"Pharm" animals (transgenic livestock)

- Bioreactors whose cells have been engineered to synthesize marketable proteins
- DNA constructs contain desired gene and appropriate regulatory sequences (tissue-specific promoters)
- More economical than producing desired proteins in cell culture

動物轉基因科技的用途

- (1)解讀基因的功能並獲得新的知識；
- (2)研究基因對生理調控與胚胎發育的影響；
- (3)建立人類疾病的動物模式；
- (4)製造醫療用的醫藥蛋白產品；
- (5)增進動物製品的優良特性；
- (6)增加動物之體表型變化與觀賞價值。

螢光魚之生產與應用 (Transgenic Fishes)

在魚類胚胎時期的基因轉殖科技，近幾年來已開創出相當龐大的市場商機。就像會發光的螢光魚，其實原本只是水族館常見到的金斑馬魚、青將魚，但是因為要在水族觀賞市場上打下一片天地，於是有了各式各樣的螢光基因轉殖魚類，從綠色到紅色螢光有「夜明珠」的稱號，從全身發光到肌肉發光，人們可以將魚兒變得五彩繽紛。

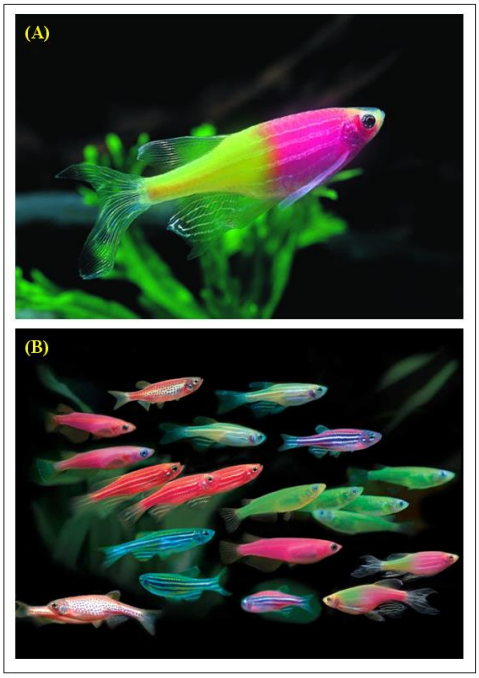


圖1、螢光魚五彩繽紛的螢光條紋。(A)全球第一條的雙色螢光基因魚TK-3，透過不同體節分化基因的調控，將橘紅色與黃色螢光基因表現於魚體兩端；(B)於水族箱中呈現色彩亮麗的各式螢光魚。

螢光魚變紅了！　全球第一最佳風水招財魚

2004/03/31 13:58 自由時報

螢光小鼠之生產與應用 (Transgenic Mice)

不同顏色及亮度的螢光普遍存在於生物界，例如螢火蟲、深海生物、腔腸動物（珊瑚蟲類、水螅類）等。源自水母綠色螢光蛋白，因其非侵入性、即時及原位特性，使其應用備受矚目。原生型GFP蛋白是由238個胺基酸折疊成一中空圓筒狀的三級結構，其中65Ser-Tyr-Gly67結構是發色團最主要的分子。

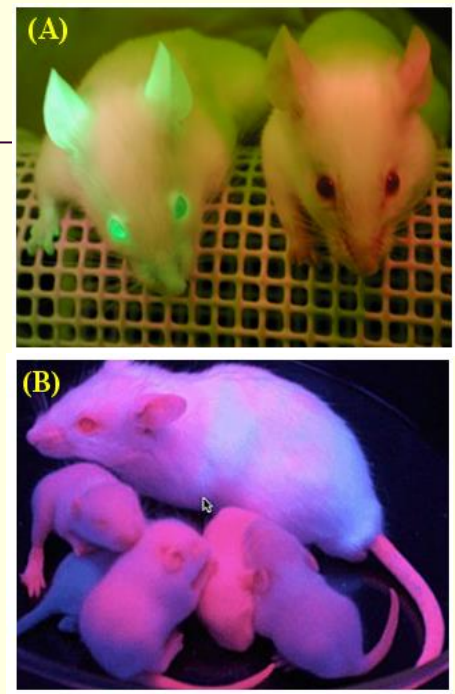
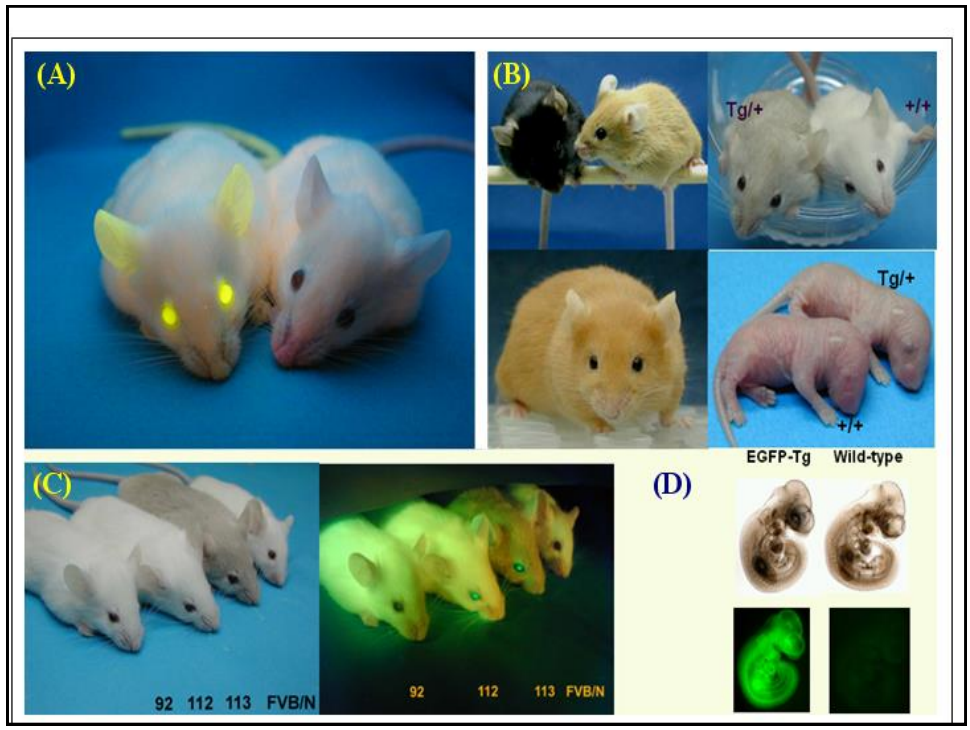


圖3、不同螢光蛋白基因轉殖小鼠。(A) 水母綠色螢光蛋白基因表現於左側邊基因轉殖小鼠全身，右側邊為對照組正常小鼠；(B)珊瑚紅色螢光蛋白基因表現於一整窩的基因轉殖小鼠全身。



螢光豬之生產與應用 (Transgenic Pig)

豬是相當適合人類生物醫學研究的模式動物，豬的體重、心臟大小、血液生化數值、以及許多生理功能，均與人類的生理相當吻合。因此在臨床醫學上，常用豬的皮膚作為各式藥物與化妝品的過敏性測試、燒燙傷的人工皮膚之敷料、心臟瓣膜的取代物、甚至為異種心臟移植的來源。

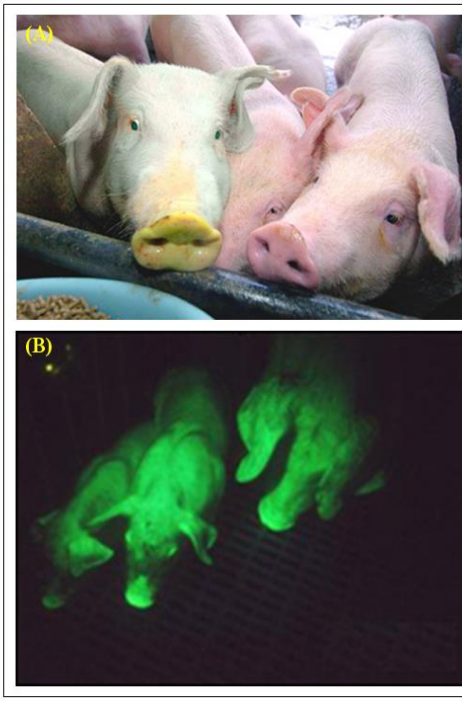
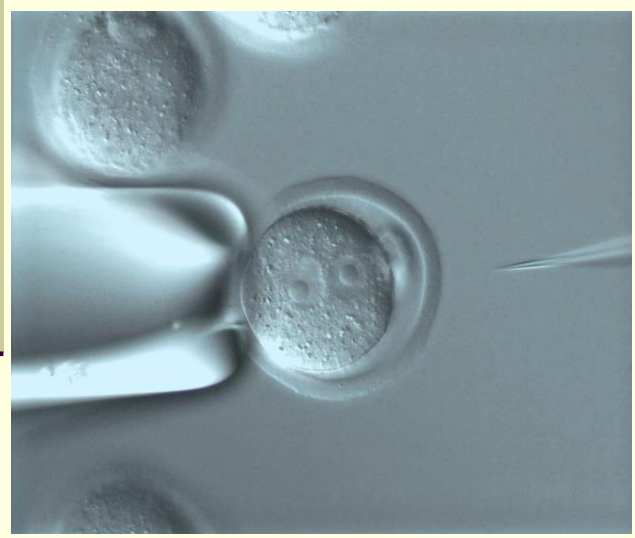


圖4、全身型綠色螢光豬作為再生醫學的研究題材。(A) 綠色螢光基因表現於豬隻體表上，於明視野即可分辨基因轉殖螢光豬的個體，在豬鼻明顯呈現黃色之膚色；(B) 於螢光視野下，基因轉殖螢光豬全身發出亮麗的螢光色澤。

Gene Transfer Methods for Creation of Transgenic Animals

- Direct pronuclear microinjection
- Virus mediated gene transfer
- Embryonic stem cells
- Sperm mediated gene transfer
- Nuclear transfers
- Gene editing (CRISPR/Cas9)

Direct Pronuclear Microinjection: Inject DNA directly into male pronucleus



An one-cell stage embryo is ready to be injected and the 2 distinct pronuclei can be clearly obtained. The DNA will be microinjected into one or, if possible, both pro-nuclei.

