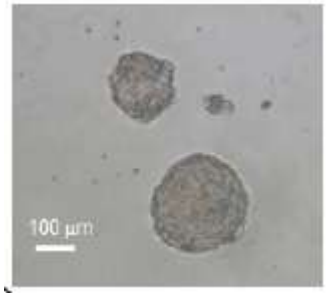
Anti-cancer stem cell drug discovery by tumorsphere platform

張文瑋 教授

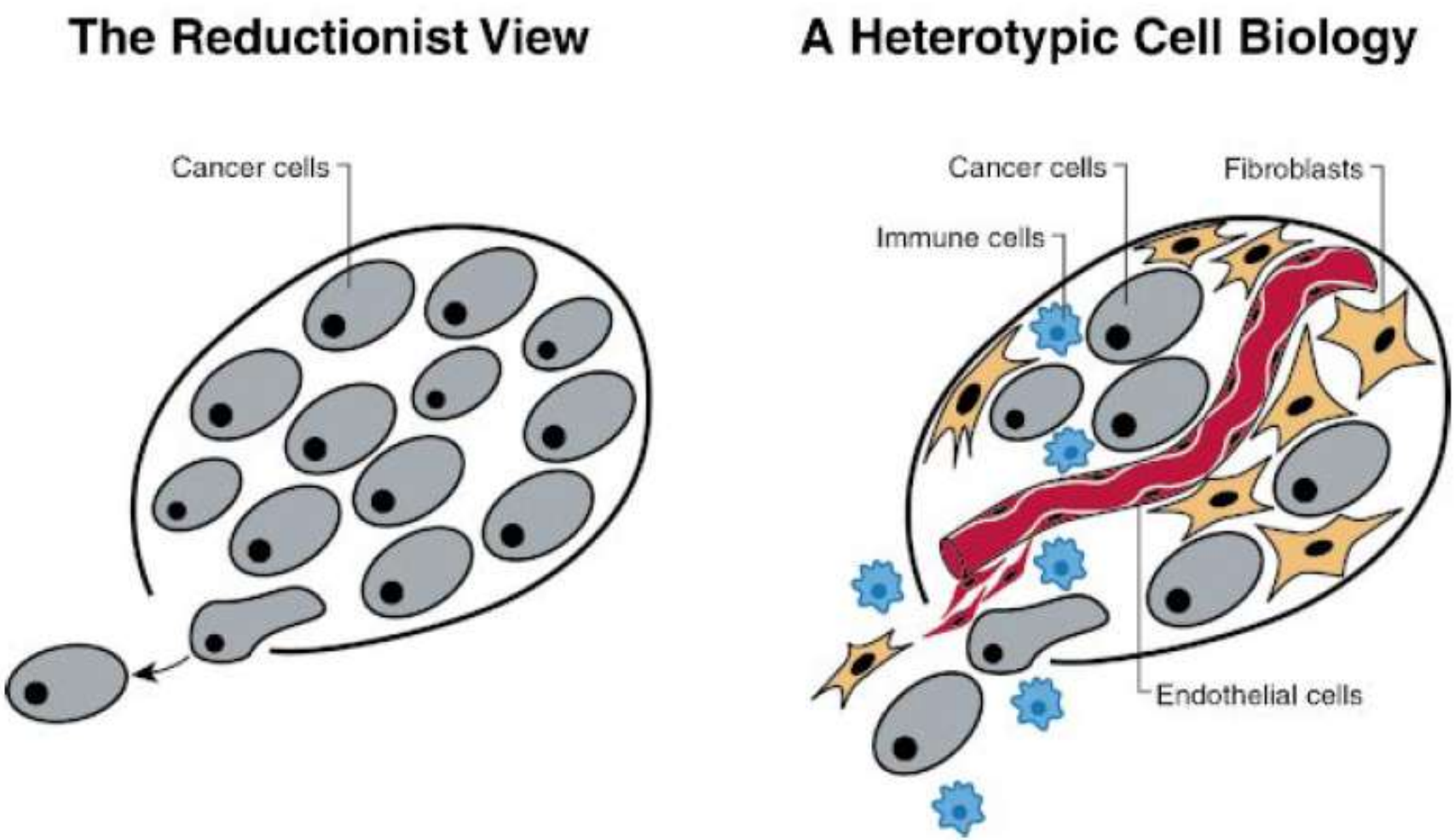
中山醫學大學

生物醫學科學系

changww@csmu.edu.tw



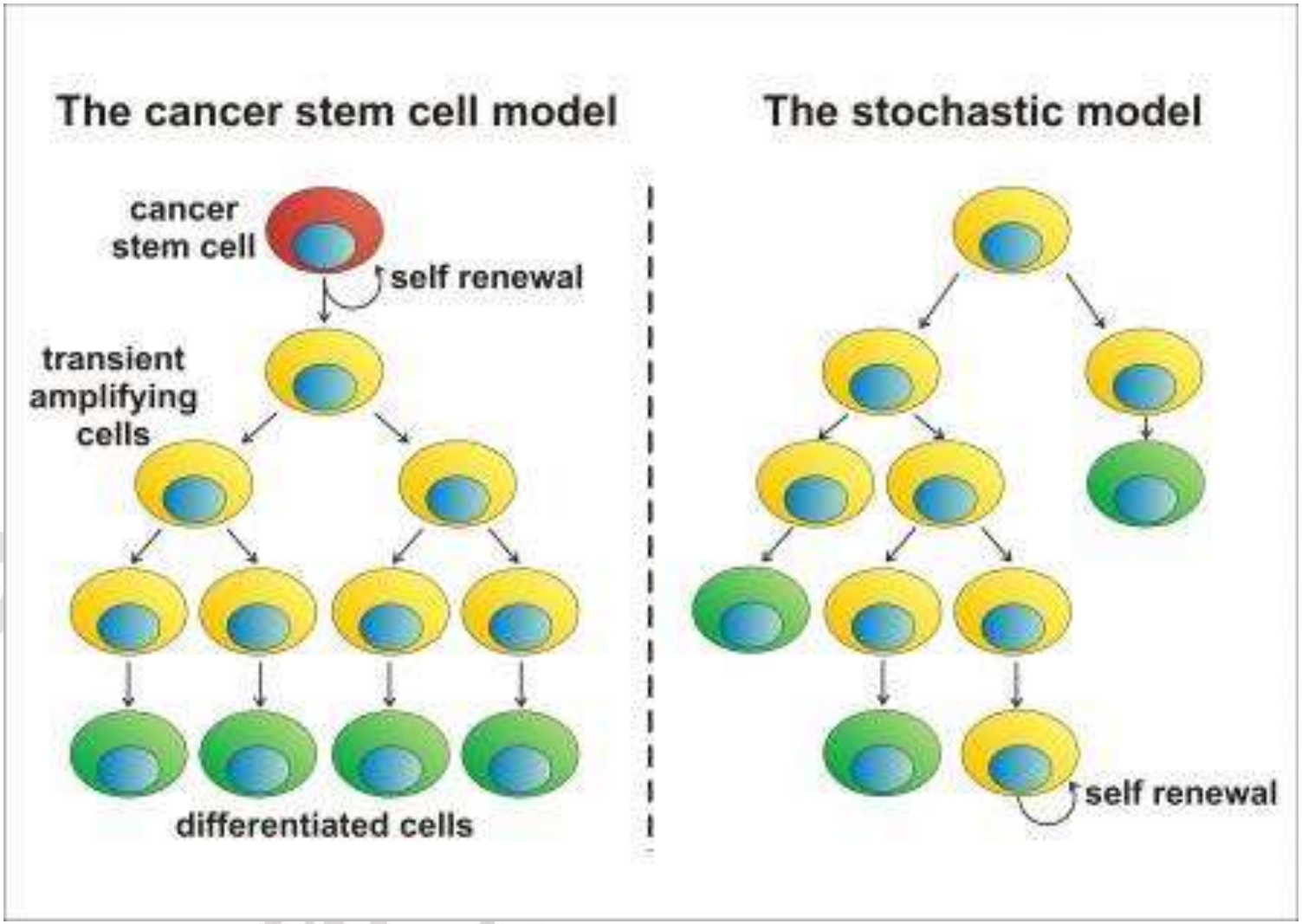
癌症可視為一個複雜性的組織



Cell. 2000. 100:57-70.

