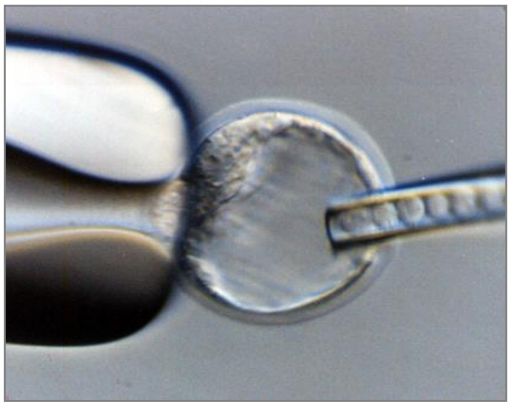


胚胎干细胞技术



—无限增殖

—分化全能性



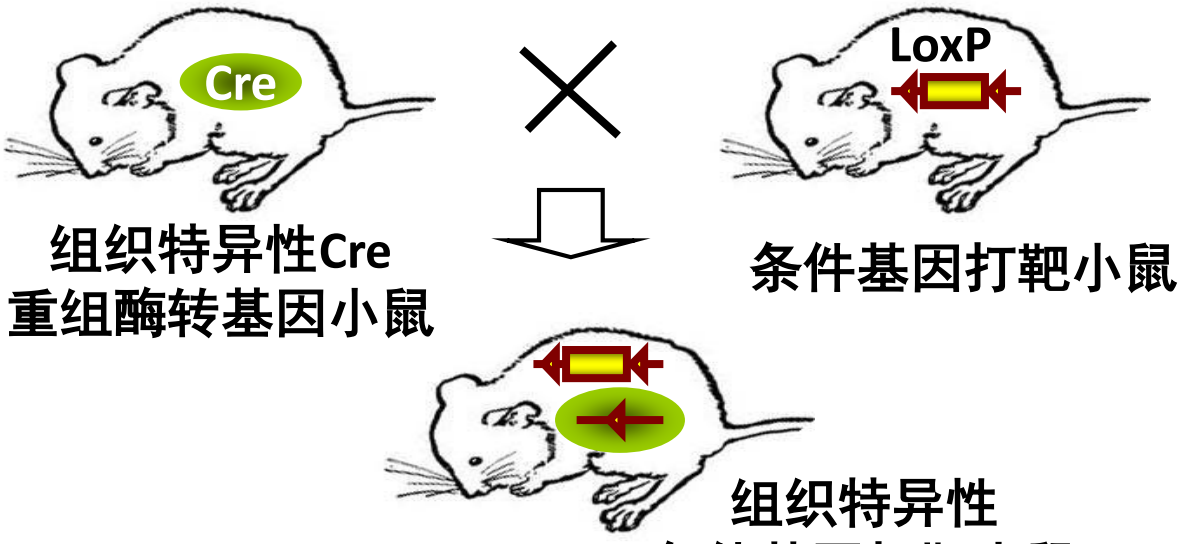
Evans MJ and Kaufman MH. (1981). Nature 292, 154–156.

条件基因打靶技术

(1994-至今)

—时间和空间可控的基因打靶

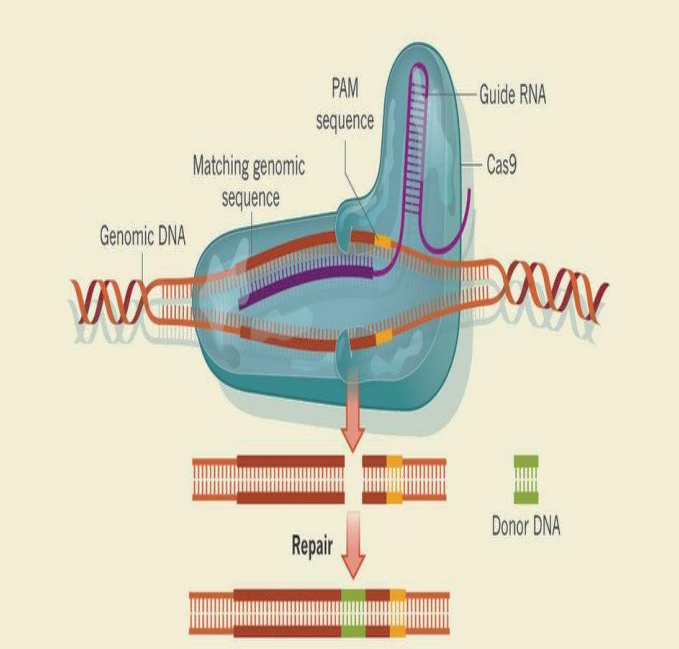
—研究组织稳态维持机制的最佳技术手段



Gu H et al. Science 1994

基于人工核酸内切酶的CRISPR技术

（2013-至今）



- 成本低廉、高效、操作简便
- 无基因序列、细胞类型和物种的限制
- 无需胚胎干细胞
- 同时进行多基因修饰
- 基因治疗

Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA and Charpentier E. (2012). Science 337, 816–821.

CRISPR-Cas9发现者荣获2015年生命科学突破奖

Jennifer Doudna　　Emmanuelle Charpentier

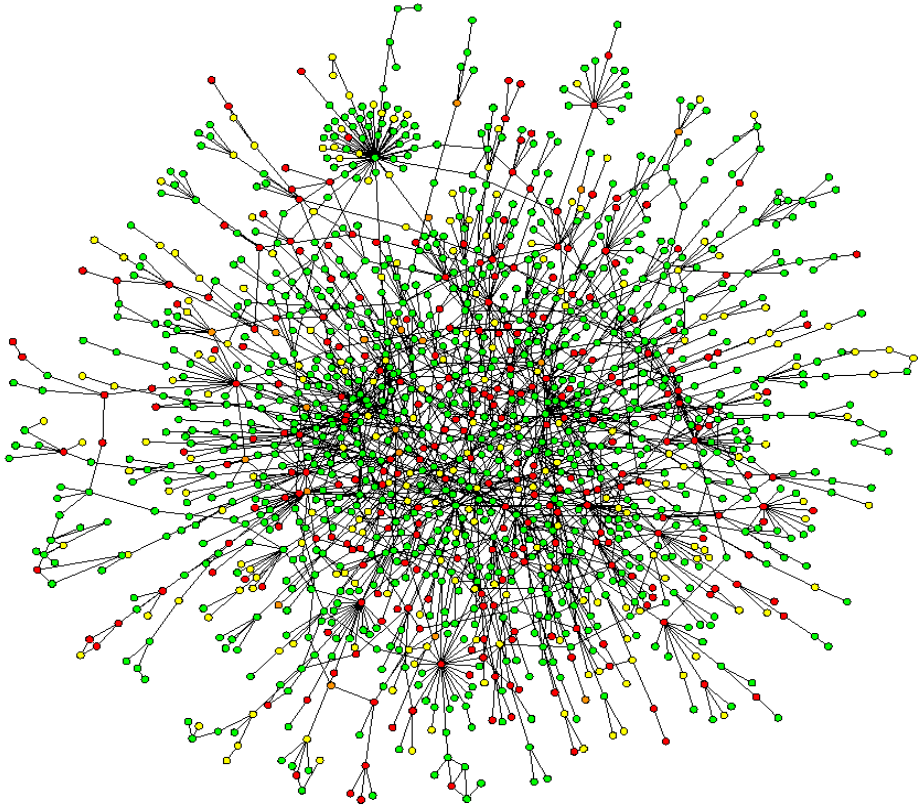
基因打靶技术的发展趋势

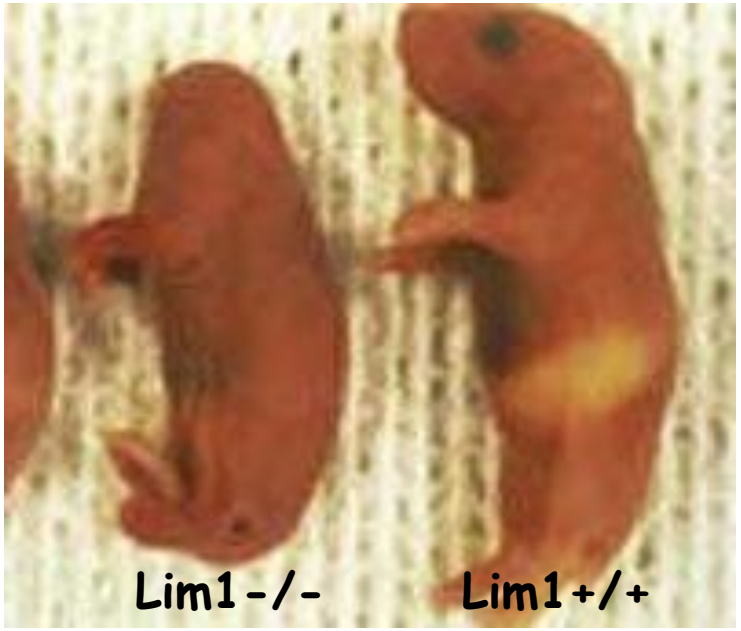
完全基因敲除	➡	条件基因敲除-空间和时间可控
单基因基因敲除	➡	全基因组基因敲除
简单基因敲除	➡	精细突变
编码基因敲除	➡	非编码基因敲除
小鼠	➡	其他模式生物