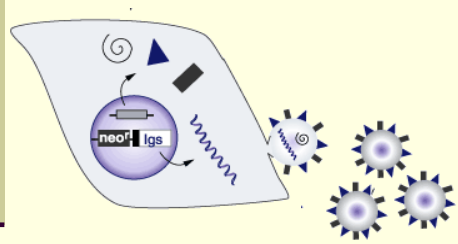
Establishing Transgenic Mice with DNA Direct Microinjection




Virus mediated gene transfer:

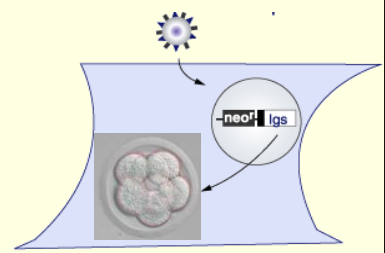
Virus particle package and embryo infection

I. Recombinant virus particle produced in packaging cells

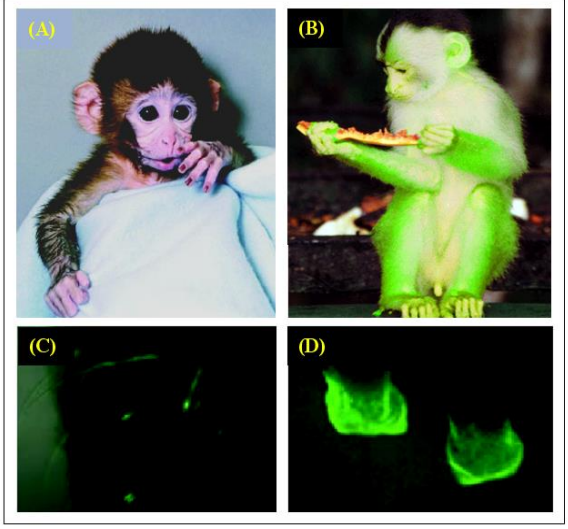


II. Harvested and Concentrated recombinant virus particles

III. Embryonic infections of recombinant virus particle

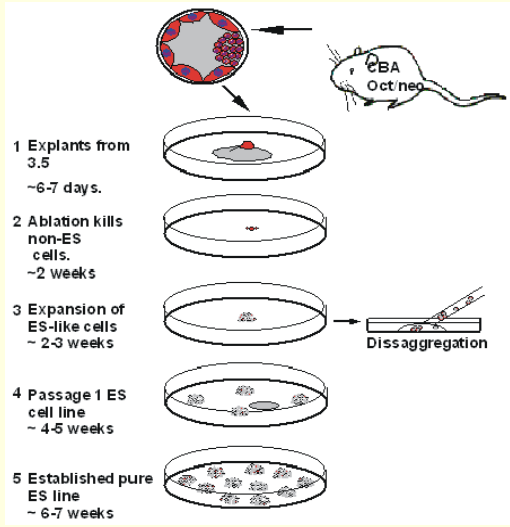


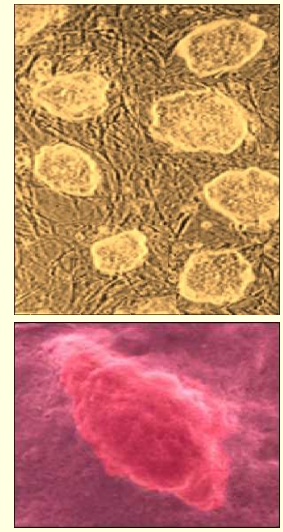
Establishing Transgenic Monkey with Retroviral Vectors



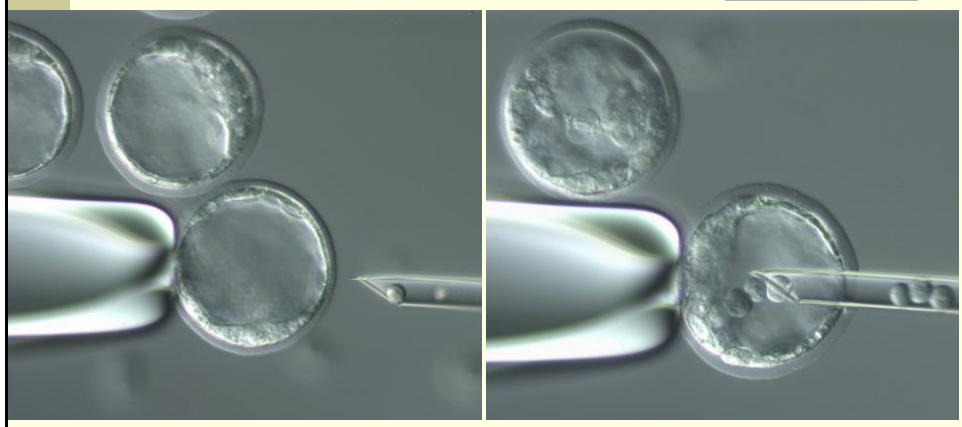
Chan , A. W. S. *et al*. **Transgenic monkeys produced by retroviral gene transfer into mature oocytes**. ***Science*** **291**, 309–312 (**2001**)

Embryonic Stem Cells:
Derived from ICM of blastocyst embryos





ES Cells Injection into Blastocyst



A Day 4 blastocyst is being injected. Each blastocyst will be injected with 12-15 healthy-looking ES Cells.

Knockout mice produced by ES cells

'Knockout mice' study wins Nobel Medicine Prize

Posted Mon Oct 8, 2007 10:00pm AEST

Three scientists have won the Nobel Medicine Prize for their work in creating "knockout mice", now used as the 21st-century testbed for biomedical research.

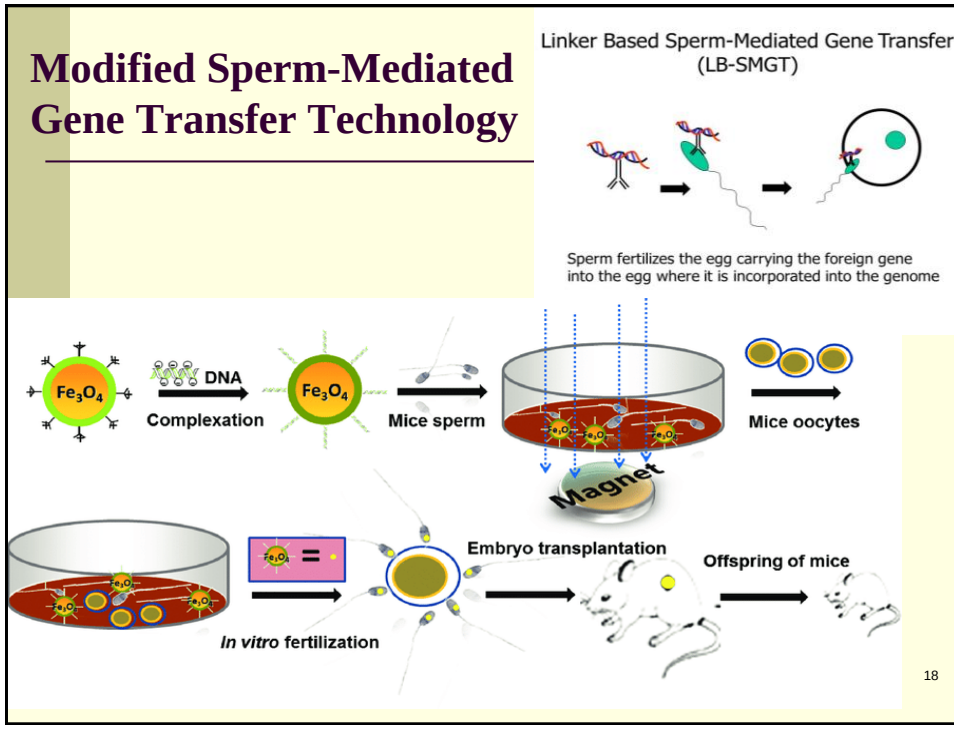
Mario Capecchi and Oliver Smithies of the United States and Martin Evans of Britain won the prize for their embryonic stem cell discoveries.