癌症異質性發展模式假說

癌症幹細胞模式　　克隆演化模式



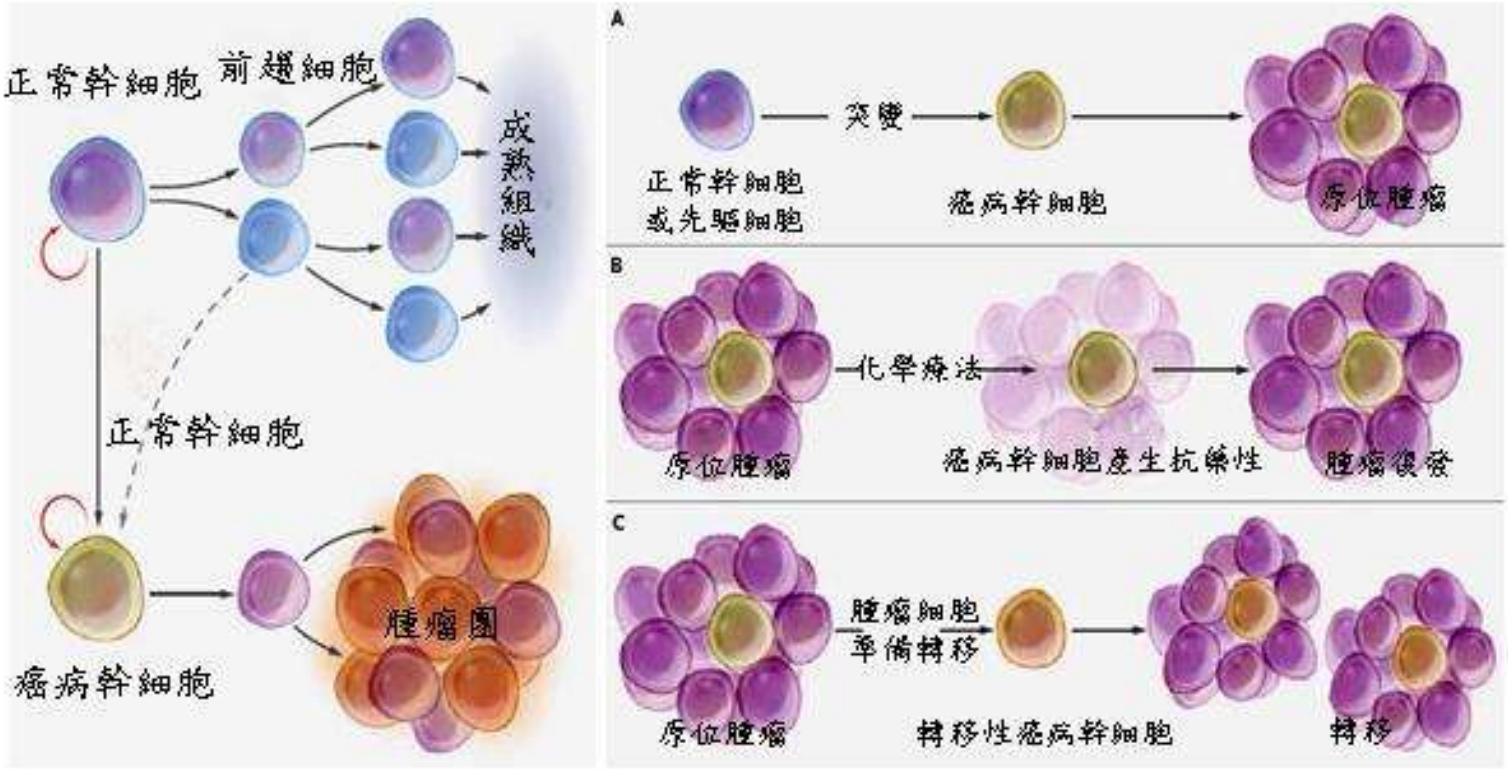
http://www.eurostemcell.org/de/node/16637

癌症幹細胞已在多種癌症中被發現

Tumour type	CSC marker	Tumour cells expressing CSC marker, %	Minimal number of cells expressing CSC markers for tumour formation	Injected in Matrigel	Transplantation site	Strain	Refs
Breast	$CD44^+/CD24^{-/low}$	11–35	200	+	Mammary fat pad	NOD-SCID	19
Breast	$CD44^+/CD24^-$	ND	2×10^3	–	Mammary fat pad	NOD-SCID	77
Breast	$ALDH1^+$	3–10	500	+*	Mammary fat pad	NOD-SCID	33
Brain	$CD133^+$ (GBM) $CD133^+$ (MB)	19–29 6–21	100 100	– –	Brain Brain	NOD-SCID NOD-SCID	20 20
Brain	$CD133^+$	2–3	500	–	Brain	nu/nu	24
Colon	$CD133^+$	1.8–25	200	+	Kidney capsule	NOD-SCID	26
Colon	$CD133^+$	0.7–6	3×10^3	–	Subcutaneous	SCID	27
Colon	$EpCAM^{hi}/CD44^+$	0.03–38	200	+	Subcutaneous	NOD-SCID	32
Head and neck	$CD44^+$	0.1–42	5×10^3	+	Subcutaneous	Rag2/$\gamma^{-/-}$DKO, NOD-SCID	117
Pancreas	$CD44^+/CD24^+/ESA^+$	0.2–0.8	100	+	Pancreas	NOD-SCID	65
Pancreas	$CD133^+$	1–3	500	–	Pancreas	NMRI-nu/nu	28
Lung	$CD133^+$	0.32–22	10^4	–	Subcutaneous	SCID	42
Liver	$CD90^+$	0.03–6	5×10^3	–	Liver	SCID/Beige	53
Melanoma	$ABCB5^+$	1.6–20	10^6	–	Subcutaneous	NOD-SCID	35
Mesenchymal	Side population (Hoechst dye)	0.07–10	100	–	Subcutaneous	NOD-SCID	118

*Also injected with fibroblasts. ALDH, aldehyde dehydrogenase; CSC, cancer stem cell; EpCAM, epithelial cell adhesion molecule; ESA, epithelial specific antigen; GBM, glioblastoma multiforme; MB, medulloblastoma, ND, not determined; NOD-SCID, non-obese diabetic-severe combined immunodeficient; Rag2/$\gamma^{-/-}$DKO, Rag 2 common cytokine receptor γ-chain double knockout.