基因打靶技术在生物医学研究中的应用

- 解析基因的生理功能
- 研制人类疾病动物模型

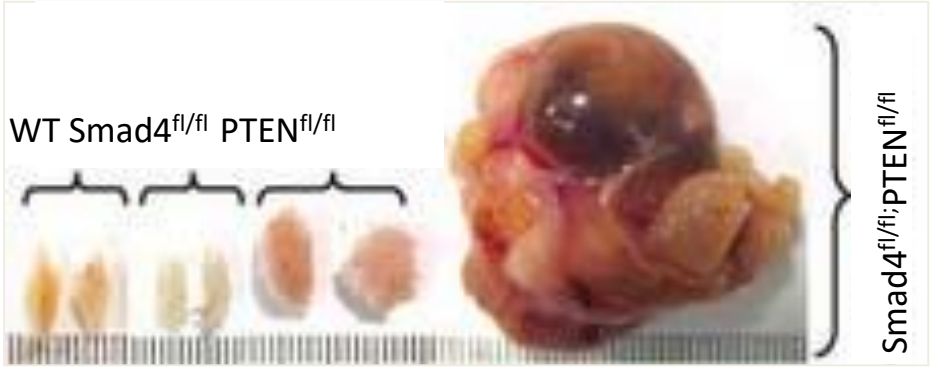
提供基因生理功能的实验证据

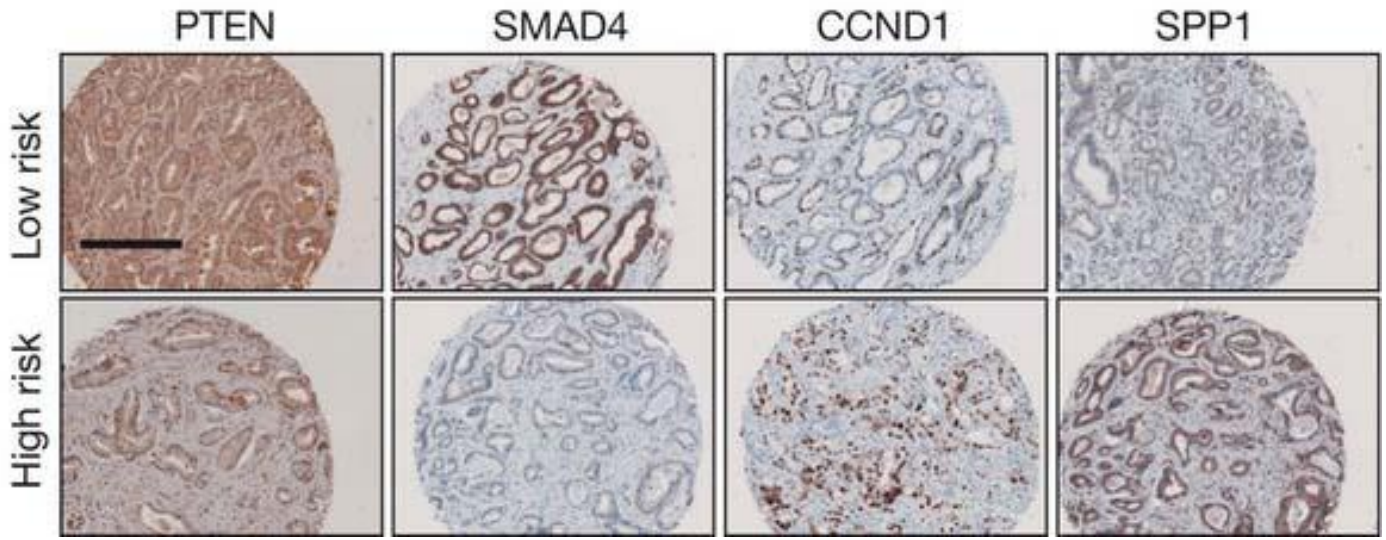




Shawlot W & Behringer RR:
Nature, 1995, 374:425

建立基因突变和疾病间的因果关系





Ding Z et al: Nature, 2011

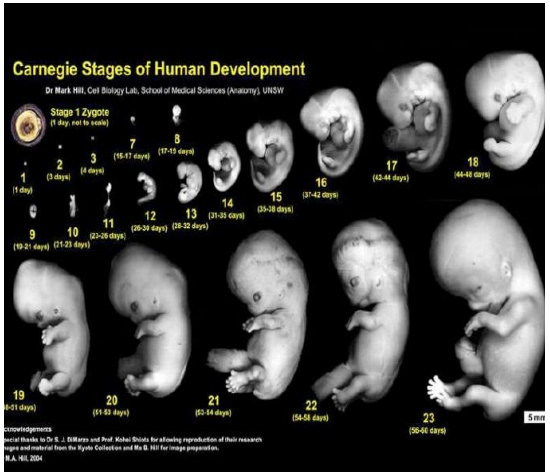
基因打靶技术对生命科学和医学研究的深远影响

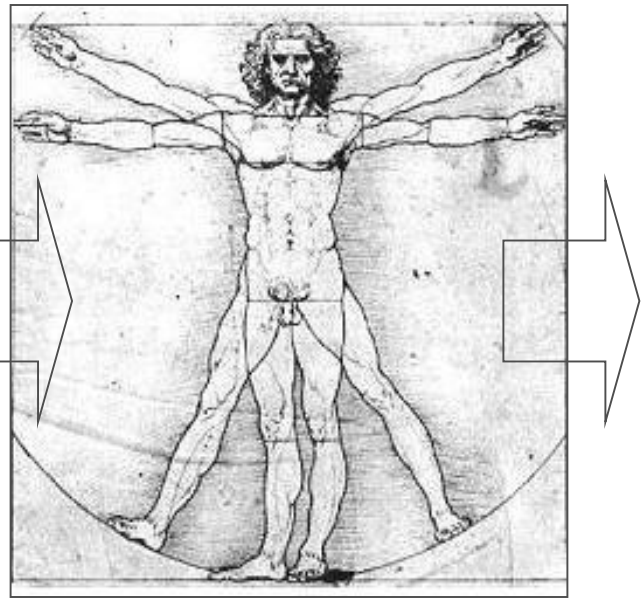
- 研究基因的生理功能（对关于特定基因生理功能的假设进行实验验证）。
- 研制人类疾病动物模型（真正建立基因突变和疾病间的因果关系）。
- 基因打靶技术的应用使得许多古老的学科如发育生物学和胚胎学焕发出新的异彩，并直接催生了许多新的学科如遗传生理学。

提　纲

- 基因打靶技术的发展历史及其应用
- 利用基因打靶技术研究组织稳态和疾病发生机制
- 关于科学与人生的几点感悟

组织稳态失衡导致疾病发生





肿瘤
心血管疾病
糖尿病
关节炎
骨质疏松
肥胖症
……

发 育 ⇒ 稳 态 ? ⇔ 疾 病

组织稳态维持机制

中枢稳态调控

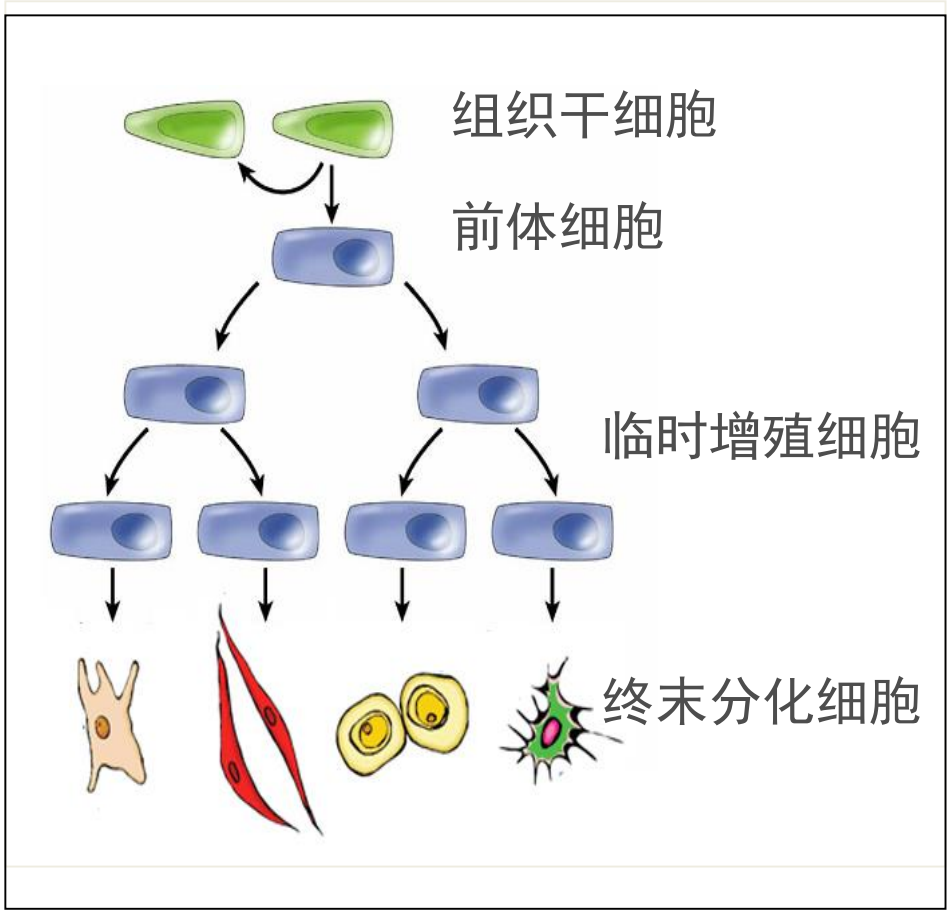
神经系统

内分泌系统

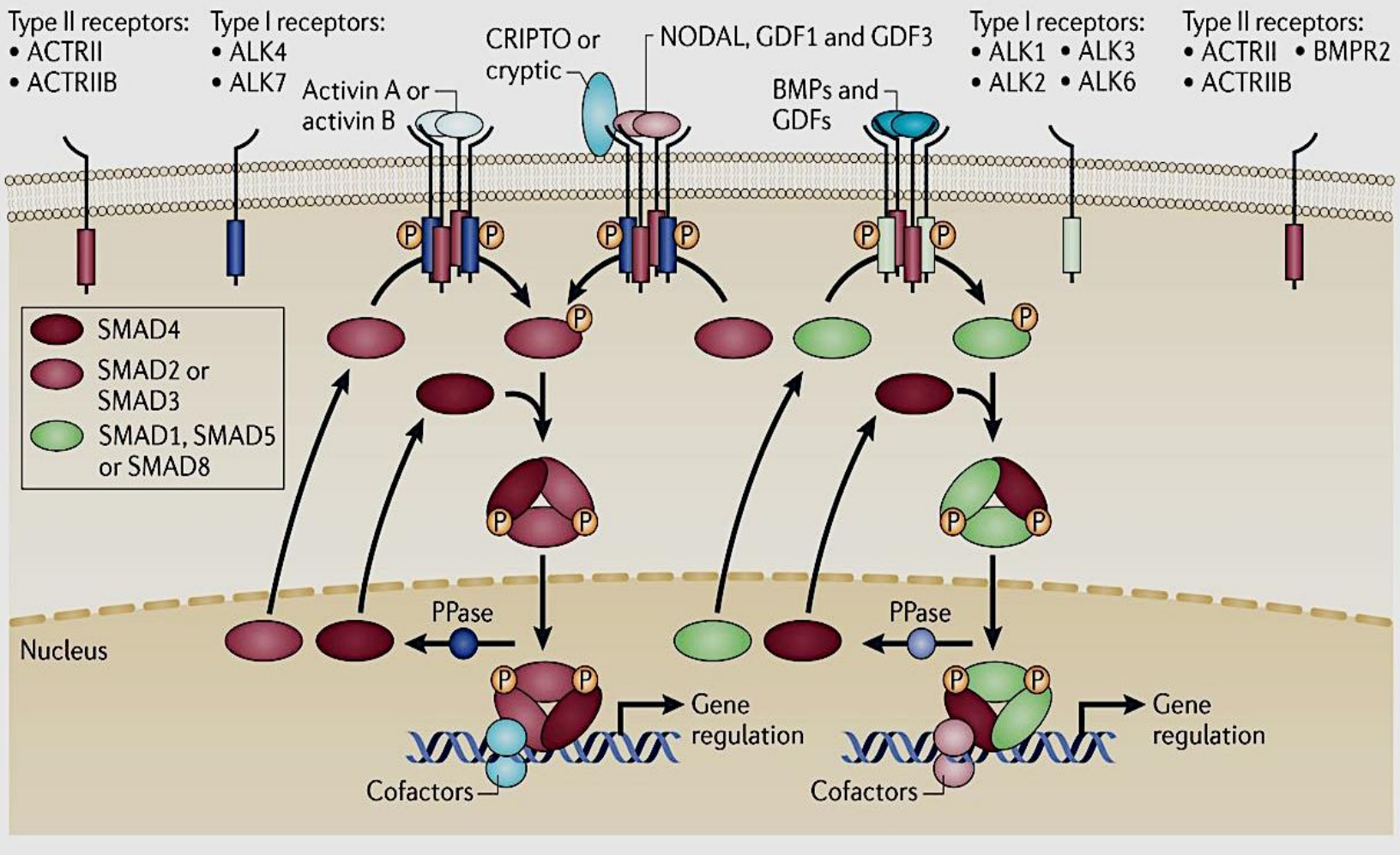
组织水平稳态调控

自分泌信号

旁分泌信号



TGF-β/Smad信号通路



Wakefield L & Hill C: Nat Rev Cancer, 2013

TGF-β通路异常与疾病

突变基因	疾病类型	文献
受体		
TGFβRI	结肠癌	Science 2008
TGFβRI/II	动脉瘤	NEJM 2006
TGFβRII	结肠癌	Science 1995
ALK1	肺动脉高压/血管扩张	NEJM 2001
胞内信号转导分子		
Smad4	青少年息肉症/血管扩张	Lancet 2004
Smad4	青少年息肉症	Science 1998
Smad4	胰腺癌	Science 1996
Smad3	白血病	NEJM 2004
Smad2	结肠癌	Cell 1996