The Nobel jury said in its citation that the trio was honoured for discovering how to genetically manipulate mouse embryonic stem cells, leading to lab rodents that replicate human disease.

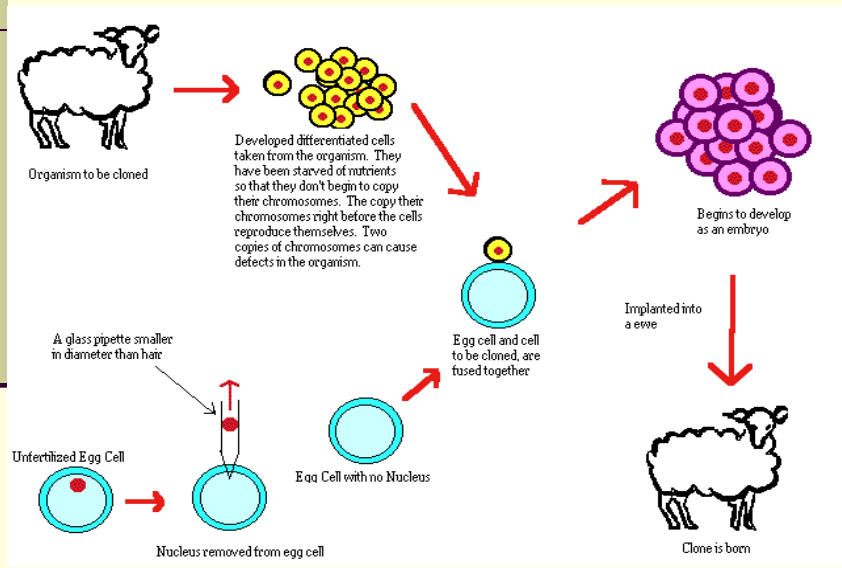
Sperm mediated gene transfer

- Started by Lavitrano et al in 1989 (Cell 57: 717-723)
- Sperm co-incubated with constructed and used for IVF (or AI)
- Can be a very powerful technology
- Transfected sperm may be injected (ICSI) with high rate transgenesis
- Need more research in other species

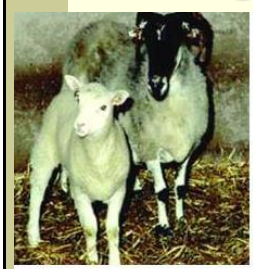
Modified Sperm-Mediated Gene Transfer Technology



Animal Cloning Technology:

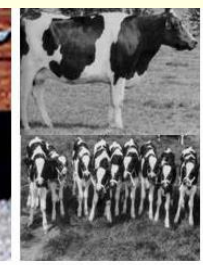


Cloning Animals by Nuclear Transfer


(Wilmut *et al.*, 1997)


(Wakayama *et al.*, 1998)


(Baguisi *et al.*, 1999)


(Wells *et al.*,1999)


(Kubota *et al.*, 2000)


(Chan *et al.*, 2000)

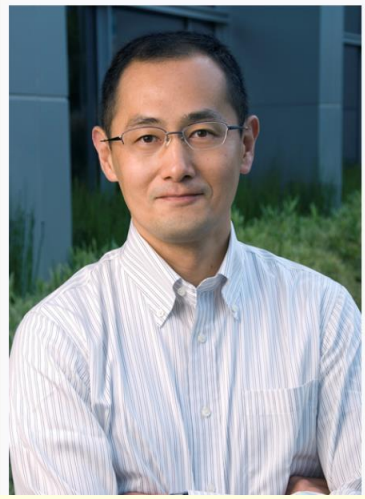

(Shin *et al.*, 2002)


(Vanderwall *et al.*, 2003)

2012 諾貝爾醫學獎 Nobel Medicine Prize

【2012 諾貝爾獎特別報導】生理醫學獎的啟示：誘導多能幹细胞與再生醫學

受精卵是一個奇蹟，它不僅可以從一個細胞發育成由一百多兆個細胞組成的個體。在細胞分裂的過程中，還有另一套精緻的分化程式同時展開：從看似完全相同的胚胎細胞，逐漸分化成皮膚、神經、肌肉等結構、功能各異的細胞，組成特定的器官。是誰在發號施令，指揮這一系列生長、分化程式的進行？是細胞選擇性刪除那些不相關的基因，讓剩下來的基因分決定它分化的命運？還是所有細胞都帶著相同的基因資訊，分化過程中，不同細胞會依循環境和內在的指令，開啟一組特定的基因，來建構特定分化的細胞？那個理論才對？最簡單的

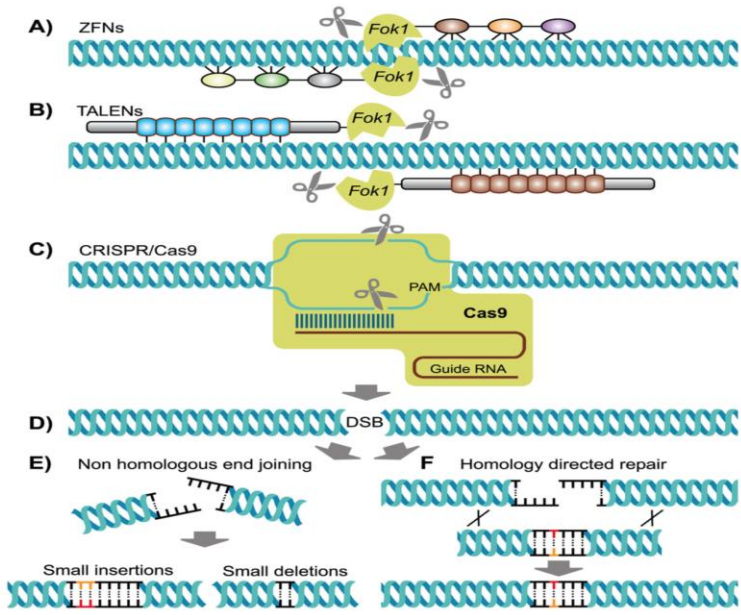


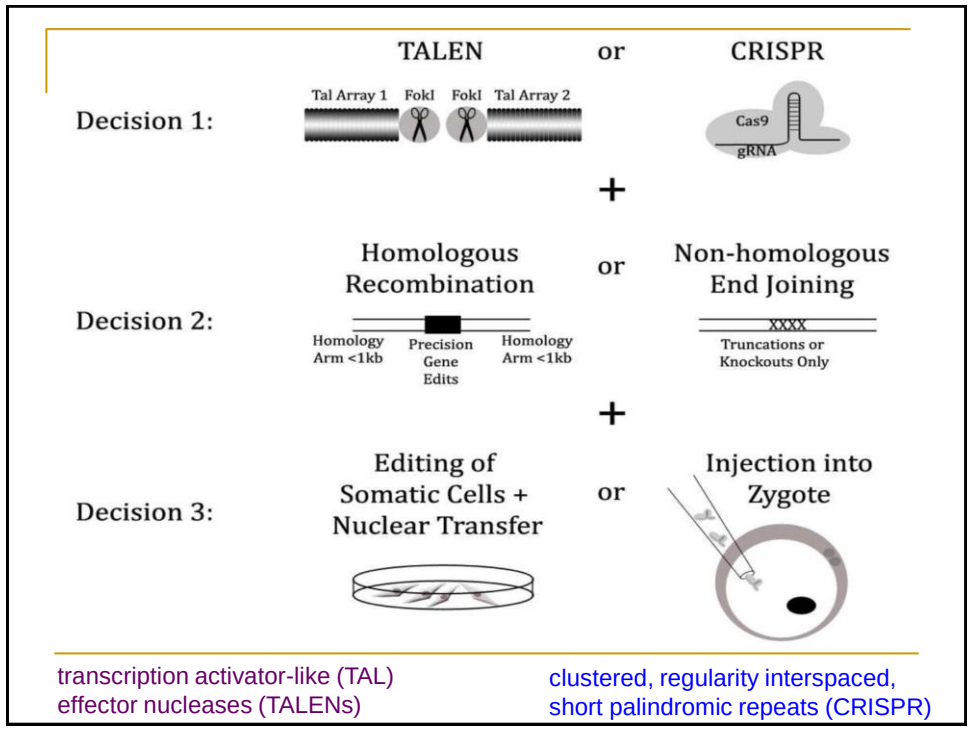
日本籍的山中伸彌(Shinya Yamanaka)

英國籍的古爾登(John Gurdon)