癌病幹細胞理論

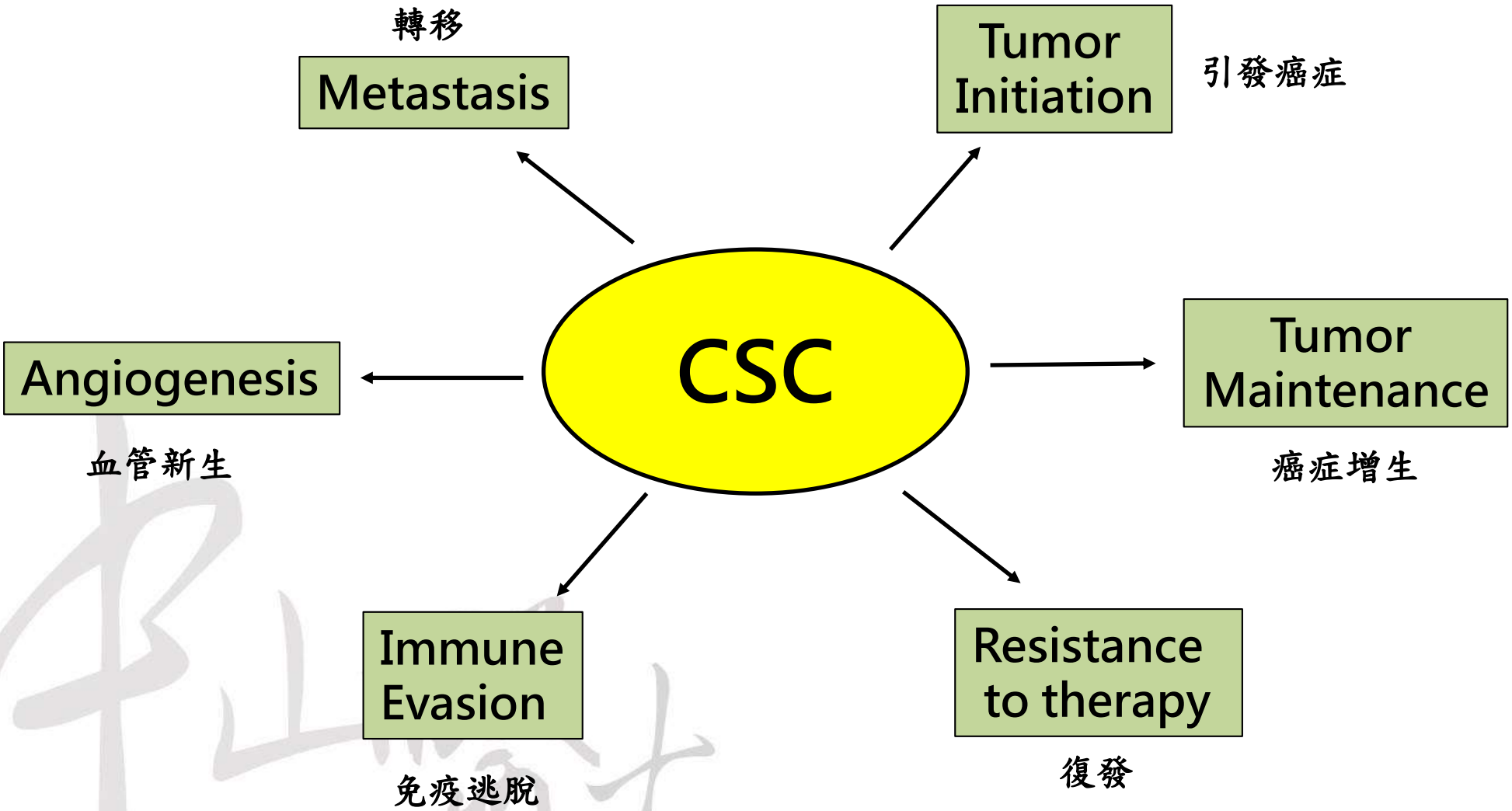


新英格蘭醫學期刊

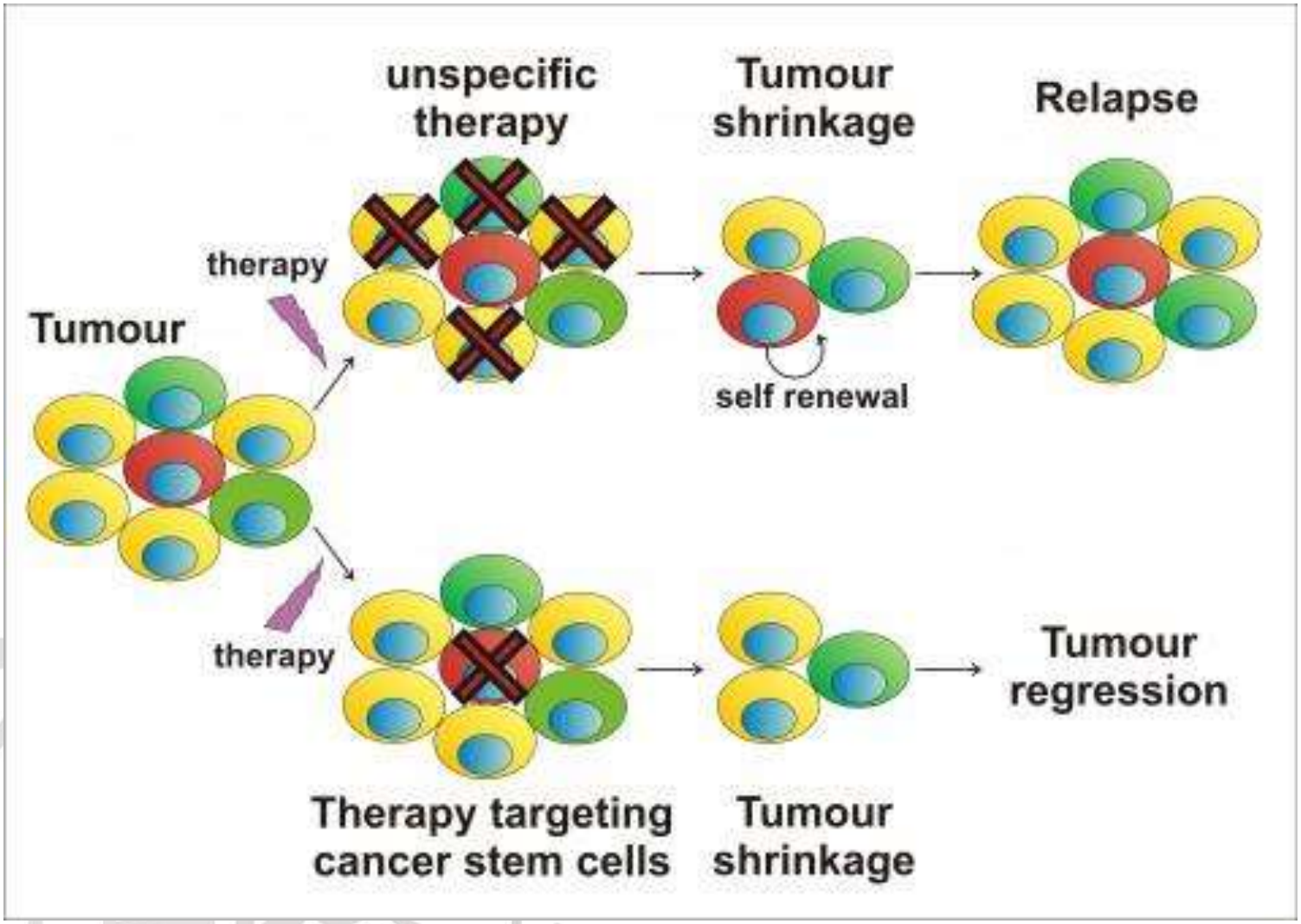
N Engl J Med 2006;355:1253-61.