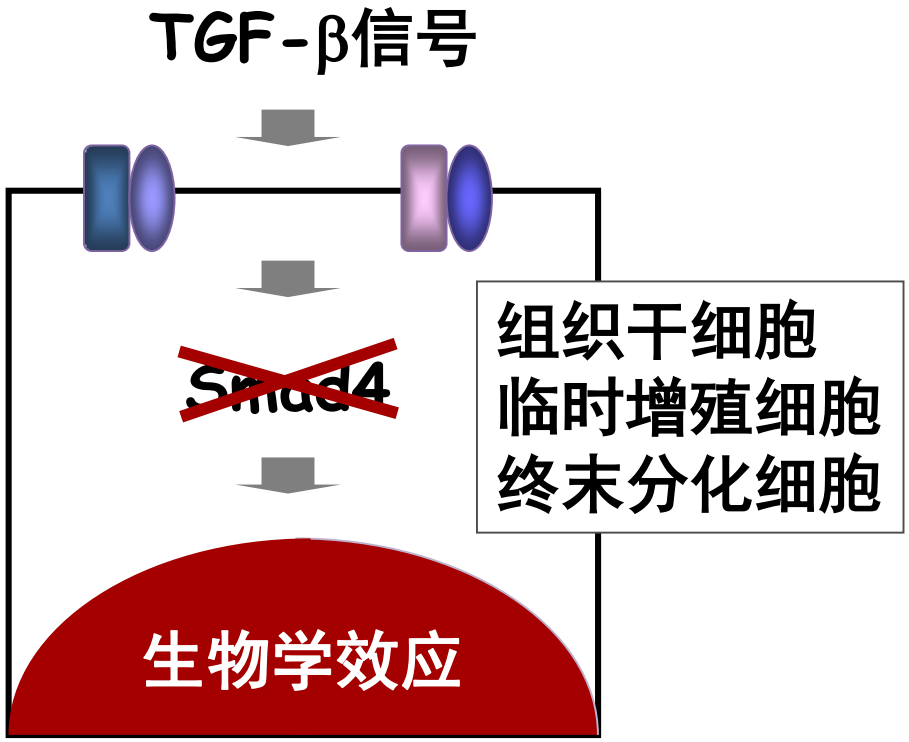
科学问题

- 不同类型组织细胞自主性TGF-β信号通路在组织稳态维持中的生理功能？
- TGF-β信号通路异常导致疾病发生的分子机制？

TGF-β/Smad通路在发育中的功能

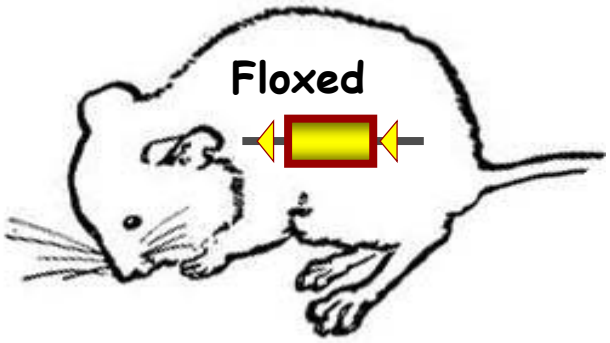
突变基因	表型	发表论文	引用次数 google scholar
Smad2	胚胎致死	PNAS 1998	266
Smad3	免疫缺陷	EMBO J 1999	870
Smad3	骨关节炎	J Cell Biol 2001	533
Smad3	创伤愈合加快	Nat Cell Biol 1999	784
Smad4	胚胎致死	PNAS 1998	367
Smad5	胚胎致死	Development 1999	413

TGF-β/Smad通路维持组织稳态?



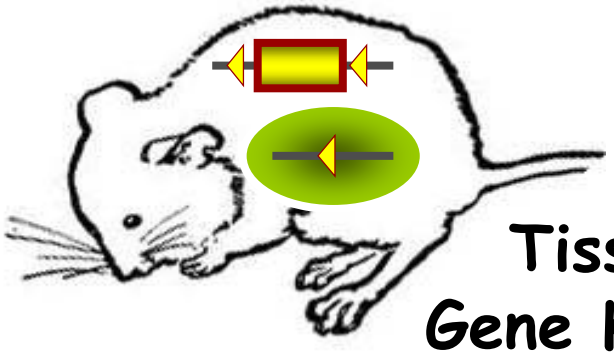
阻断细胞对TGF-β反应性 → 组织稳态失衡 → 疾病发生 → 病理和分子机制

基于Cre-LoxP系统的条件基因敲除策略



Conditional Gene
Targeting Mouse

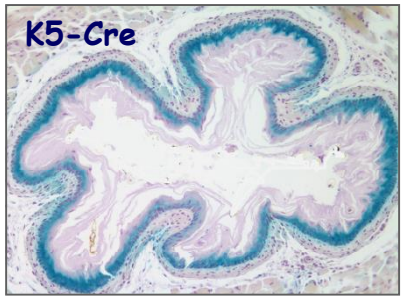
Tissue Specific Cre
Transgenic Mouse



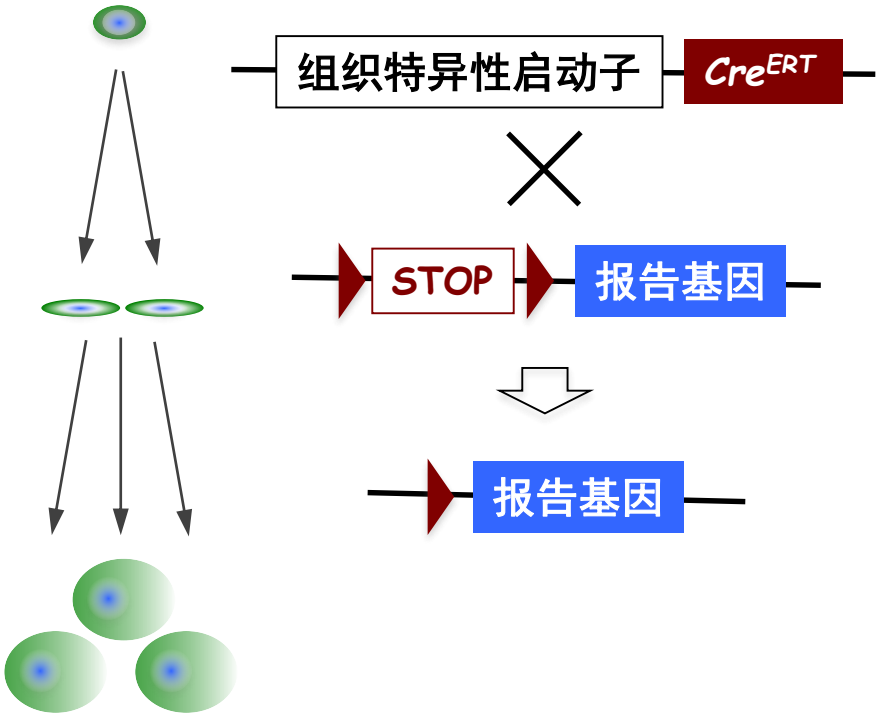
Tissue Specific
Gene Knockout Mouse

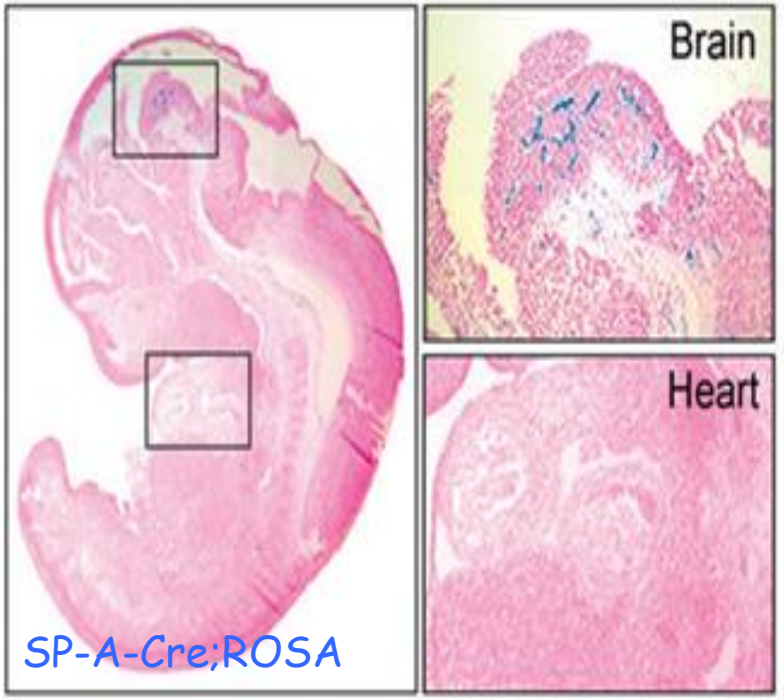
组织特异性Cre重组酶转基因小鼠

- OC-Cre（成骨细胞，JCS，2007）
- Col1a1-Cre（成骨细胞，JGG，2008）
- Col2a1-Cre（软骨细胞，Dev Biol，2005）
- Col10a1-Cre（肥大软骨细胞，Genesis，2005）
- Alb-Cre（肝细胞，CJH，2004）
- Keratin 5-Cre（角质细胞，Cancer Res，2005）
- Insulin-Cre（胰岛细胞，CJB，2002）
- Capn8-Cre（胃顶细胞，Genesis，2009）
- Atp4b-Cre（胃壁细胞，JGG，2010）
- Pgc-Cre（胃主细胞）
- PC1-Cre（前列腺细胞）
- MHC-Cre （心肌细胞，Circ Res，2005）
- SMA-Cre（平滑肌细胞，Dev Biol，2007）
- Tie2-Cre（血管内皮细胞，MCB，2007）
- SPA-Cre（脑血管内皮细胞，Matrix Biol，2007）