21

Gene Editing in Animals: CRISPR/Cas9





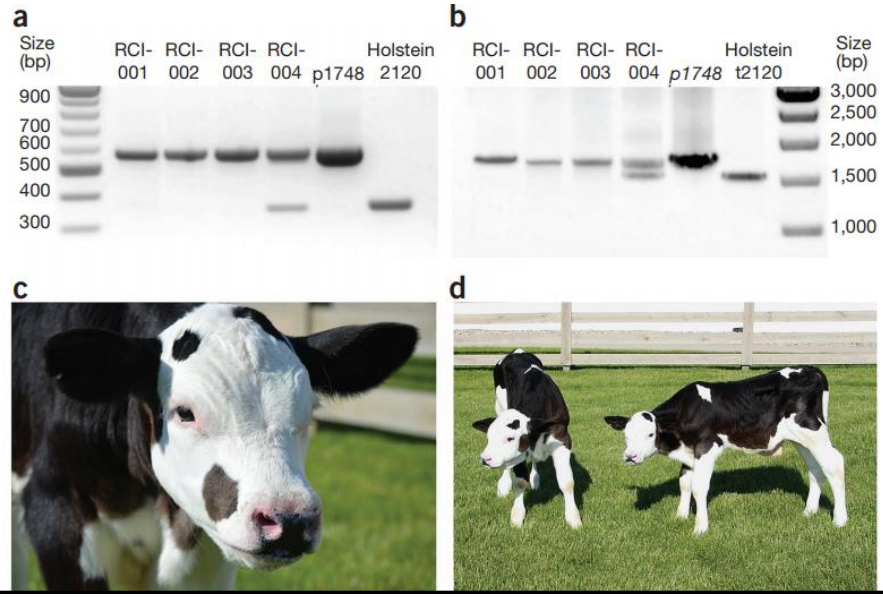
transcription activator-like (TAL) effector nucleases (TALENs)

clustered, regularity interspaced, short palindromic repeats (CRISPR)

應用基因編輯技術產製家畜成果

類型	標的基因	編輯技術	方法	動物	文獻
動物福祉	Polled locus	TALEN	SCNT	牛	Carlson *et al.* (2016)
抗病能力	SP110	CRISPR/Cas9	SCNT	牛	Wu *et al.* (2015)
	NRAMP1	CRISPR/Cas9	CMI	牛	Gao *et al.* (2017)
	CD163	CRISPR/Cas9	CMI	豬	Whitworth *et al.* (2016)
	CD163 SRCR5	CRISPR/Cas9	SCNT	豬	Burkard *et al.* (2017)
	CD163 SRCR5	CRISPR/Cas9	SCNT	豬	Yang *et al.* (2018)
生產性能	BLG	ZFN	SCNT	乳牛	Yu *et al.* (2011)
	GDF8	ZFN	SCNT	牛	Luo *et al.* (2014)
	UCP1	CRISPR/Cas9	SCNT	豬	Zheng *et al.* (2017)
	FGF,GDF8	CRISPR/Cas9	CMI	山羊	Wang *et al.* (2015)
	NANOS2	CRISPR/Cas9	CMI	豬	Park *et al.* (2017)
	BMPR-IB	CRISPR/Cas9	CMI	綿羊	Zhang *et al.* (2017)
生物醫學	EGFP	ZFN	SCNT	豬	Whyte *et al.* (2011)
	PPAR-γ	ZFN	SCNT	豬	Yang *et al.* (2011)
	LDLR	TALEN	SCNT	豬	Carlson *et al.* (2012)
	B2M	TALEN	CMI	豬	Wang *et al.* (2016)
	vWF	CRISPR/Cas9	CMI	豬	Hai *et al.* (2014)
	SLA-1,2,3	CRISPR/Cas9	SCNT	豬	Reyes *et al.* (2014)
	α 1,3GT	CRISPR/Cas9	SCNT	豬	Butler *et al.* (2016)
	CMAH,B4GalNT2 *po*	CRISPR/Cas9	SCNT	豬	Niu *et al.* (2017)

Production of hornless dairy cattle from genome-edited cell lines (*Nature Biotechnology 2016)*



Comparison of different methodologies in the production of transgenic goats

Method	Success to obtain...		
DNA microinjection	[4]		
Somatic cell nuclear transfer	[16]		
Lentiviral injection	[15]		
Sperm-mediated gene transfer	[14]		

Figure 3: Success to obtain transgenic goat embryos, fetuses or offspring according to the different methods used

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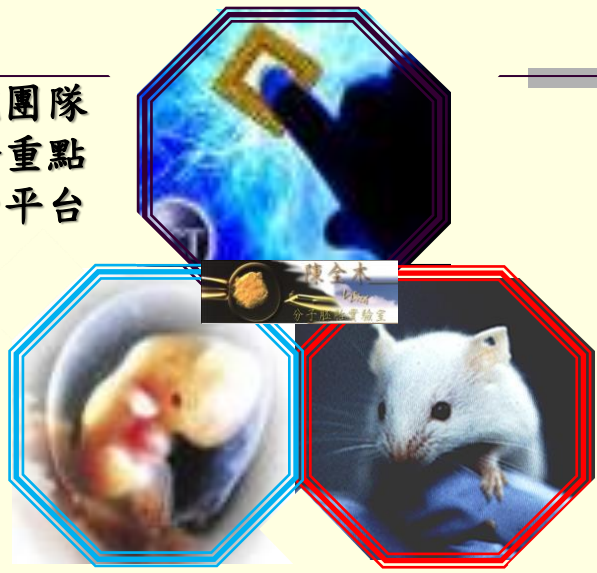
本研究團隊的研發重點與核心平台



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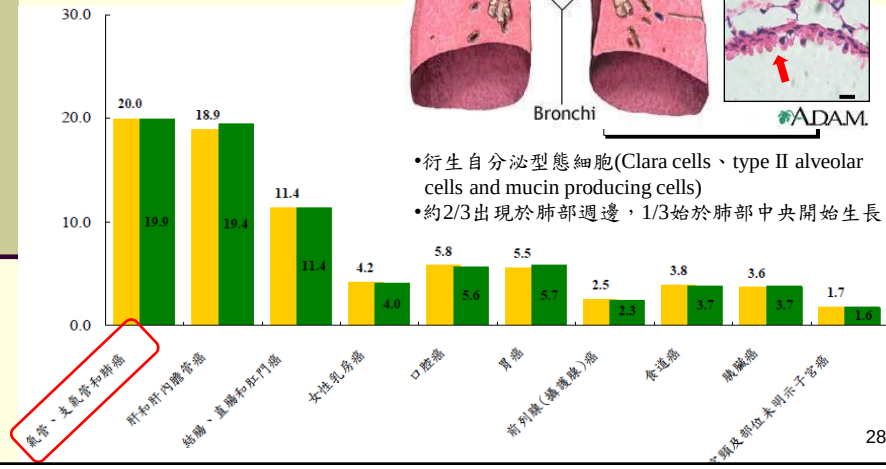
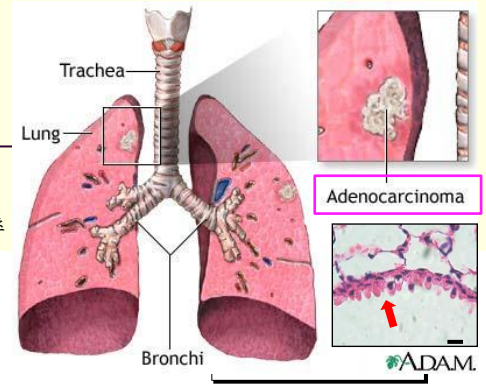
1 提出研究議題的重要性

Lung cancer

99及100年主要癌症死亡人數占率





- 衍生自分泌型態細胞(Clara cells、type II alveolar cells and mucin producing cells)
- 約2/3出現於肺部週邊，1/3始於肺部中央開始生長

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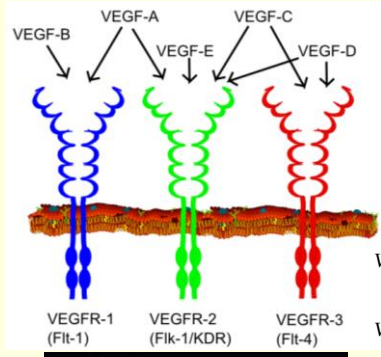
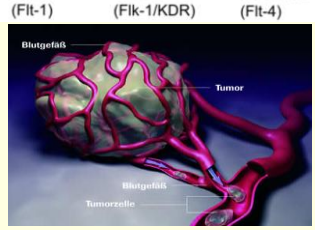
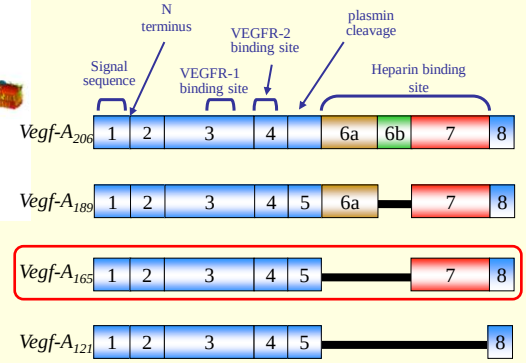
VEGF (vascular endothelial growth factor)





VEGF promotes tumor angiogenesis and is an important target in various malignancies, including non-small cell lung cancer (NSCLC).