http://www.vghtpe.gov.tw/cnews_detail.jsp?newsid=2496

癌症幹細胞在癌症中的角色



癌症幹細胞在未來癌症治療藥物發展的重要性



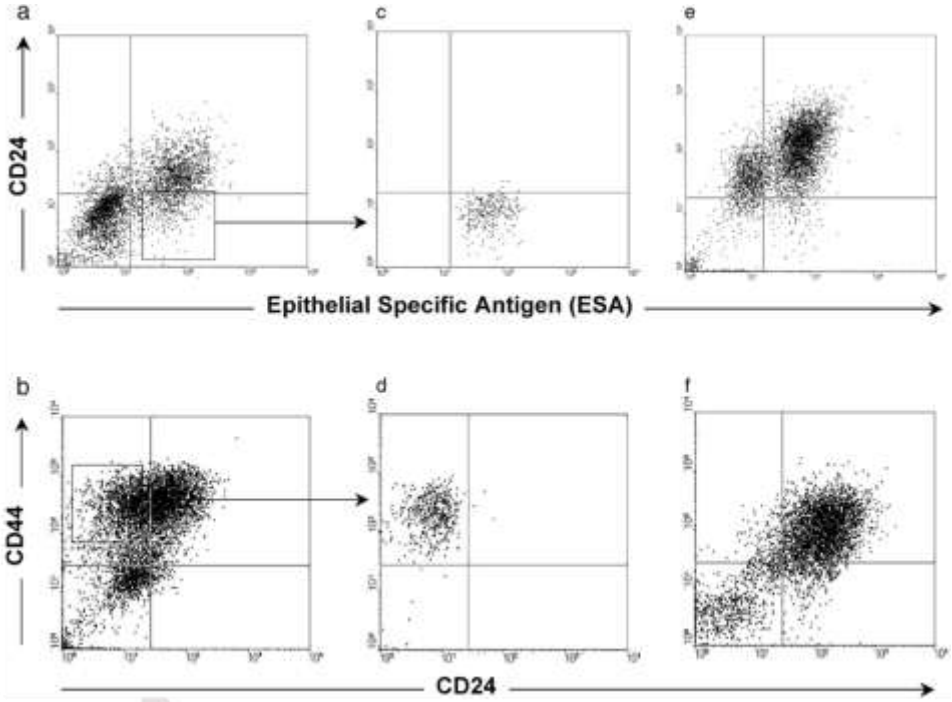
20120424 癌症幹細胞分離技術 成為治癌大突破

https://www.youtube.com/watch?v=lyNVc3Ip4h0

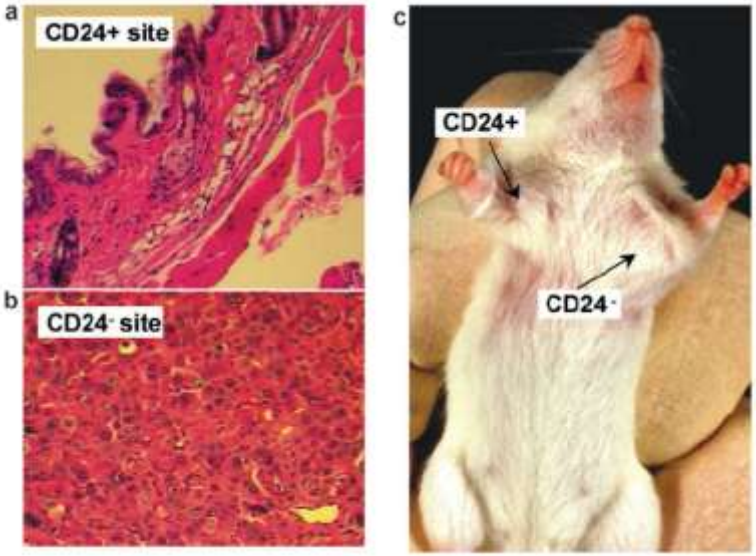
20120424 癌症幹細胞分離技術 成為治癌大突破

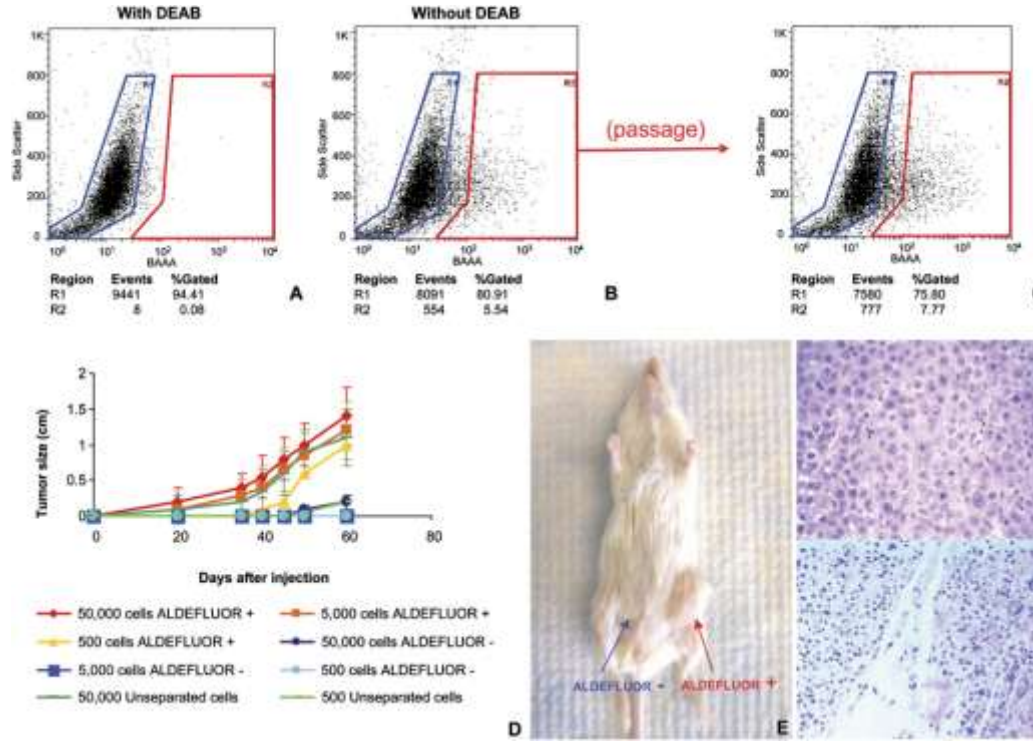
https://www.youtube.com/watch?v=lyNVc3Ip4h0

Breast cancer stem cells



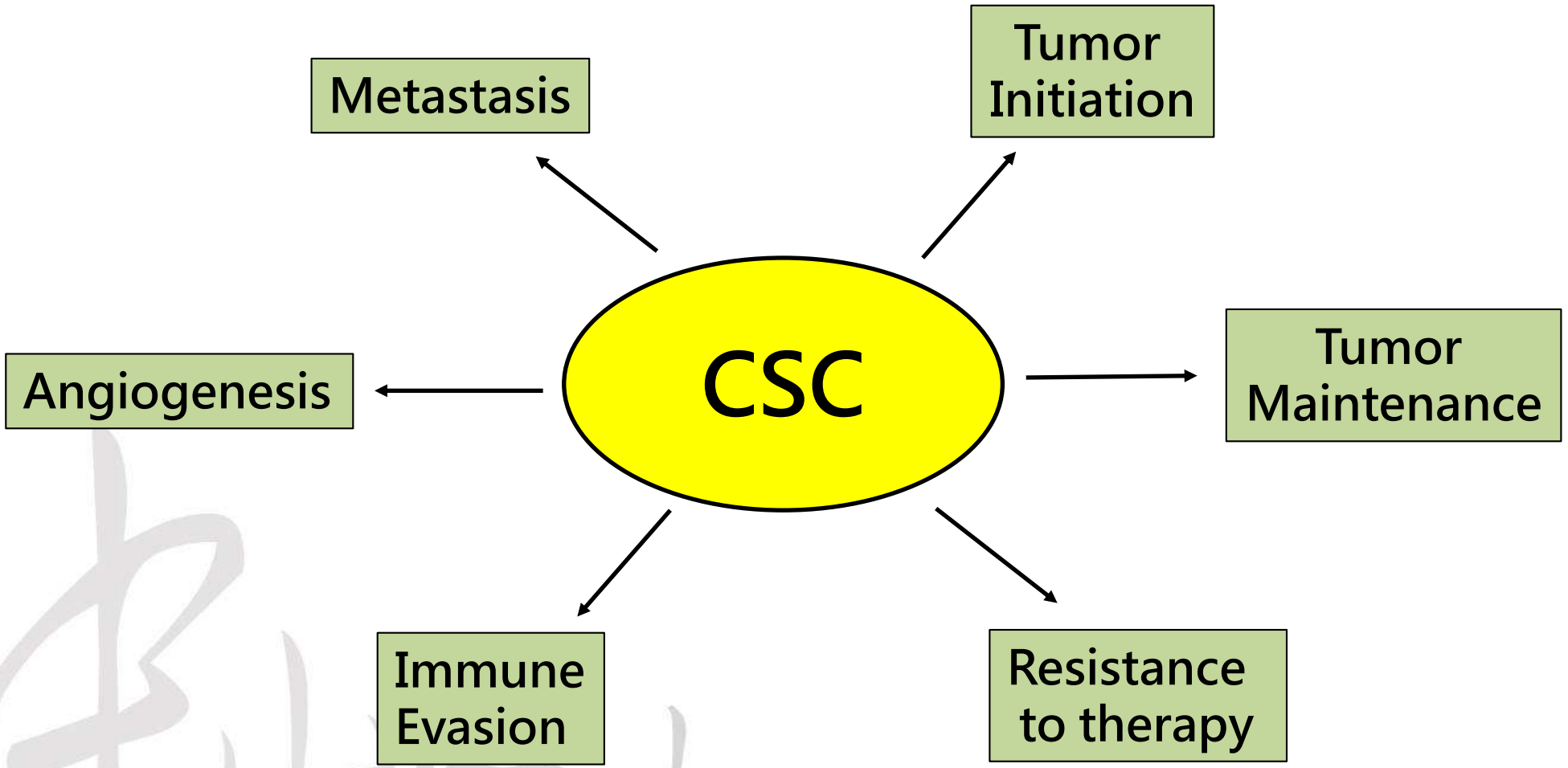
Muhammad Al-Hajj et al. PNAS 2003;100:3983-3988