组织特异性Cre重组酶转基因小鼠用于细胞谱系分析

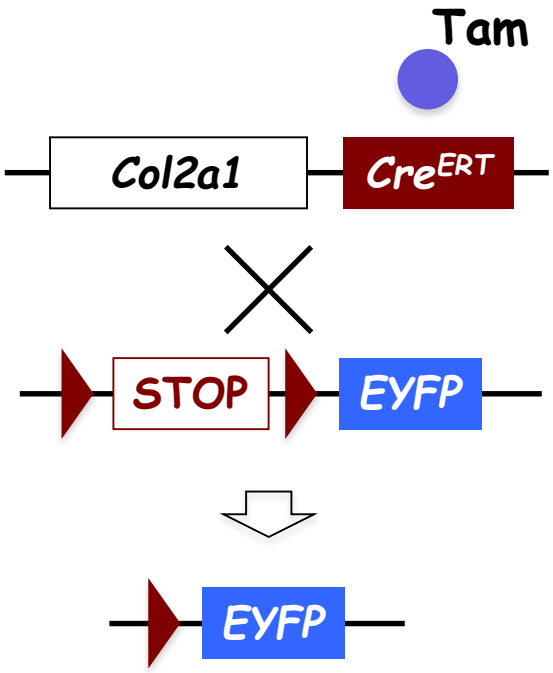


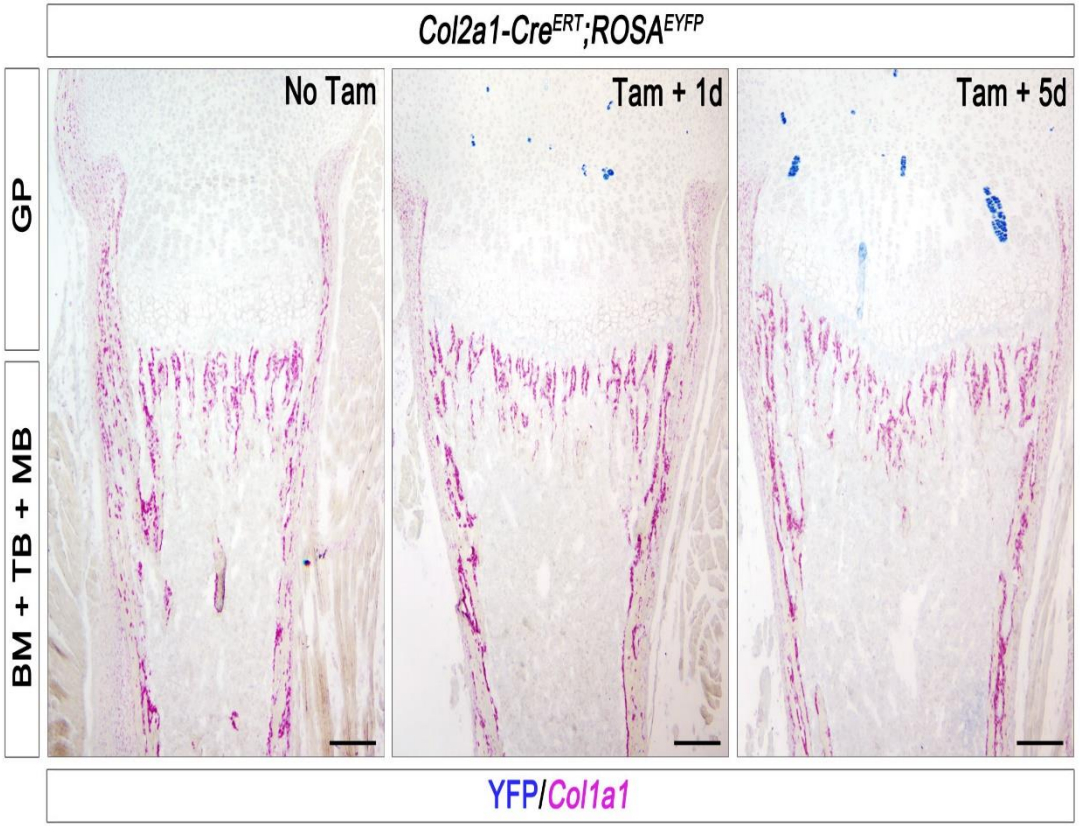


Meng et al. Matrix Biol, 2008

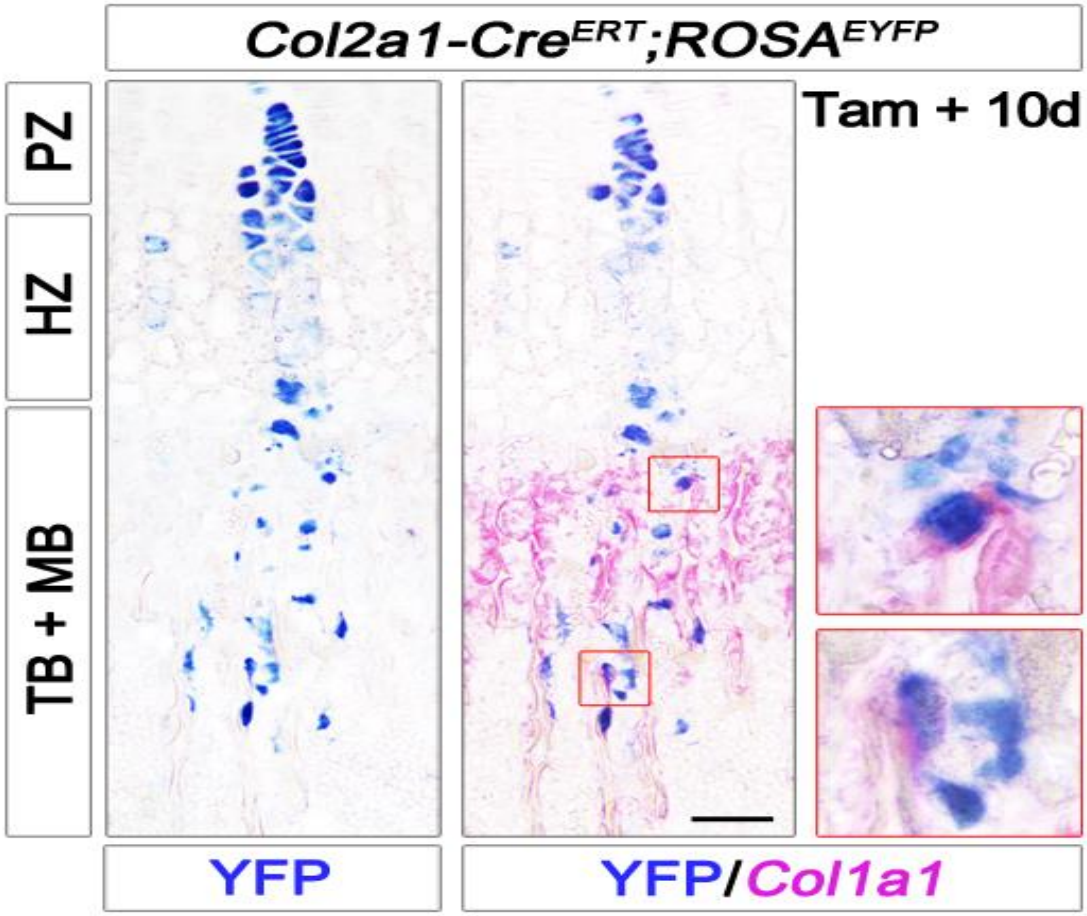
Li et al. Dev Cell, 2011

单个软骨细胞命运示踪

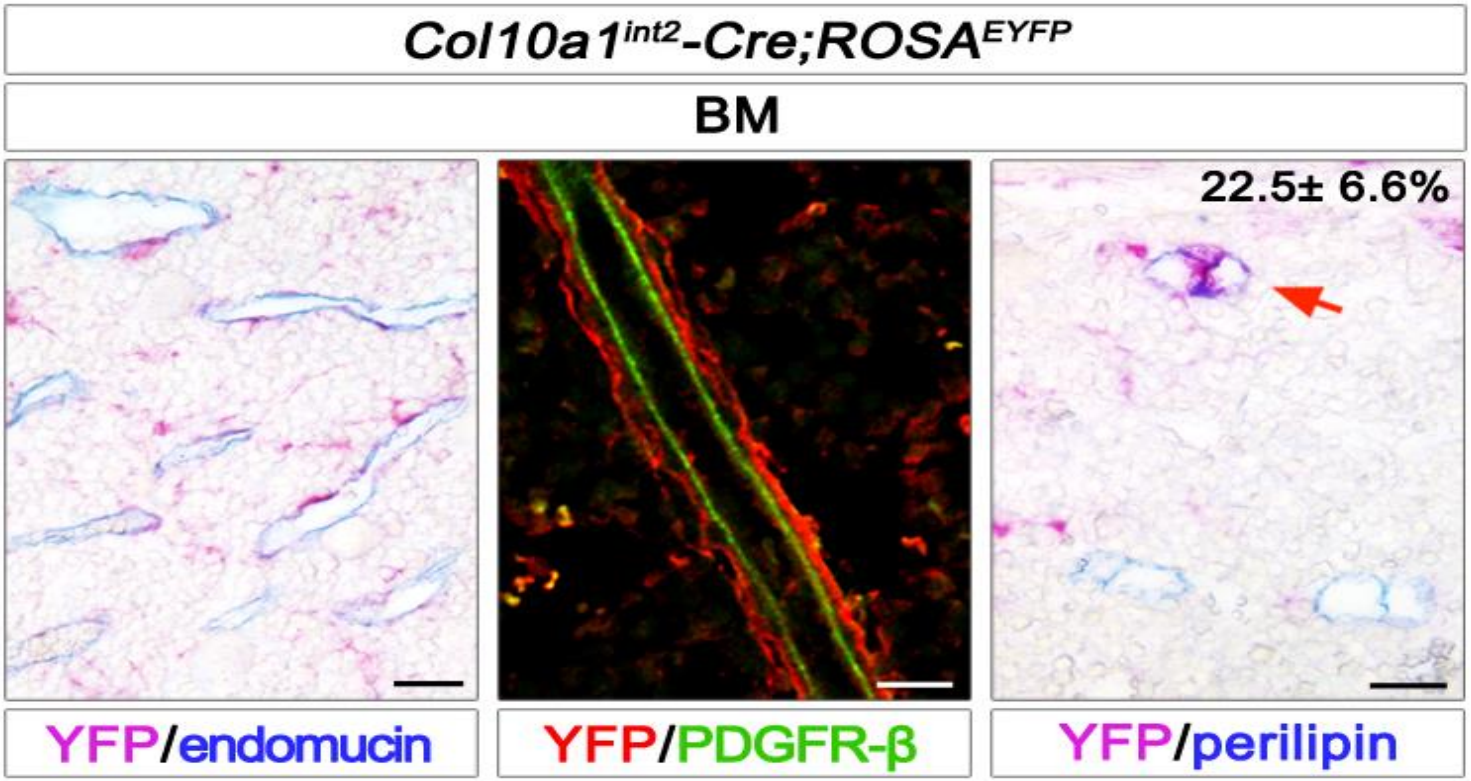

Tam
Col2a1
CreERT
STOP
EYFP
EYFP


Col2a1-CreERT;ROSAEYFP
GP
BM + TB + MB
No Tam
Tam + 1d
Tam + 5d
YFP/Col1a1

克隆分析显示肥大型软骨细胞变为成骨细胞



肥大型软骨细胞转分化为骨髓中血管周细胞和脂肪细胞



Yang G et al: Cell Res, 2014

肥大型软骨细胞的命运

——争议了一个世纪的科学问题

- 终末分化的肥大型软骨细胞能存活并在软骨内成骨过程中变成成骨细胞和骨细胞。
- 肥大型软骨细胞具有多分化潜能。

肥大型软骨细胞转分化的分子机制?