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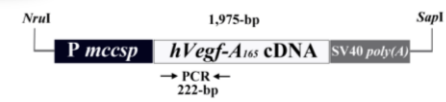
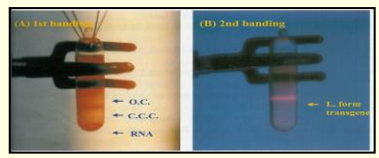
2 建立正確的研究模式

Production of Clara cell-specific hVEGF-A165 transgenic mice



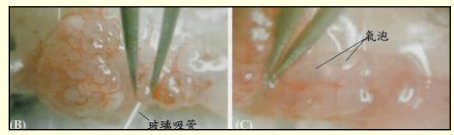
DNA Purification

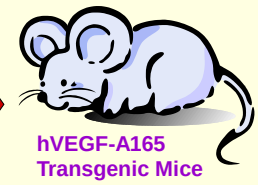


Microinjection

Transgenic Mice

Embryo Transfer





hVEGF-A165 Transgenic Mice

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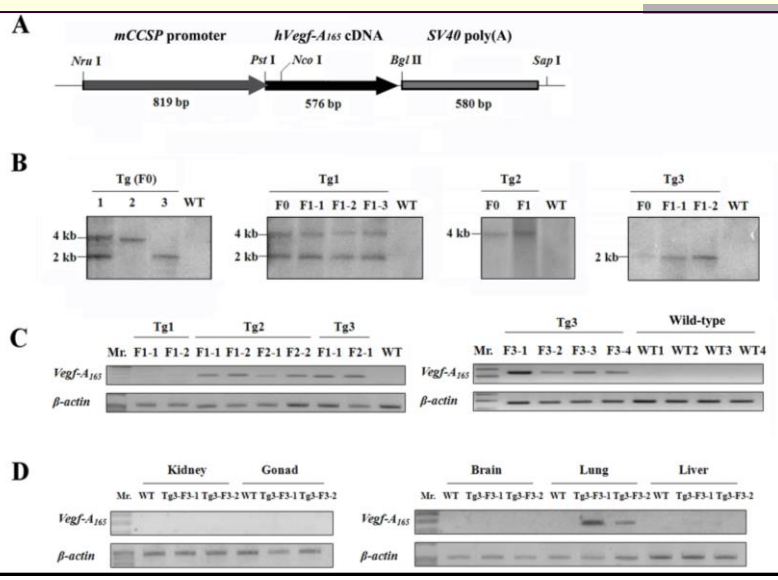
Generation of lung-specific hVEGF-A165-overexpression of transgenic mice



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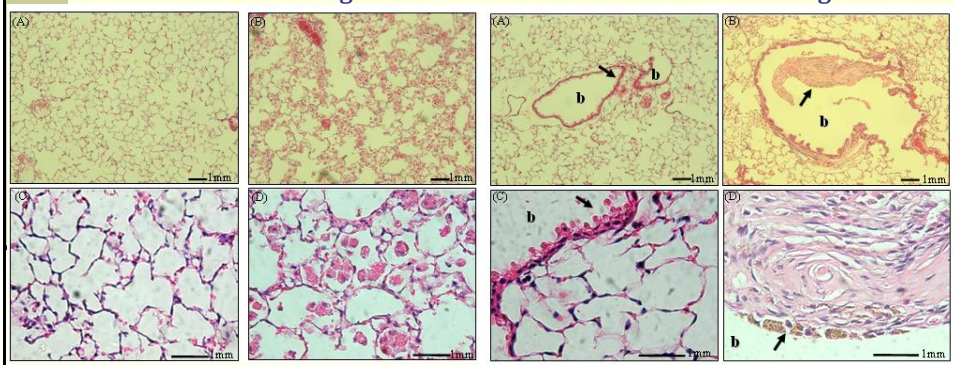
Histopathological examination of the lung tissues in Wt & Tg mice: Inflammation & Neoplasia

> 6 months of age | > 8 months of age

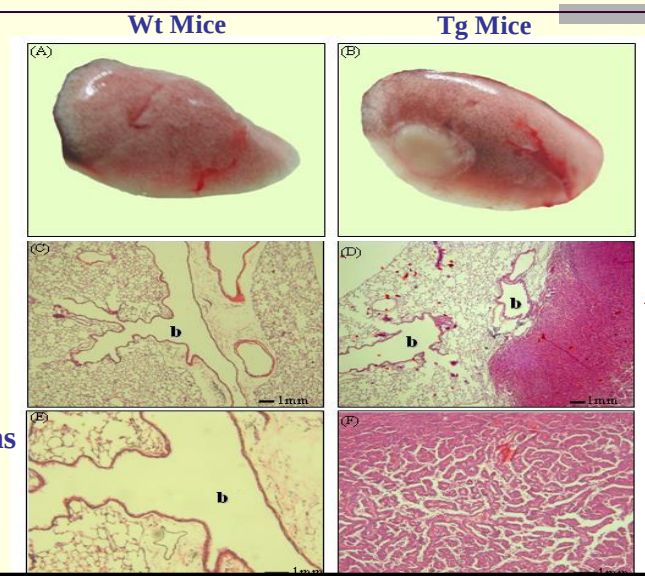
Wt Mice | Tg Mice | Wt Mice | Tg Mice



Inflammation | Neoplasia

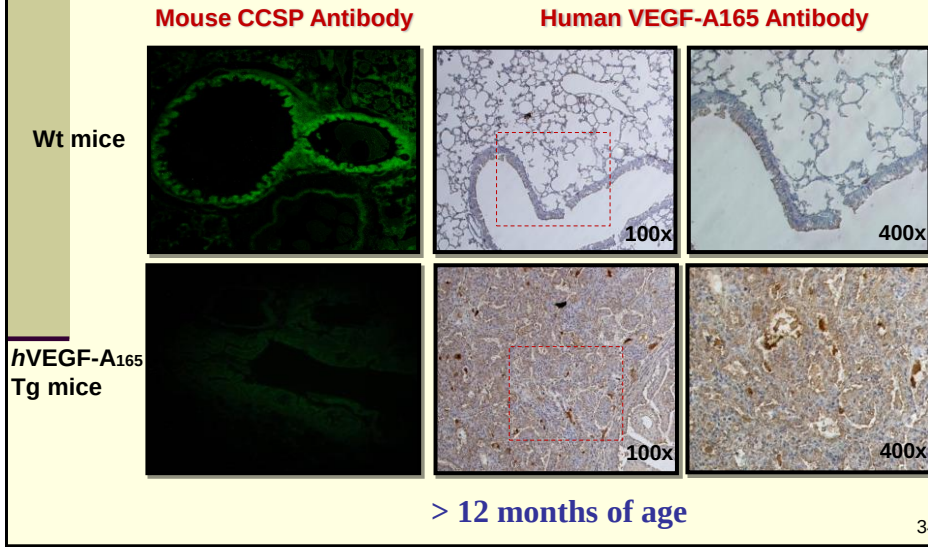
32

Histopathological examination of the lung tissues in Wt & Tg mice： Adenomas



33

Expression of endogenous mouse CCSP and exogenous human VEGF-A_{165} in the lung tissues of Wt & Tg mice



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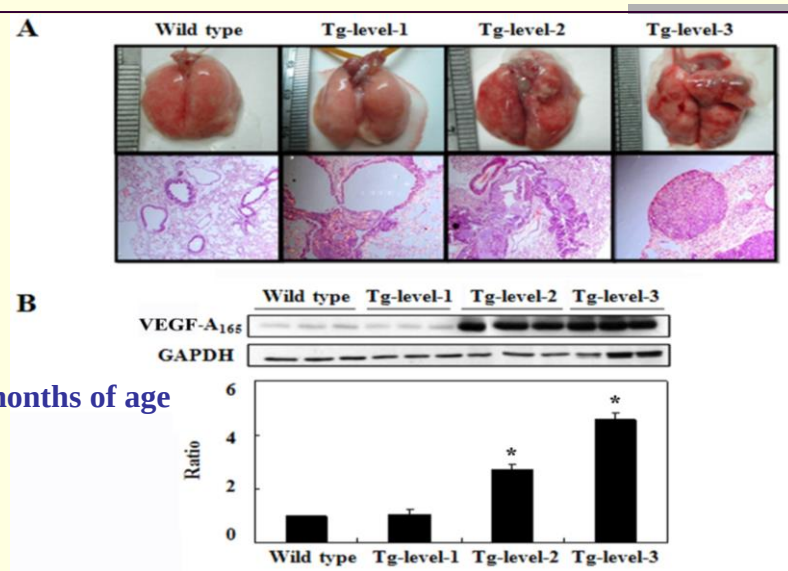
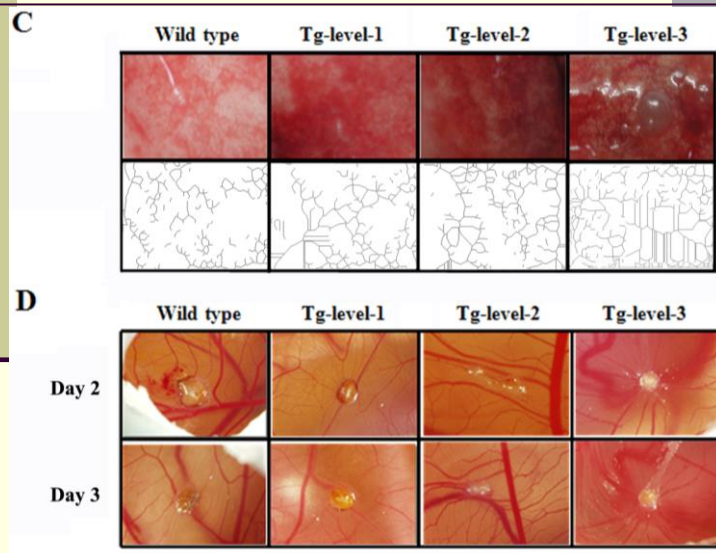
Histopathology and Western blotting analyses of the lung tissues in three tumorigenesis levels of hVEGF-A165-overexpressing transgenic mice