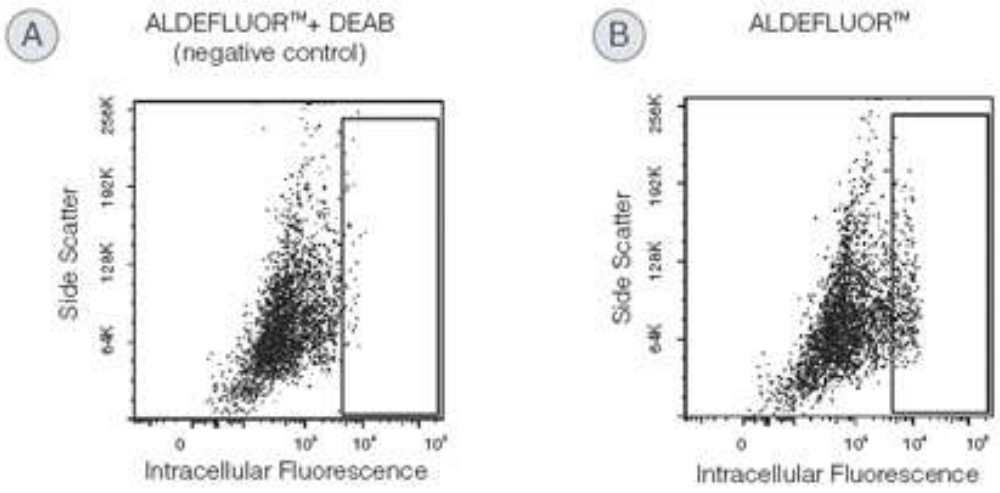
Cell Stem Cell 1, 555–567, November 2007

Impact of CSC in cancer biology

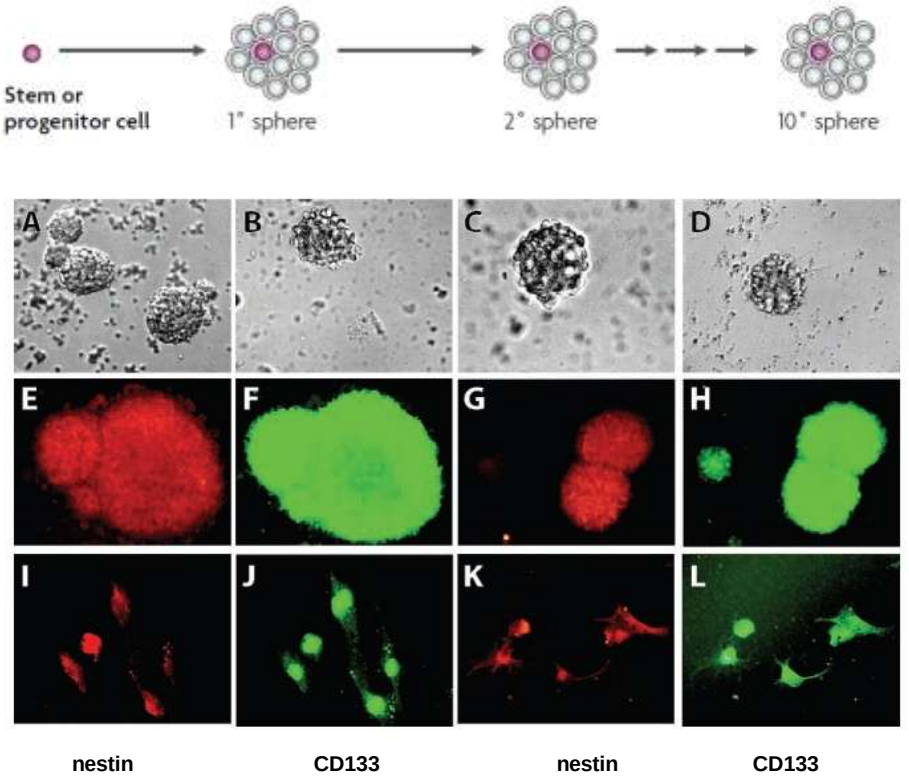


如何分析癌症幹細胞(量或活性)

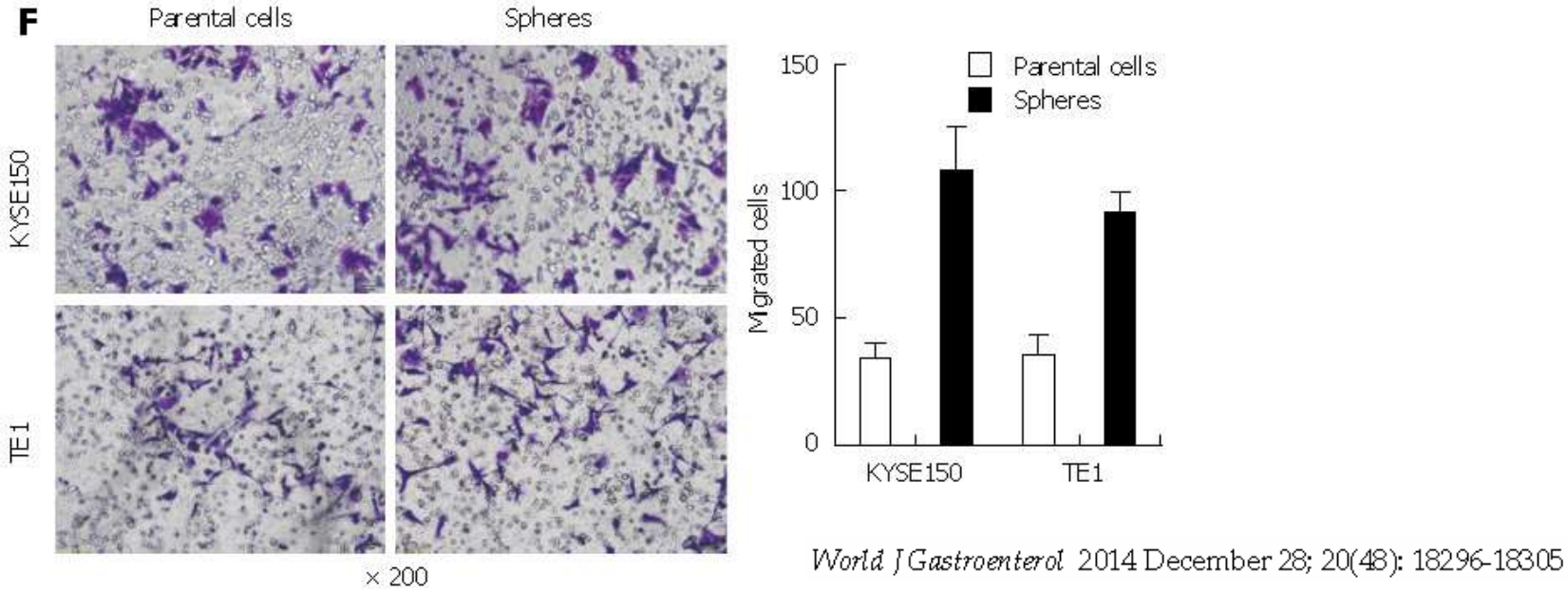
1. 螢光分子標記(ALDEFLUOR assay)