软骨细胞来源的骨髓间质细胞的生理功能?

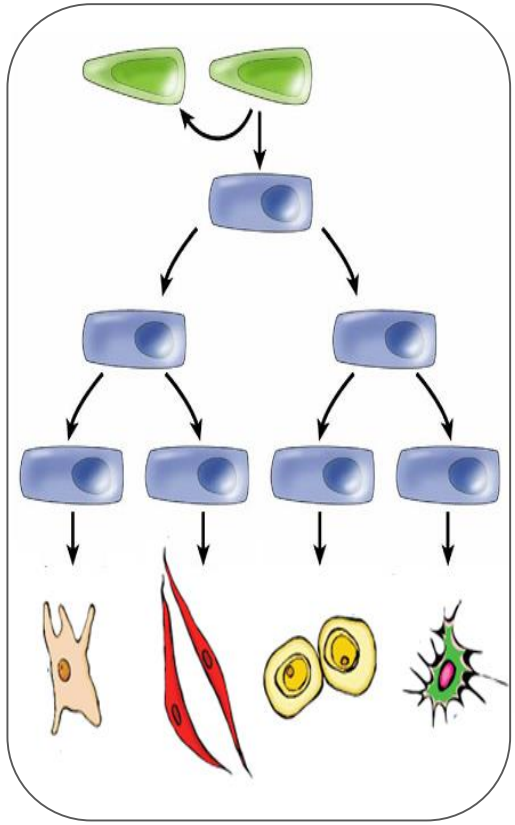
TGF-β信号通路失调导致组织稳态失衡与疾病发生

调节干细胞动员和EHT
Mol Biol Cell, 2009; Blood, 2014

抑制细胞增殖
Development, 2008
Cancer Res, 2005; 2006
Dev Biol, 2005

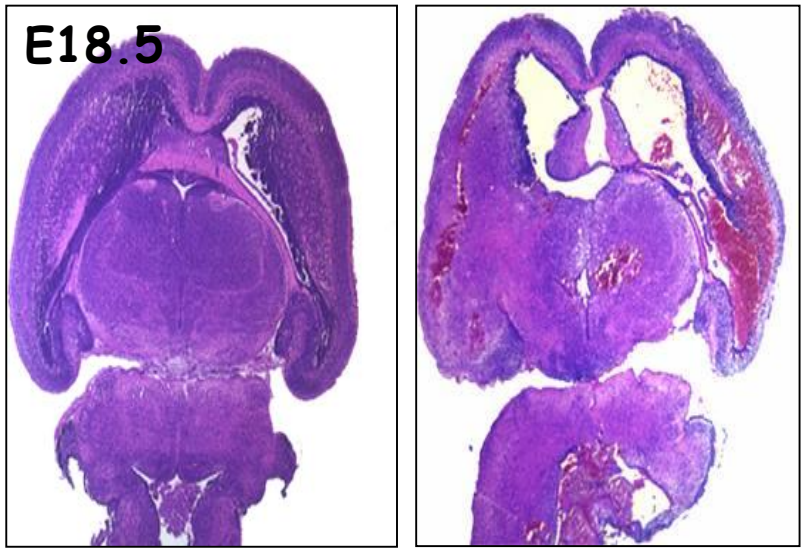
维持终末分化细胞稳态
Mol Cell Biol, 2009
J Cell Sci, 2006
Circ Res, 2005