> 12 months of age

35

*h*VEGF-A165 induces neovascular developments in the lung tissues of transgenic mice

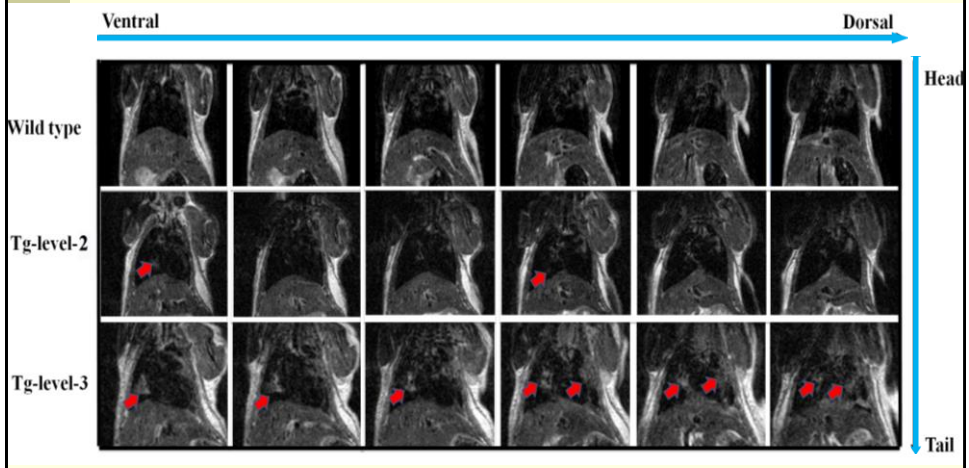


Angiogenesis Image Analyzer (AIA)

Chick Chorioallantoic Membrane (CAM)

36

The MRI living images of lung adenocarcinomas in two different tumorigenesis levels of transgenic mice



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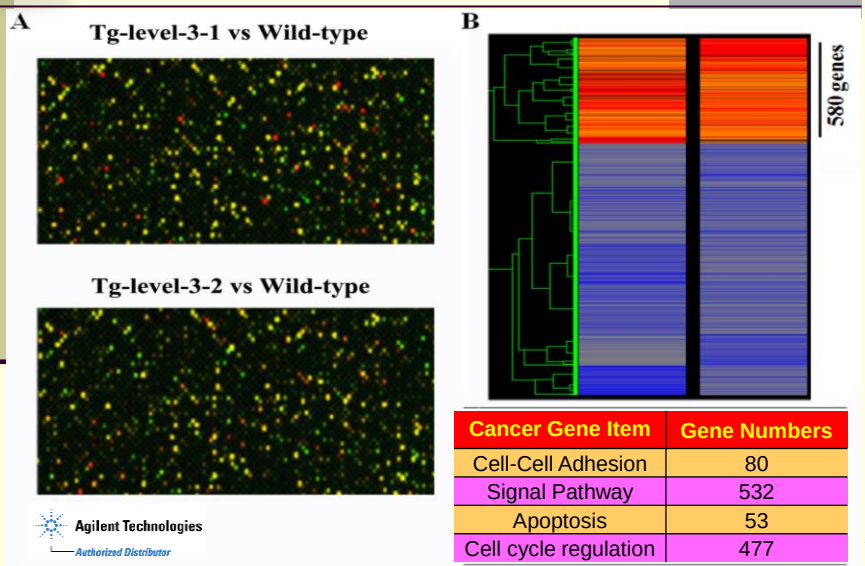
3 挖掘重要的分子機制

Gene expression profiling of hVEGF-A165 transgenic mice using a 44K cDNA microarray



Cancer Gene Item	Gene Numbers
Cell-Cell Adhesion	80
Signal Pathway	532
Apoptosis	53
Cell cycle regulation	477

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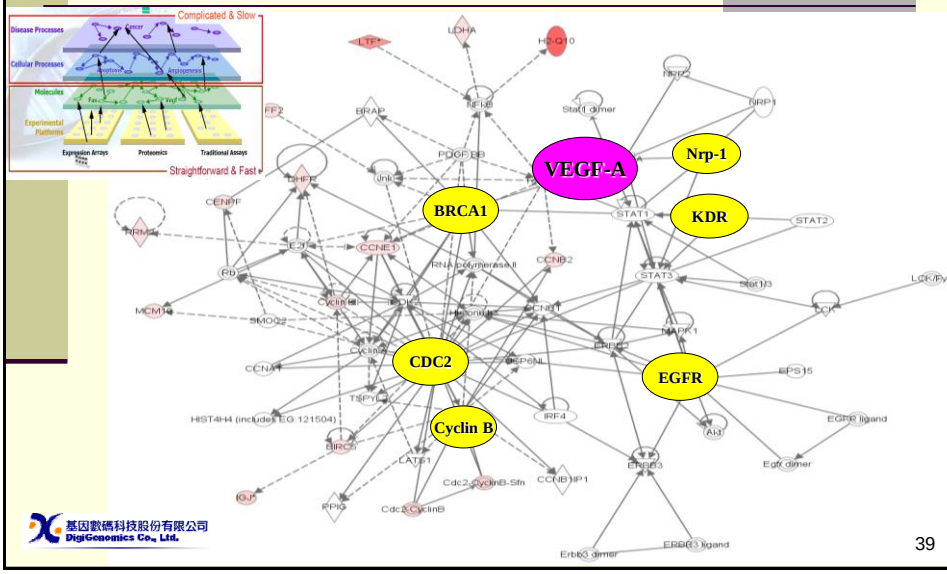
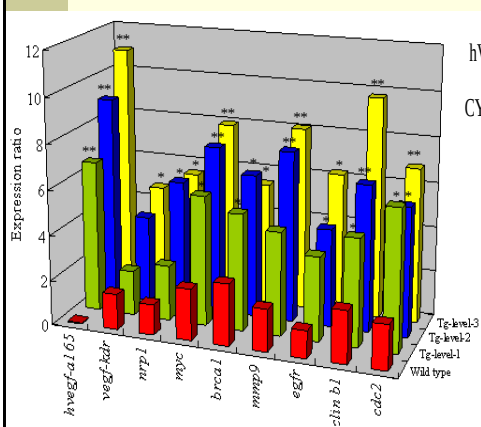
The Ingenuity Pathway Analysis (IPA) of mouse cDNA microarray data



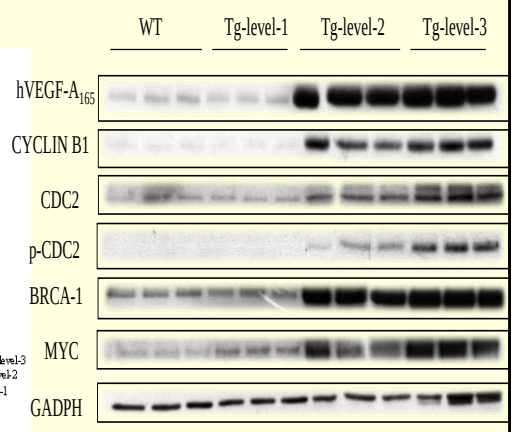
39

Validations of mRNA and protein expressions in the lung tissues of three different tumorigenesis levels of transgenic mice

mRNA levels

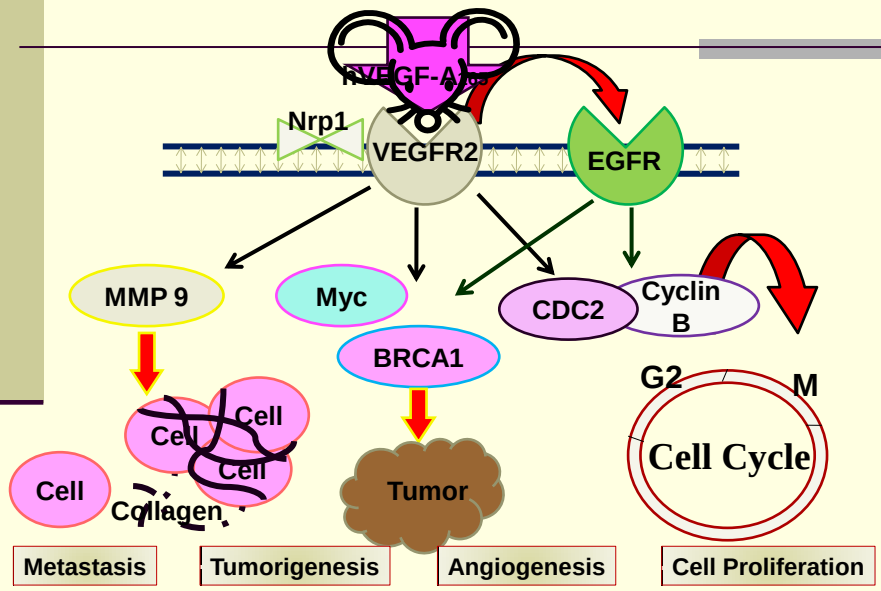


Protein levels



40

Mechanism of lung adenocarcinoma induced by hVEGF-A_{165} overexpression in transgenic mice