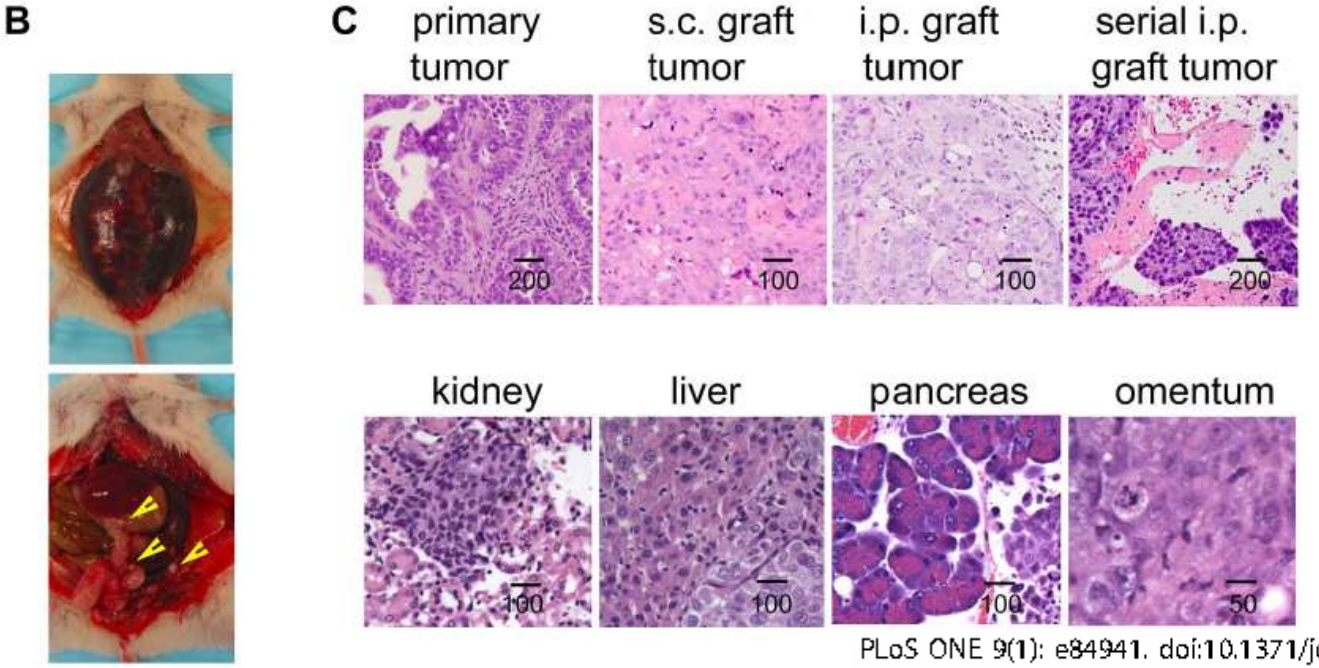
2. 癌症球體 (spheroid)



癌症球體較二維培養之癌細胞具侵襲性(轉移)



World J Gastroenterol 2014 December 28; 20(48): 18296-18305



PLoS ONE 9(1): e84941. doi:10.1371/journal.pone.0084941

癌症球體較二維培養之癌細胞具抗藥性

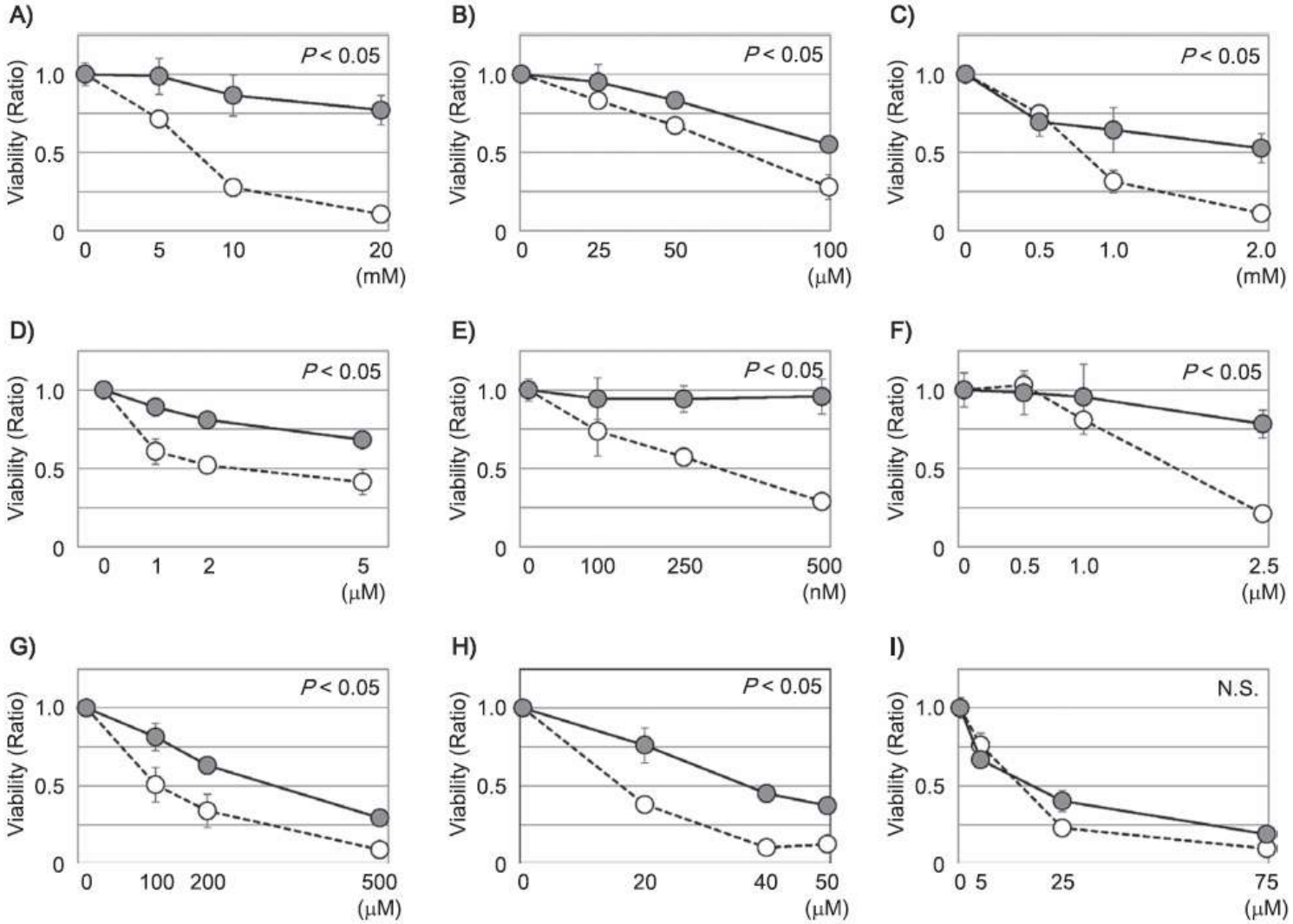
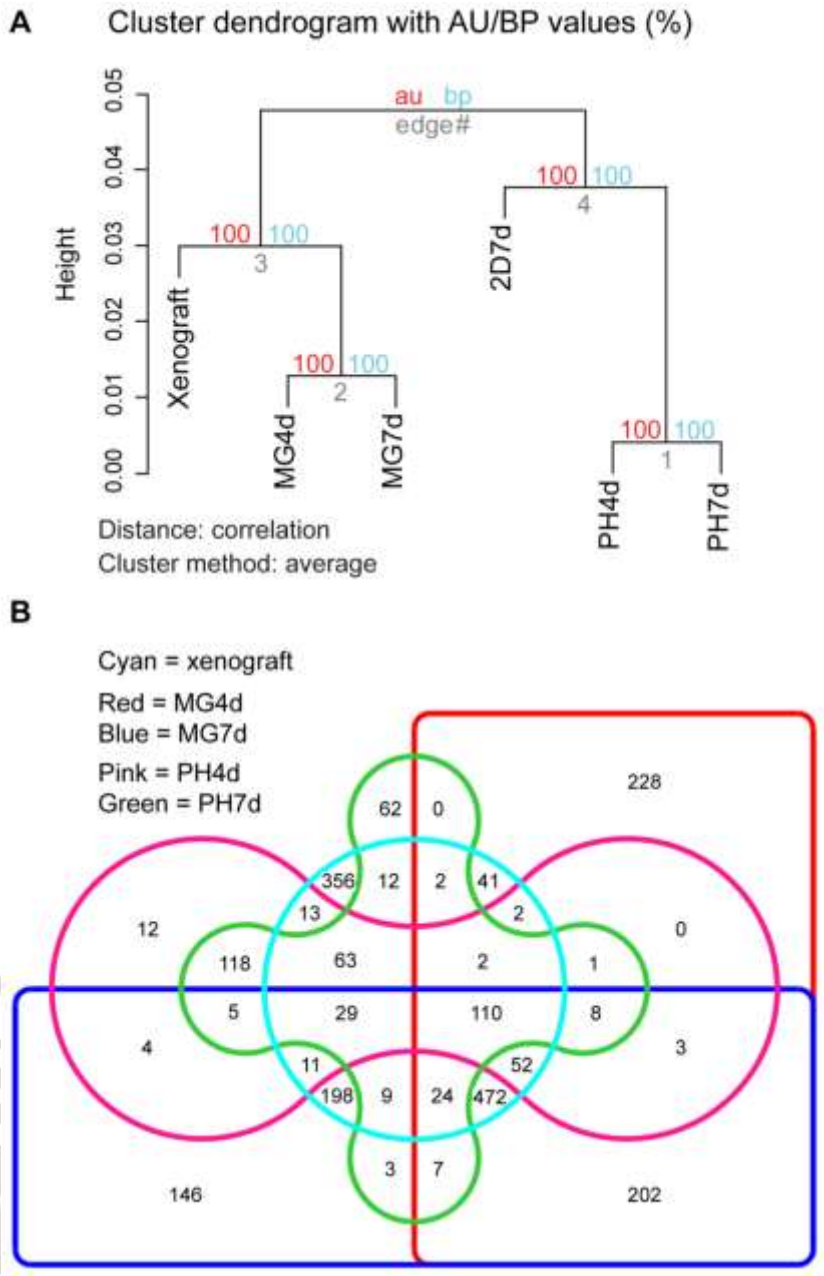