稳定细胞间相互作用
Dev Cell, 2011
Mol Cell Biol, 2007

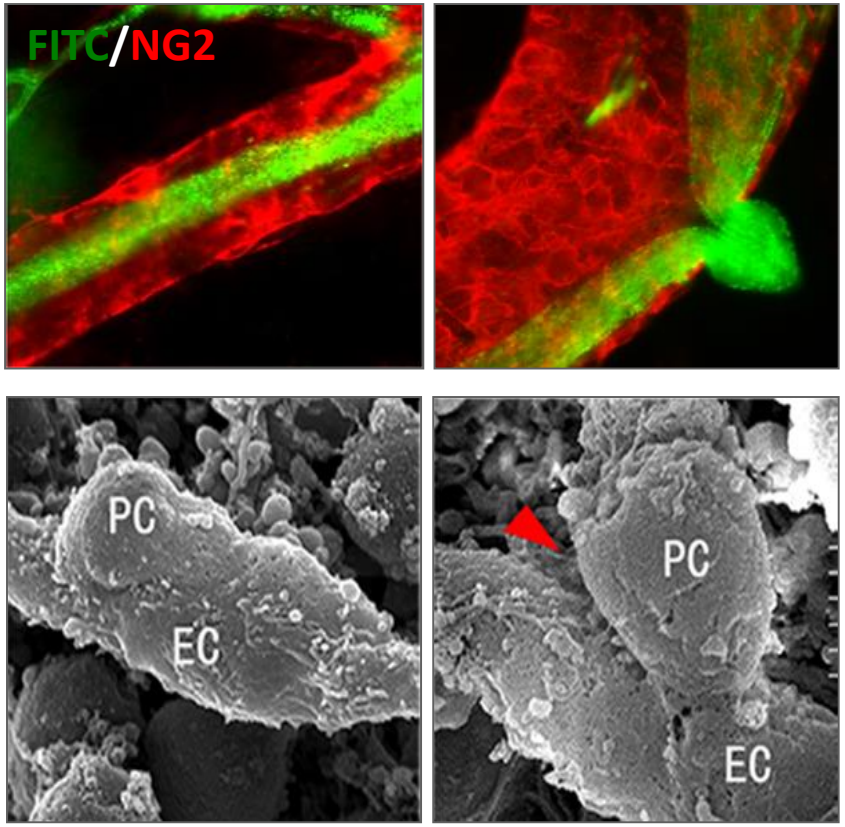


皮肤癌
食管癌
软骨瘤
心肌肥厚
骨质疏松
血管扩张
颅内出血

脑血管内皮Smad4基因敲除导致脑内皮–周细胞相互作用缺陷和颅内出血

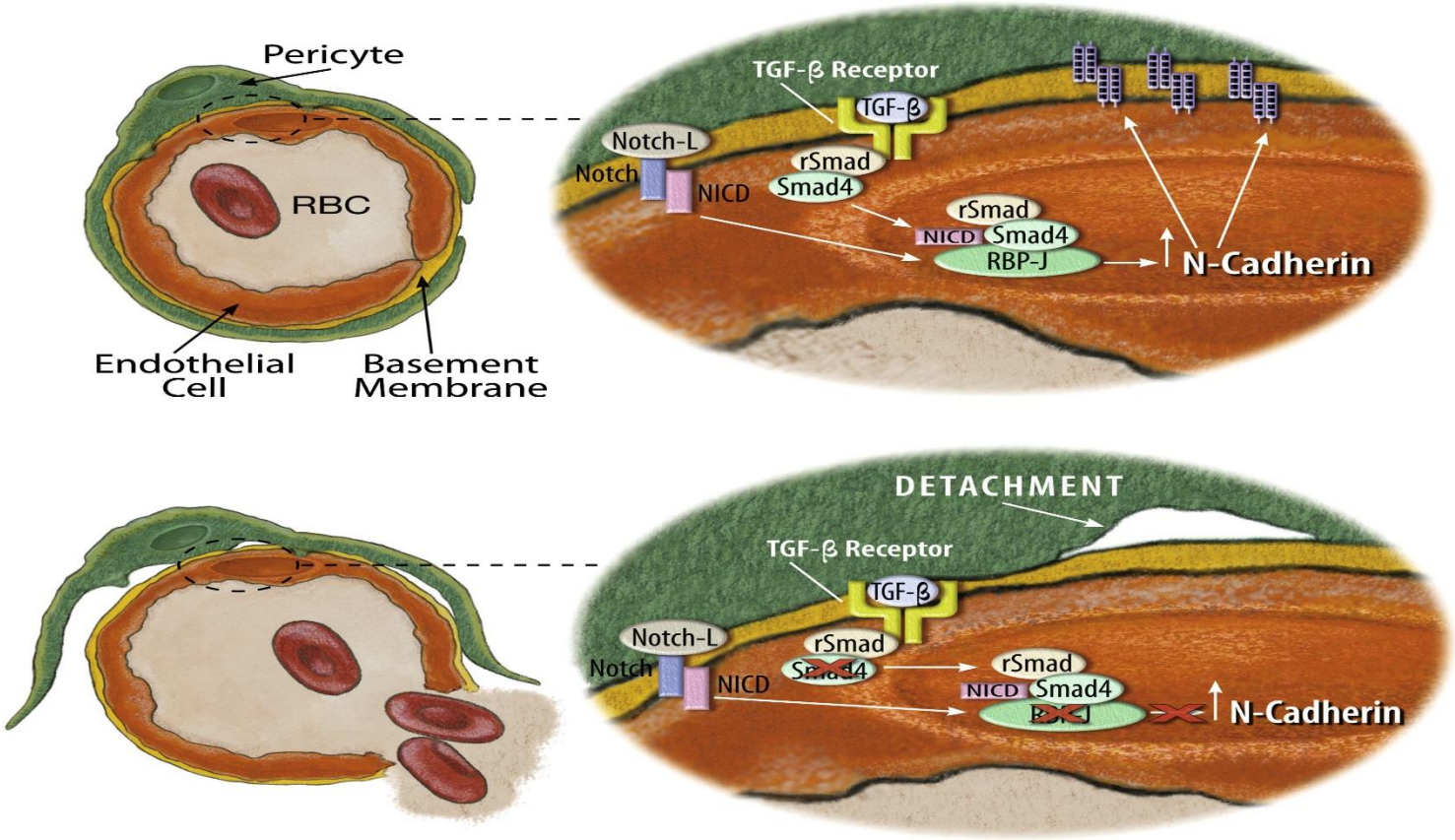


Control　　Smad4$^{fl/fl}$;SP-A-Cre

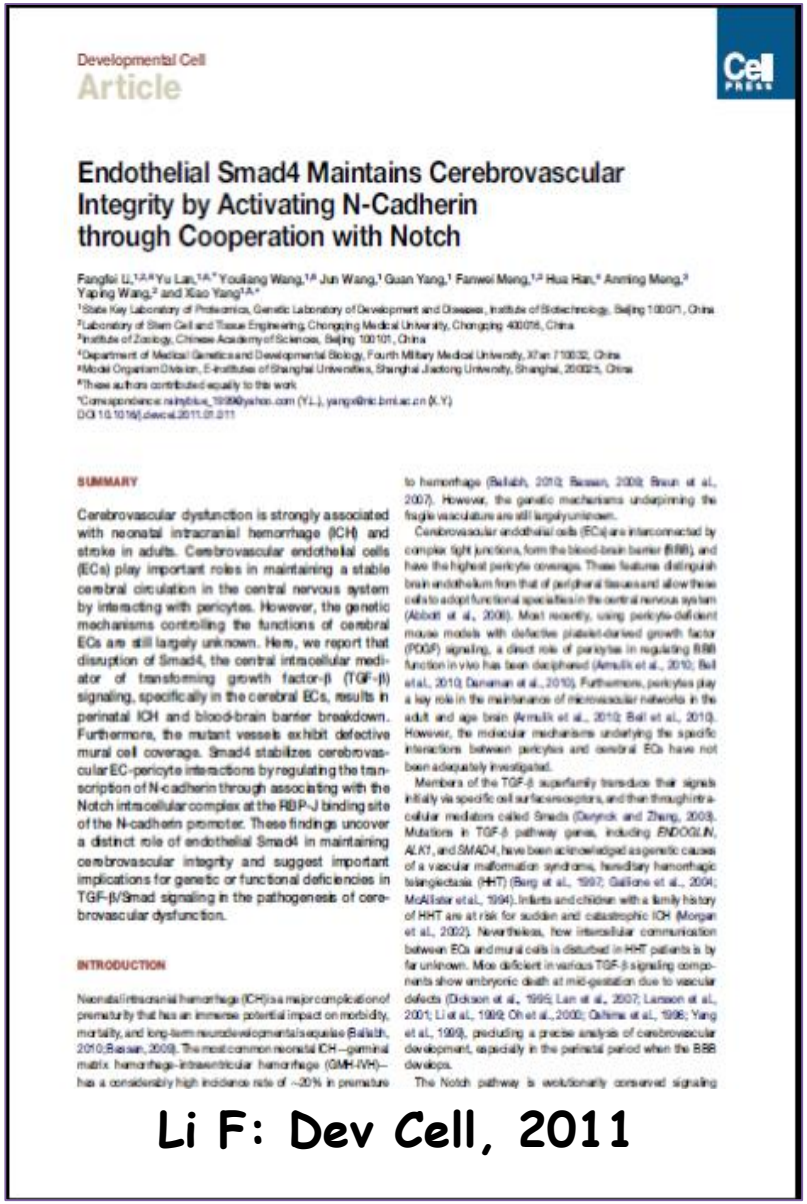


Control　　Smad4$^{fl/fl}$;SP-A-Cre

Smad4与Notch协同上调N-Cadherin维持脑血管稳态



Li F et al: Dev Cell, 2011



Developmental Cell

Article

Cell PRESS

Endothelial Smad4 Maintains Cerebrovascular Integrity by Activating N-Cadherin through Cooperation with Notch

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SUMMARY

Cerebrovascular dysfunction is strongly associated with neonatal intracranial hemorrhage (ICH) and stroke in adults. Cerebrovascular endothelial cells (ECs) play important roles in maintaining a stable cerebral circulation in the central nervous system by interacting with pericytes. However, the genetic mechanisms controlling the functions of cerebral ECs are still largely unknown. Here, we report that disruption of Smad4, the central intracellular mediator of transforming growth factor-β (TGF-β) signaling, specifically in the cerebral ECs, results in perinatal ICH and blood-brain barrier breakdown. Furthermore, the mutant vessels exhibit defective mural cell coverage. Smad4 stabilizes cerebrovascular EC-pericyte interactions by regulating the transcription of N-cadherin through associating with the Notch intracellular complex at the RBP-J binding site of the N-cadherin promoter. These findings uncover a distinct role of endothelial Smad4 in maintaining cerebrovascular integrity and suggest important implications for genetic or functional deficiencies in TGF-β/Smad signaling in the pathogenesis of cerebrovascular dysfunction.

INTRODUCTION

Neonatal intracranial hemorrhage (ICH) is a major complication of prematurity that has an immense potential impact on morbidity, mortality, and long-term neurodevelopmental sequelae (Ballabh, 2010; Bassan, 2009). The most common neonatal ICH—germinal matrix hemorrhage-intraventricular hemorrhage (GMH-IVH)—has a considerably high incidence rate of ~20% in premature to hemorrhage (Ballabh, 2010; Bassan, 2009; Braun et al., 2007). However, the genetic mechanisms underpinning the fragile vasculature are still largely unknown.

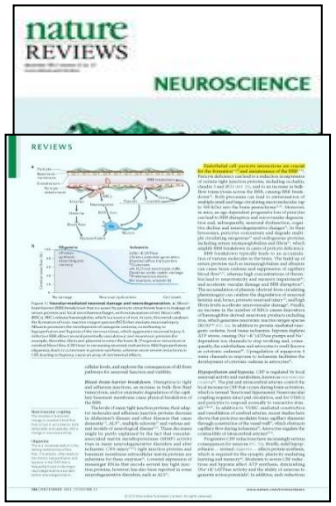
Cerebrovascular endothelial cells (ECs) are interconnected by complex tight junctions, form the blood-brain barrier (BBB), and have the highest pericyte coverage. These features distinguish brain endothelium from that of peripheral tissues and allow these cells to adopt functional specialties in the central nervous system (Abbott et al., 2006). Most recently, using pericyte-deficient mouse models with defective platelet-derived growth factor (PDGF) signaling, a direct role of pericytes in regulating BBB function in vivo has been deciphered (Armulik et al., 2010; Bell et al., 2010; Daneman et al., 2010). Furthermore, pericytes play a key role in the maintenance of microvascular networks in the adult and age brain (Armulik et al., 2010; Bell et al., 2010). However, the molecular mechanisms underlying the specific interactions between pericytes and cerebral ECs have not been adequately investigated.

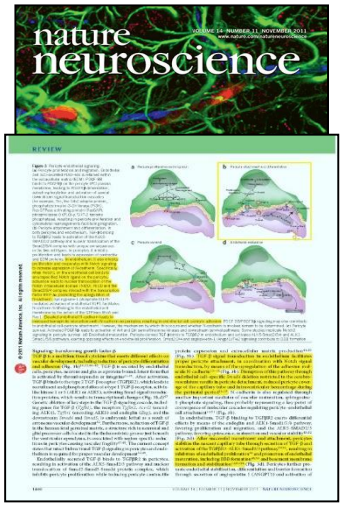
Members of the TGF-β superfamily transduce their signals initially via specific cell surface receptors, and then through intracellular mediators called Smads (Derynck and Zhang, 2003). Mutations in TGF-β pathway genes, including *ENDOGLIN*, *ALK1*, and *SMAD4*, have been acknowledged as genetic causes of a vascular malformation syndrome, hereditary hemorrhagic telangiectasia (HHT) (Berg et al., 1997; Gallione et al., 2004; McAllister et al., 1994). Infants and children with a family history of HHT are at risk for sudden and catastrophic ICH (Morgan et al., 2002). Nevertheless, how intercellular communication between ECs and mural cells is disturbed in HHT patients is by far unknown. Mice deficient in various TGF-β signaling components show embryonic death at mid-gestation due to vascular defects (Dickson et al., 1995; Lan et al., 2007; Larsson et al., 2001; Li et al., 1999; Oh et al., 2000; Oshima et al., 1996; Yang et al., 1999), precluding a precise analysis of cerebrovascular development, especially in the perinatal period when the BBB develops.

The Notch pathway is evolutionarily conserved signaling

Li F: Dev Cell, 2011

Nat Rev Neurosci等杂志引用136次；
Nat Neurosci综述单篇14次引用并配图详细阐述；
北美血管生物学会选为“Vascular Biology Publications Alert”论文；
被Faculty of 1000推荐。