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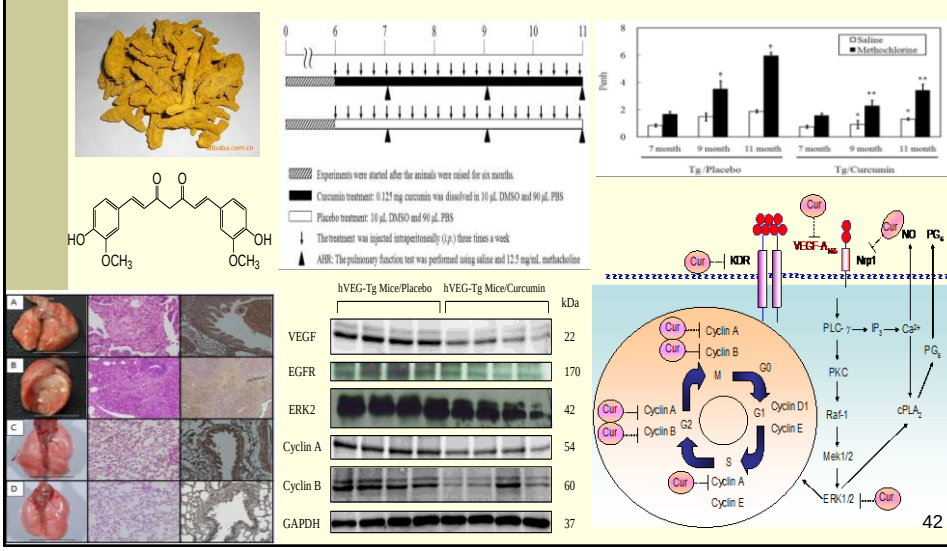
4 找尋治療的新策略

Curcumin reduces pulmonary tumorigenesis in vascular endothelial growth factor (VEGF)-overexpressing transgenic mice

Mol. Nutr. Food Res. 2011, *55*, 1036–1043

Yu-Tang Tung[1], Hsiao-Ling Chen[2]*, Cheng-Wei Lai[1], Chih-Jie Shen[1], Yi-Wen Lai[1] and Chuan-Mu Chen[1]*

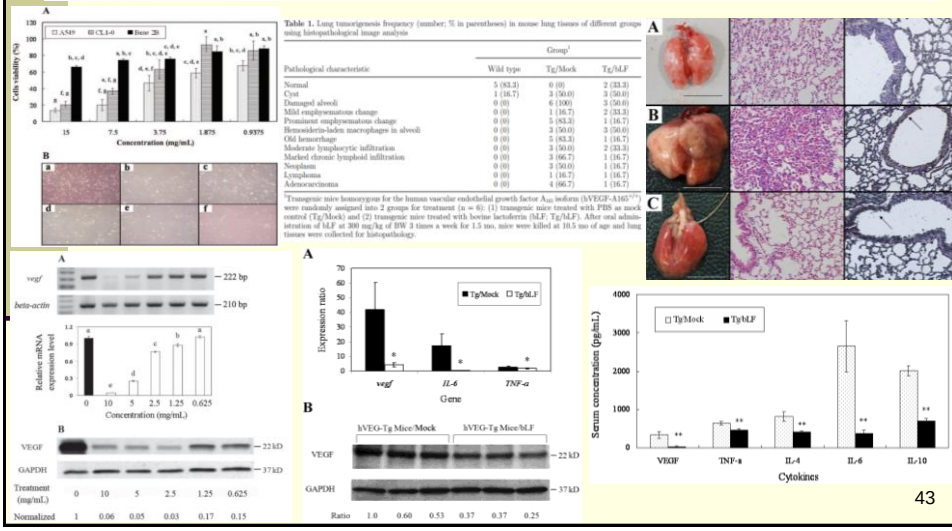


42

J. Dairy Sci. 96:2095–2106
http://dx.doi.org/10.3168/jds.2012-6153

Bovine lactoferrin inhibits lung cancer growth through suppression of both inflammation and expression of vascular endothelial growth factor

Yu-Tang Tung,*[1] Hsiao-Ling Chen,†[1] Chih-Ching Yen,*‡[1] Po-Ying Lee,§ Hsin-Chung Tsai,*# Ming-Fong Lin,*# and Chuan-Mu Chen*[2]



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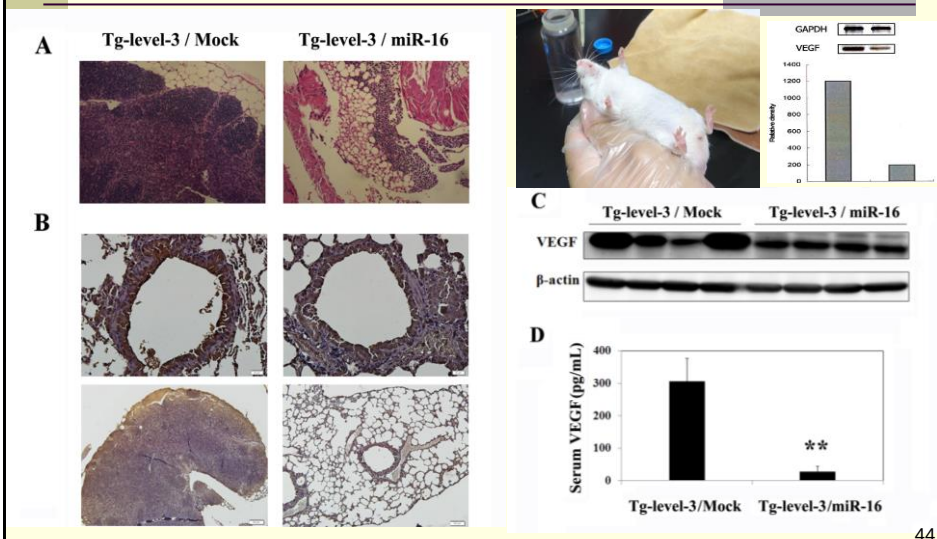
Lung tumorigenesis induced by human vascular endothelial growth factor (hVEGF)-A165 overexpression in transgenic mice and amelioration of tumor formation by miR-16

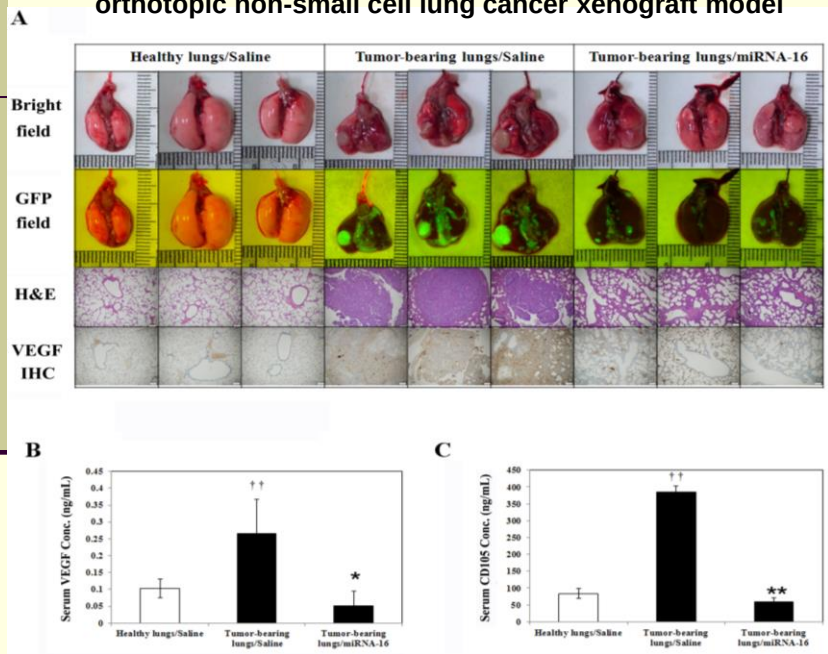
Oncotarget

miR-16: 5′-UAGCAGCACGUAAAUAUUGGCG-3′



44

MicroRNA-16 reduces pulmonary tumorigenesis in human orthotopic non-small cell lung cancer xenograft model