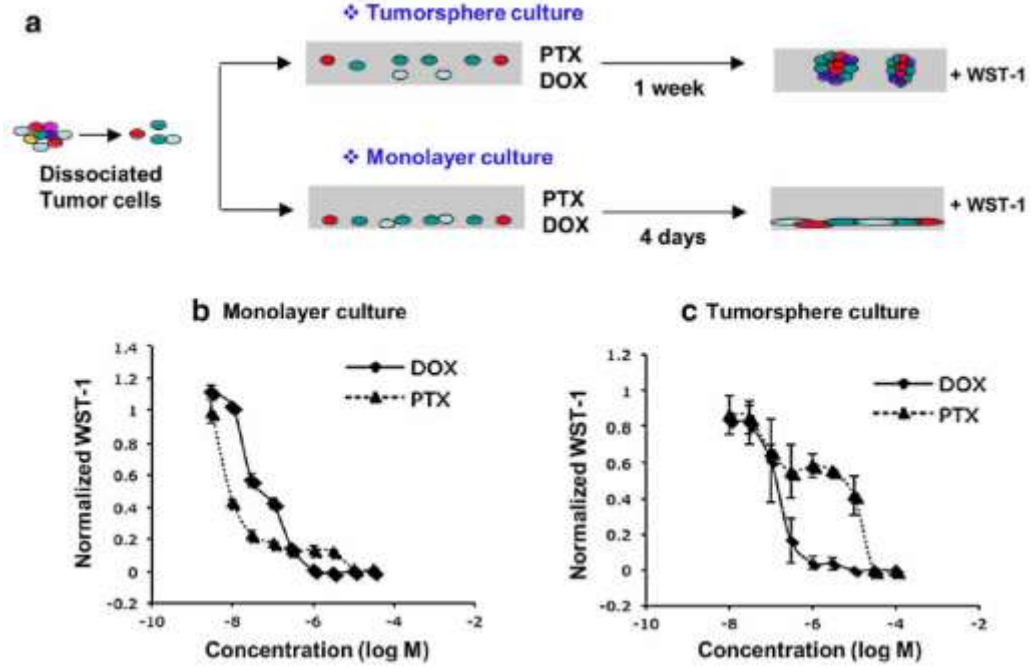
Figure 6 Susceptibility of SK-HEP-1 derivative cells to anti-cancer drugs. Cells were subjected to an MTS assay to evaluate the viability of SK-HEP-1 (open circles) and SK-sphere (closed circles) cells in the presence of anti-cancer drugs (***A***, 5-FU; ***B***, cisplatin; ***C***, carboplatin, ***D***, doxorubicin; ***E***, docetaxel; ***F***, SAHA; ***G***, irinotecan; ***H***, sunitinib; and ***I***, sorafenib). Except for sorafenib, SK-sphere cells showed higher viability in the presence of the tested anti-cancer drugs. *P* values were calculated with repeated-measures ANCOVA.