TGF-β/Smad4维持组织稳态的表观遗传机制

发现第一个受TGF-β抑制的miRNA簇以及miR-24促进细胞分化的功能

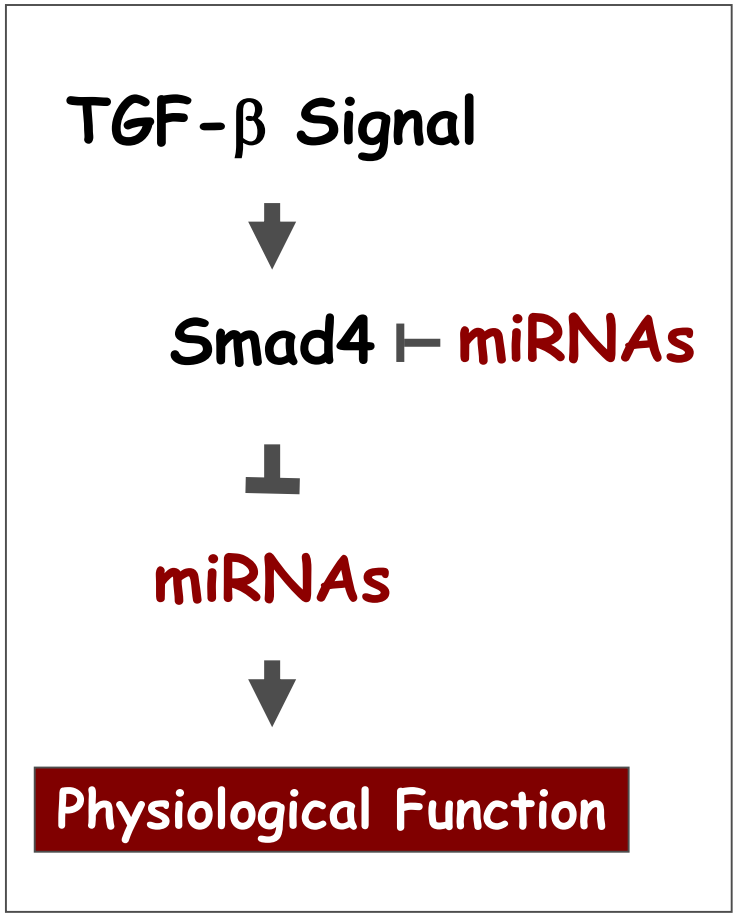
Nucleic Acids Res, 2008

系统筛选靶向Smad4的miRNA

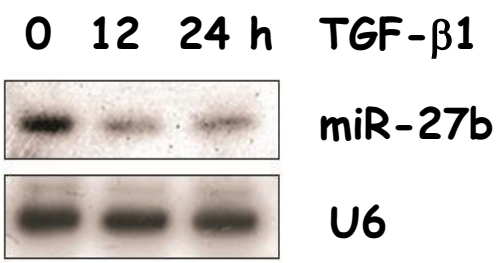
Nucleic Acids Res, 2012

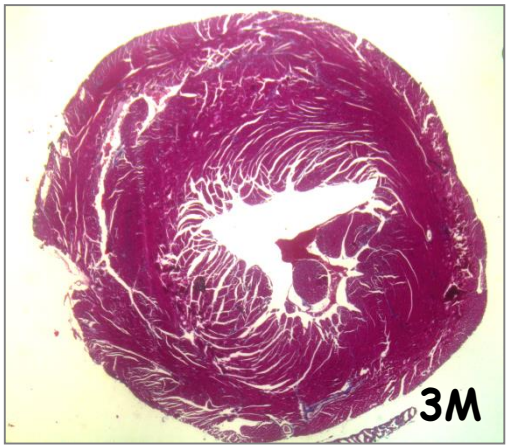
发现TGF-β调节的miNRA可作为心肌肥厚的治疗靶标

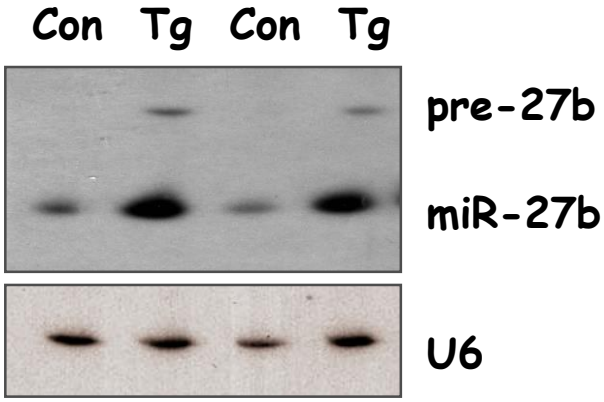
Cell Res, 2012; J Biol Chem, 2013



miR-27b过表达诱发心肌肥厚



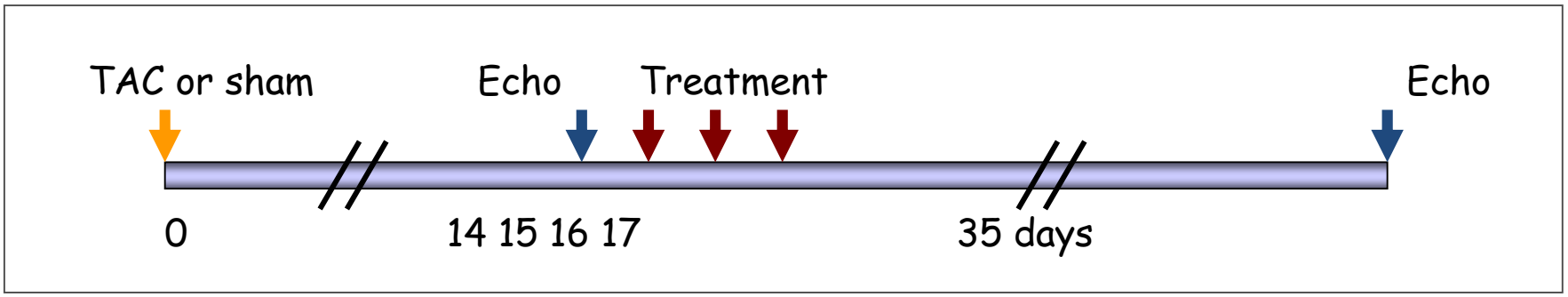


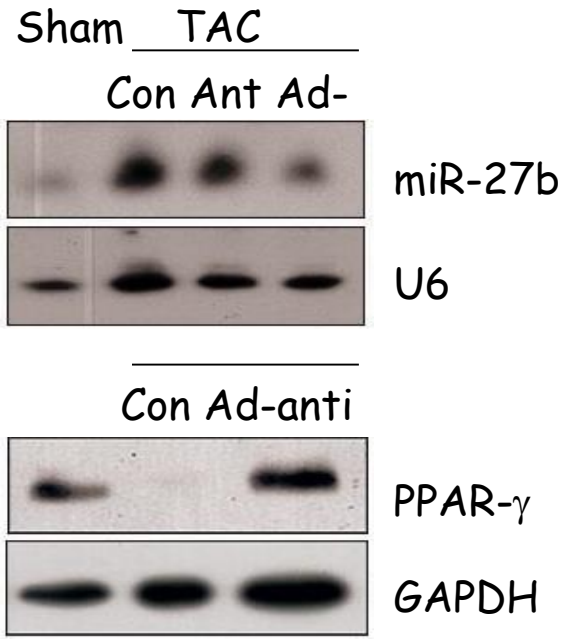


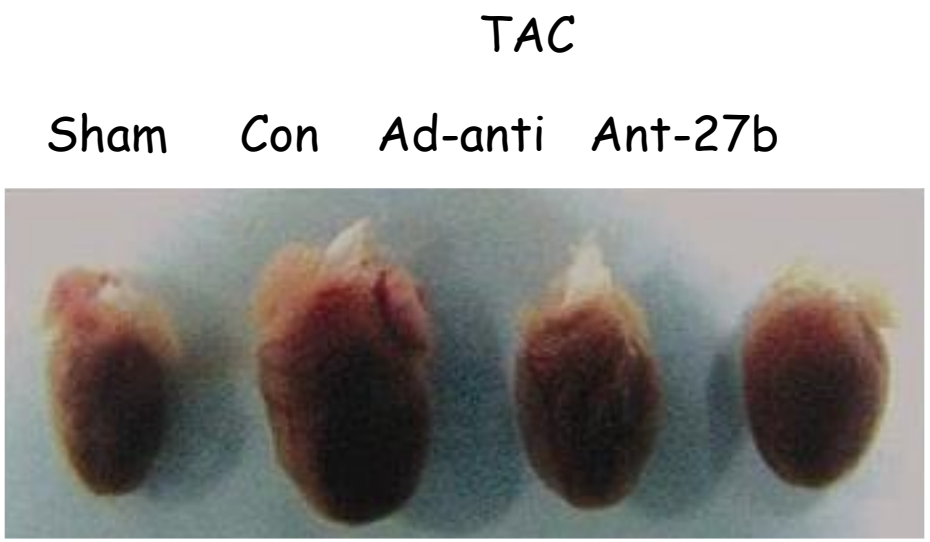
Control

miR-27b Tg

miR-27b敲减挽救TAC诱发的心肌肥厚

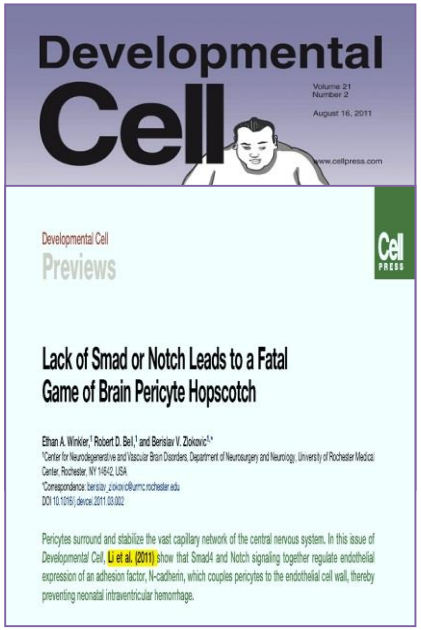


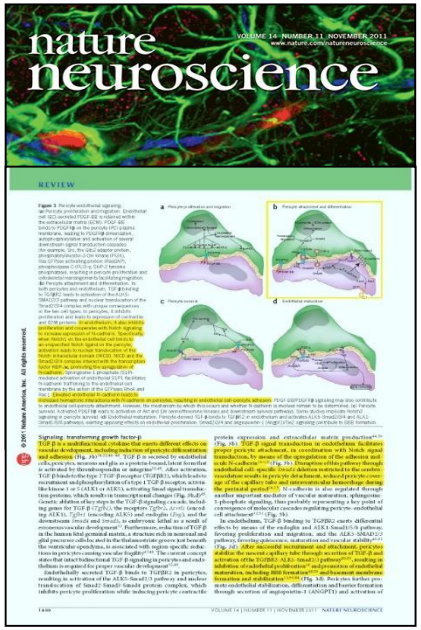




对重要生理过程和多种疾病发生发展的机制提出了新的观点

颅内出血新机制 Dev Cell 2011





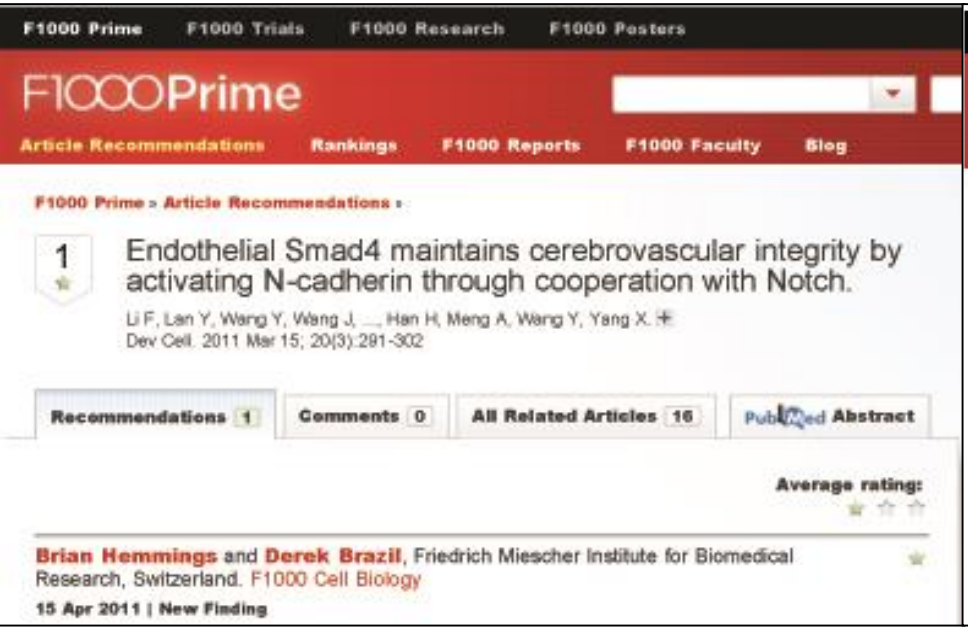
These exciting insights into the complex molecular signaling cascades governing endothelial-pericyte interactions have raised several important questions.

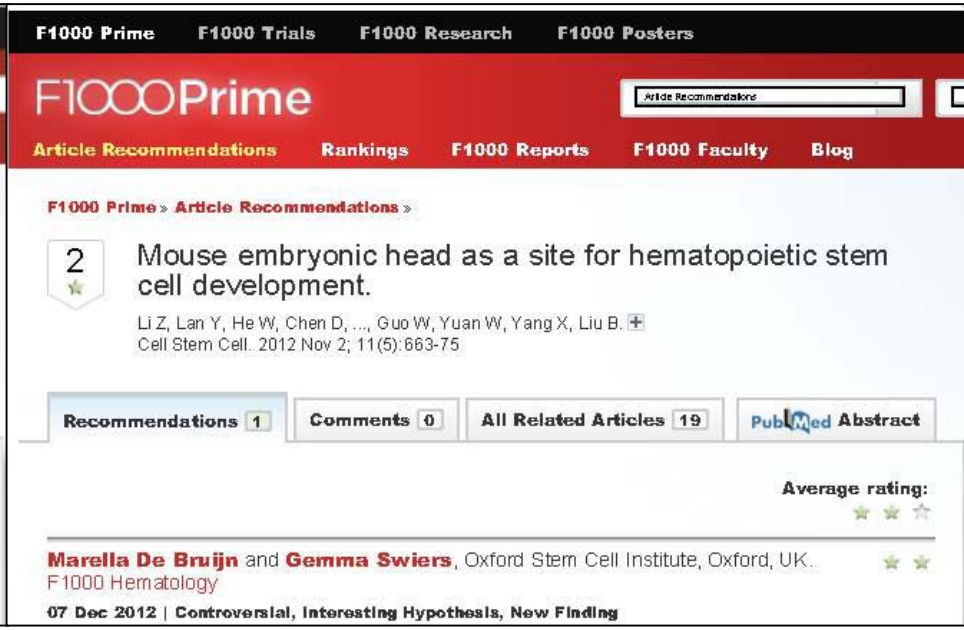
—Dev Cell, 2011

Pericyte, although long speculated to fill critical position in BBB, have only recently been demonstrated to help form and maintain the BBB in vivo…

—Nat Neurosci, 2011

被F1000推荐





Thus, an elegant molecular mechanism describing why deletion of Smad4 in brain ECs causes hemorrhage and lethality is presented.