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5 專利佈局與論文發表

Paper publications related to the studies of VEGF-lung carcinoma

1. Y. T. Tung, H. L. Chen, C. W. Lai, C. J. Shen, Y. W. Lai, and **C. M. Chen***. 2011. Curcumin reduces pulmonary tumorigenesis in vascular endothelial growth factor (VEGF)-overexpressing transgenic mice. ***Mol. Nutri. Food Res.*** 55(7): 1036-1043. [IF=**4.909**; Food Science & Technology, 2/128= **Top 1.6%**]
2. Y. T. Tung, H. L. Chen, C. Y. Lee, Y. C. Chou, P. Y. Lee, H. C. Tsai, Y. L. Lin, and **C.M. Chen***. 2013. Active component of Danshen (*Salvia miltiorrhiza* Bunge), Tanshinone I, attenuates lung tumorigenesis via inhibitions of VEGF, Cyclin A and Cyclin B expression. ***Evid. Complement. Alter. Med.*** 2013: e319247. [Integrative & complementary medicine, 6/22= **Top 27%**]
3. Y. T. Tung, C. C. Yen, H. L. Chen, P. Y. Lee, H. C. Tsai, M. F. Lin, and **C.M. Chen***. 2013. Bovine lactoferrin inhibits lung cancer growth through suppression of inflammation and VEGF expression. ***J. Dairy Sci.*** 96(4): 2095-2106. (SCI) [Agriculture, Dairy & Animal Science, **2/52 = Top 3.8%**] **[Cancer Commons: Angiogenesis特別報導; 02/22/2013]** **[High Cited Paper: 2015]**
4. Y. T. Tung, H. L. Chen, H. C. Tsai, S. H. Yang, Y. C. Chang, and **C.M. Chen***. 2013. Therapeutic potential of andrographolide isolated from the leaves of *Andrographis paniculata* Nees for treating lung adenocarcinomas. ***Evid. Complement. Alter. Med.*** 2013: e305898. [Integrative & complementary medicine, 6/22= **Top 27%**]
5. H. L. Chen, C. C. Yen, S. M. Wang, T. C. Tsai, Z. L. Lai, J. Y. Sun, W. Lin, W. H. Hsu, and **C. M. Chen***. 2014. Aerosolized bovine lactoferrin reduces lung injury and fibrosis in mice exposed to hyperoxia. ***BioMetals*** 27: 1057-1068. (SCI) [IF=**3.284**; Biochemistry & Molecular Biology 111/290= 38.3%]
6. Y. T. Tung, P. W. Huang, Y. C. Chou, H. P. Wang, H. C. Ho, C. Y. Tu, D. C. Yeh, K. Y. Chong, and **C. M. Chen*.** 2015. Lung tumorigenesis induced by human vascular endothelial growth factor (hVEGF)-A_{165} overexpression in transgenic mice and amelioration of tumor formation by miR-16. ***Oncotarget*** 6:10222-10238. [IF=**6.627**; Oncology, 17/203= **Top 8.4%**]

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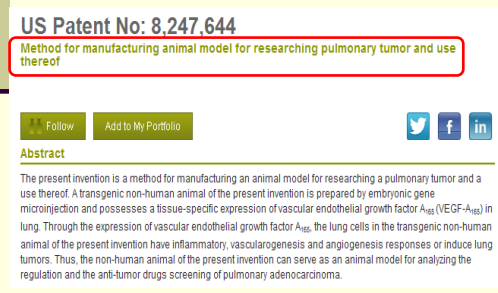
US and Taiwan Patents related to the studies of VEGF-lung carcinoma

1. **C. M. Chen**. 2012/8/21- 2030/6/30. Method for manufacturing animal model for researching pulmonary tumor and use thereof. ***USA Patent,*** patent number: ***US 8,247,644 B2.***

2. **C. M. Chen**. 2012/12/11- 2029/7/1. Method for production of non-human transgenic animals to express human vascular endothelial growth factor and use thereof. (具肺部腫瘤之動物模式的製造方法及其去氧核醣核酸構成物、用途) ***Taiwan Patent,*** Patent number: ***I 379005***.

3. **C. M. Chen** (陳全木) 2012. 7一種製造能表現人類血管內皮新生因子之肺腫瘤動物模式及其於藥物篩選之平台應用。技轉廠商：豐展生物科技股份有限公司。


US Patent No: 8,247,644

Method for manufacturing animal model for researching pulmonary tumor and use thereof

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Abstract

The present invention is a method for manufacturing an animal model for researching a pulmonary tumor and a use thereof. A transgenic non-human animal of the present invention is prepared by embryonic gene microinjection and possesses a tissue-specific expression of vascular endothelial growth factor A_{165} (VEGF-A_{165}) in lung. Through the expression of vascular endothelial growth factor A_{165}, the lung cells in the transgenic non-human animal of the present invention have inflammatory, vascularogenesis and angiogenesis responses or induce lung tumors. Thus, the non-human animal of the present invention can serve as an animal model for analyzing the regulation and the anti-tumor drugs screening of pulmonary adenocarcinoma.


(12) United States Patent
Chen

(10) Patent No.: US 8,247,644 B2
(45) Date of Patent: Aug. 21, 2012

(54) METHOD FOR MANUFACTURING ANIMAL MODEL FOR RESEARCHING PULMONARY TUMOR AND USE THEREOF

(75) Inventor: Chuan-Mu Chen, Taichung (TW)

(73) Assignee: National Chung Hsing University, Taichung (TW)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: 12/838,706

(22) Filed: Jul. 1, 2010

(65) Prior Publication Data

US 2011/0004398 A1 Jan. 6, 2011

(30) Foreign Application Priority Data

Jul. 2, 2009 (TW) ..... 98122476 A

(51) Int. Cl.
A01K 67/027 (2006.01)
A01K 67/00 (2006.01)
C12N 15/00 (2006.01)

(52) U.S. Cl. 800/18; 800/3; 800/21

(58) Field of Classification Search 800/3, 18, 800/21

See application file for complete search history.

(56) References Cited

U.S. PATENT DOCUMENTS

6,703,016 B2 * 3/2004 Tabolin et al. 424/93.2
2003/0194392 A1 * 10/2003 Tabolin et al. 424/93.2
2010/0137730 A1 * 6/2010 Eckert et al. 424/94.65

OTHER PUBLICATIONS

Hamayasu et al. Curr Protoc Cell Biol. May 2009 Chapter Unit-19. doi: 10.1002/0471143030.cb1910s42. pp. 1-12.*

* cited by examiner

Primary Examiner — Marcia S Noble
(74) Attorney, Agent, or Firm — Ming Chyou; Sinorica, LLC

(57) ABSTRACT

The present invention is a method for manufacturing an animal model for researching a pulmonary tumor and a use thereof. A transgenic non-human animal of the present invention is prepared by embryonic gene microinjection and possesses a tissue-specific expression of vascular endothelial growth factor A_{165} (VEGF-A_{165}) in lung. Through the expression of vascular endothelial growth factor A_{165}, the lung cells in the transgenic non-human animal of the present invention have inflammatory, vascularogenesis and angiogenesis responses or induce lung tumors. Thus, the non-human animal of the present invention can serve as an animal model for analyzing the regulation and the anti-tumor drugs screening of pulmonary adenocarcinoma.

6 Claims, 10 Drawing Sheets

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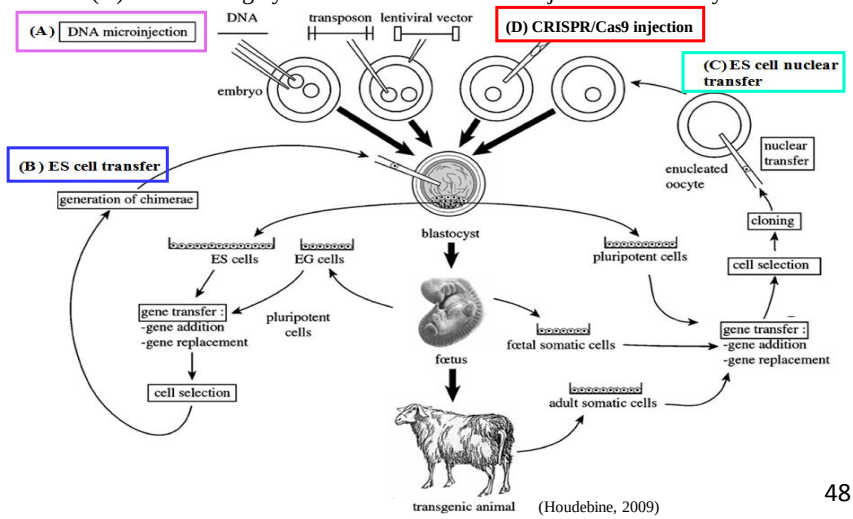
Production Methods of Transgenic Animals

(A) DNA microinjection : DNA, transposon, lentiviral vector
(B) ES cell-mediated gene transfer into blastocyst
(C) ES cell-mediated somatic nuclear transfer
(D) Gene editing by CRISPR/Cas9 and microinjection into embryo



(Houdebine, 2009)

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Acknowledgments