These data also have important implications for tissues such as kidney and lung affected by fibrosis and scarring.

为人类疾病的致病基因鉴定和遗传学诊断提供了理论基础

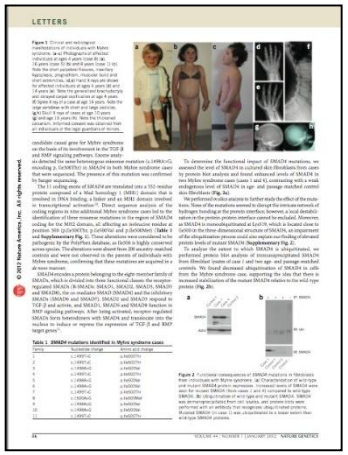
VOLUME 44 | NUMBER 1 | JANUARY 2012

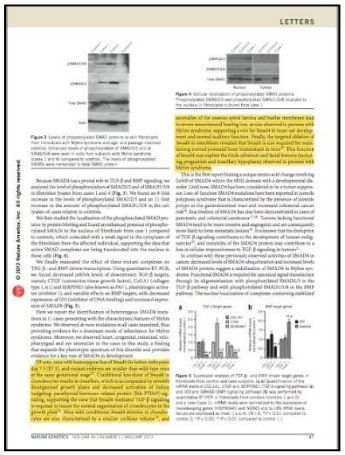
nature genetics

Mutations at a single codon in Mad homology 2 domain of SMAD4 cause Myhre syndrome

Carine Le Goff[1], Clémentine Mahaut[1], Avinash Abhyankar[2], Wilfried Le Goff[3], Valérie Serre[1], Alexandra Afenjar[4], Anne Destrée[5], Maja di Rocco[6], Delphine Héron[7], Sébastien Jacquemont[8], Sandrine Marlin[9], Marleen Simon[10], John Tolmie[11], Alain Verloes[12], Jean-Laurent Casanova[2,13], Arnold Munnich[1] & Valérie Cormier-Daire[1]

SMAD4单一密码子突变导致Myhre综合征





为疾病的诊断、预后和治疗提供新的靶标和实验模型

遗传修饰	疾病模型	原创性
PTEN	胃癌	国际首创 2013 Nat Commun
HBx/Kras	肝癌	国际首创 2013 Oncogene
miR-27b	心肌肥厚	国际首创 2012 Cell Res
Smad4	颅内出血	国际首创 2011 Dev Cell
Nlp	多发肿瘤	国际首创 2010 J Clin Invest
Gab1	骨质疏松	国际首创 2010 J Cell Sci
Smad4	角化囊性瘤	国际首创 2009 Mol Cell Biol
UCP2	肝损伤	国际首创 2009 Hepatology
CKIP-1	石骨症	国际首创 2008 Nat Cell Biol
Smad4	血管扩张	国际首创 2007 Mol Cell Biol
Smad4	骨质疏松	国际首创 2007 J Cell Sci
Smad4/PTEN	食管癌	国际首创 2006 Cancer Res
Smad4	皮肤癌	国际首创 2005 Cancer Res
Smad4	心肌肥厚	国际首创 2005 Circ Res
HBx/HBsAg	肝细胞癌	国际首创 2004 Hepatology

结 论

- 细胞自主性TGF-β信号通路维持组织稳态
- TGF-β信号通过调节miRNA维持组织稳态
- TGF-β调节的miRNA可作为疾病的治疗靶标

科技奖励

2013年 国家科技进步奖创新团队奖（署名第五）

2012年 国家自然科学二等奖（署名第一）

2008年 北京市科学技术进步一等奖（署名第一）

2005年 北京市科学技术进步二等奖（署名第一）

中国高被引学者

2014-2017年入选中国生化、遗传和分子生物学领域高被引学者（h指数45）。

- 作为第一和通讯作者发表论文的被引总次数在本学科所有中国大陆研究者中处于顶尖水平；
- 按学科配额筛选出作为通讯或第一作者发表论文被引总次数排名最高的作者；
- 排除从未发表过被引次数世界前1%论文且论文标准化影响力低于1的作者。

关于转基因动物

- 转基因动物相关研究是人类探索生命未知奥秘的利器，也将为精准医学提供理论基础和治疗策略。
- 加强对转基因动物相关研究的支持、监督、和相关伦理的系统研究将确保其造福人类。

提　纲

- 基因打靶技术的发展历史及其应用
- 利用基因打靶技术研究组织稳态和疾病发生机制
- 关于科学与人生的几点感悟

自信是成功的第一秘诀

一发现自我

一内心梦想

一天生才能

选择的智慧是成功的关键

一立志高远

一敢为天下先

一独辟蹊径

选择是放弃的艺术

—选择奋斗、放弃安逸

—选择坚强、放弃懦弱

—选择独行、放弃盲从

。。。。。。

科学之美

一发现

一简约

一永恒

持之以恒是成功的必经之路

古今之成大事业、大学问者，必经过三种之境界：

“昨夜西风凋碧树。独上高楼，望尽天涯路。”
此第一境也。

“衣带渐宽终不悔，为伊消得人憔悴。”
此第二境也。

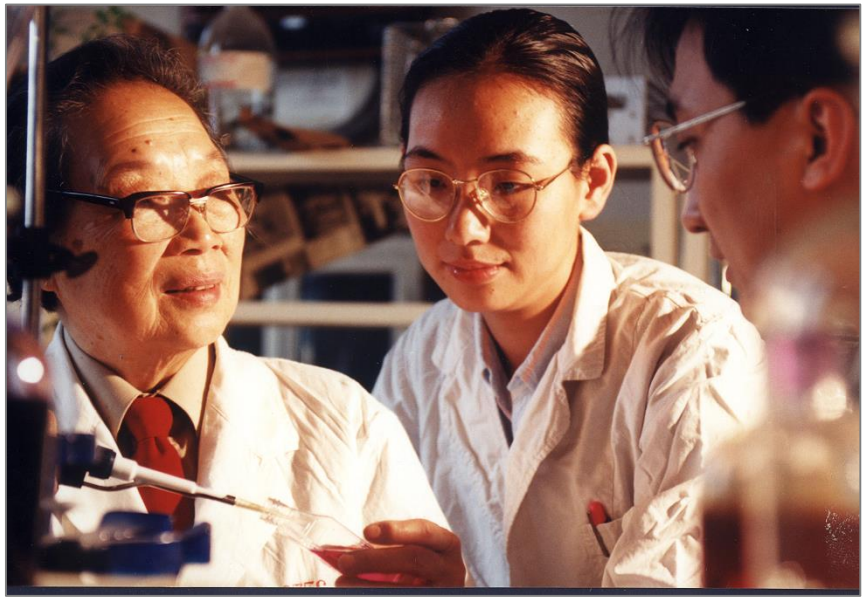
“众里寻他千百度，回头蓦见，那人正在，灯火阑珊处。”
此第三境也。

此等语皆非大词人不能道。

——王国维《人间词话》


持之以恒才能览科学顶峰胜景

致 谢



黄翠芬院士

陈添弥研究员

致 谢

Figure 8. A photograph of Chuxia Deng who worked in my laboratory as a graduate student from 1987 to 1992.

(Figure 7). The exon we chose, exon 8, encodes the active catalytic site for this enzyme. Homologous recombination between this targeting vector and the endogenous *hprt* locus would generate hprt⁻ ES cells resistant to growth in medium containing both 6TG (killing cells with untargeted hprt⁺ loci) and G418 (killing cells lacking the inserted *neo*ʳ gene, as described above). All cell lines generated from cells selected in this way, had lost *hprt* function as a result of the targeted disruptions of the *hprt* locus[10]. Thus, the *hprt* locus provided Chuxia Deng (Figure 8), then a graduate student in our laboratory, an ideal locus for further study of the parameters that influenced the targeting efficiency[31–35].

Because we foresaw that the *neo*ʳ gene would probably be used as a positive selectable gene for the disruption of many genes in ES cells, it was essential that its expression be mediated by an enhancer that would function in ES cells, regardless of the expression status of the target locus. Here our previous experience with enhancers proved of value. We knew from those experiments that the activities of promoter-enhancer configurations are very cell-type specific. To encourage strong *neo*ʳ expression in ES cells, we chose to drive its expression with a mutated polyoma virus enhancer that had been

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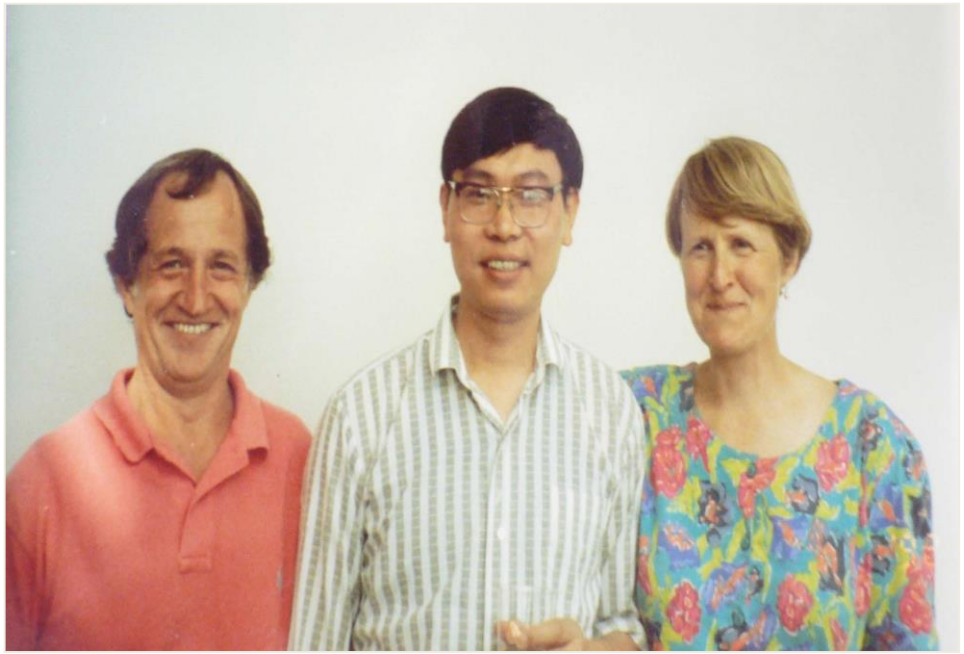

Dr. Mario Capecchi, Chuxia Deng and Mario's wife (May 1992)

GENE TARGETING 1977–PRESENT

Nobel Lecture, December 7, 2007, by Mario R. Capecchi

致 谢

致谢为人类健康献出生命的小鼠