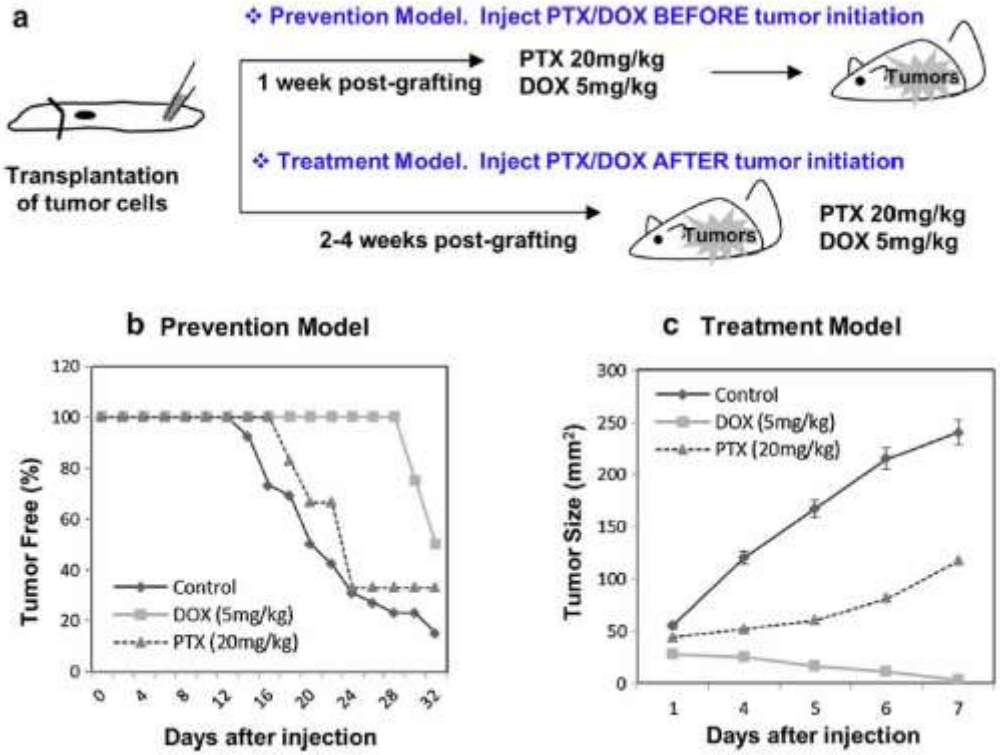
癌症球體內基因表現與異體腫瘤相近



PLoS ONE 8(10): e77232. doi:10.1371/journal.pone.0077232

癌症藥物篩選：癌症球體 V.S. 二維平面培養

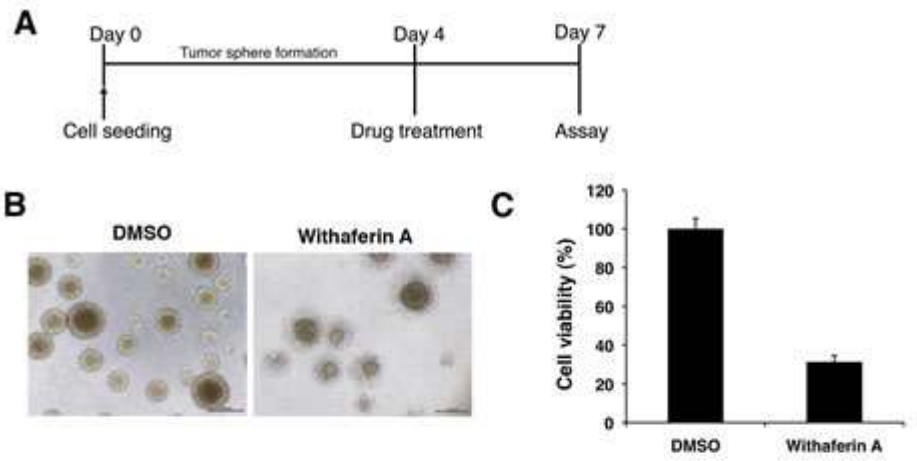


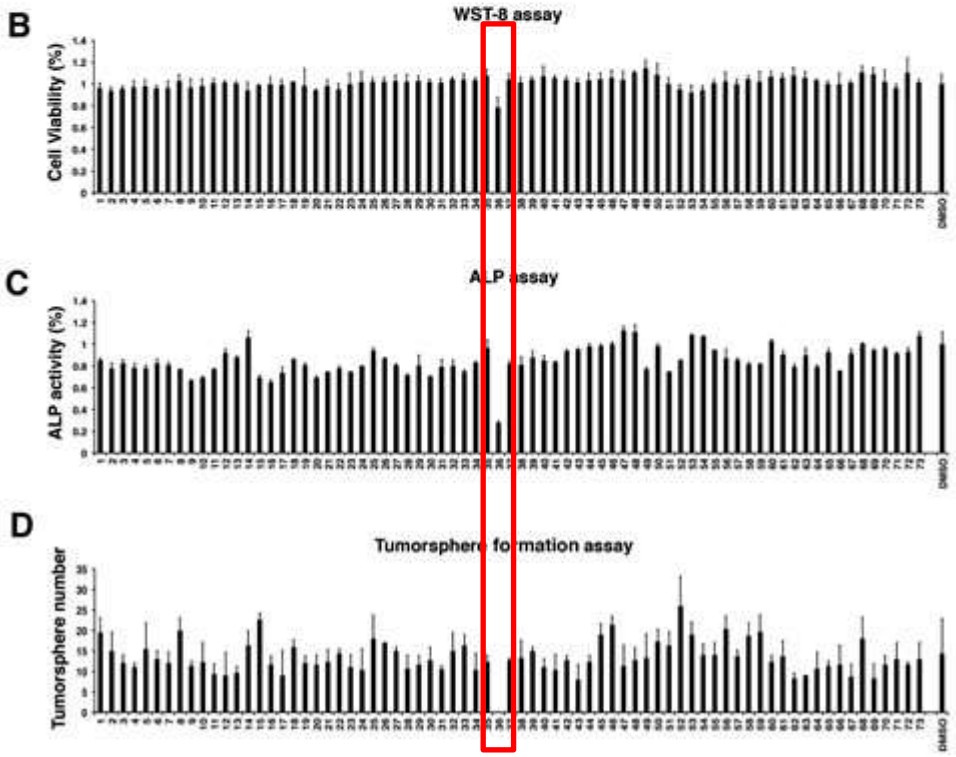


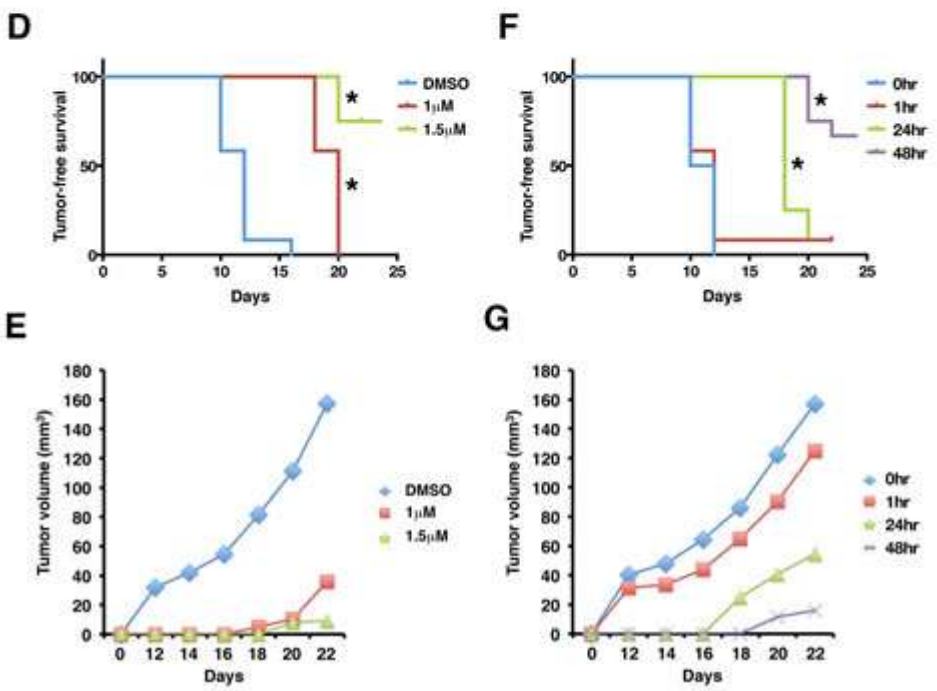
Biotechnol Lett (2014) 36:481–488
DOI 10.1007/s10529-013-1393-1

A

1	Hyperoside	26	Bilobalide	51	Cryptochlorogenic acid
2	Isoquercitrin	27	Ginkgolide A	52	Neochlorogenic acid
3	Tectoridin	28	Ginkgolide B	53	Isochlorogenic acid A
4	Tectorigenin	29	Ginkgolide C	54	Isochlorogenic acid C
5	6-Hydroxygenistein 6,7-diglucoside	30	Licoricesaponin G2	55	Pedalitin 6-glucoside
6	Tectorigenin 7-o-xylosylglucoside	31	Licoricesaponin H2	56	Simmondsin
7	Daidzin	32	Glycyrrhizinic acid	57	Plantaginin
8	Daidzein	33	Soyasaponin I	58	Calicosin 7-glucoside
9	Glycitin	34	Soyasaponin V	59	Hesperidin
10	Glycitein	35	Withanone	60	PlaeruptorinA
11	Genistin	36	Withaferin A	61	Luteolin 7-glucoside
12	Genistein	37	Pteropodine	62	Quercetin 3-arabinoside
13	Neohesperidin	38	Isopteropodine	63	Rhein
14	Luteolin	39	Mitraphylline	64	Swertiamarin
15	(+)-Catechin	40	Speciophylline	65	Deacyl gimnemic acid
16	(-)-Epicatechin	41	Uncarine F	66	Isorhamnetin-3-O-glucoside
17	(-)-Epigallocatechin	42	Rhynchophylline	67	Isorhamnetin-3-O-rutinoside
18	Quercetin 4'-glucoside	43	Geniposide	68	Quercetin
19	Quercetin 3,4'-diglucoside	44	Arctiin	69	ε-Viniferin
20	rutin dihydrate	45	Chlorogenic acid	70	trans-Resveratrol
21	3,5,7,3',4'-Pentamethoxyflavone	46	Caffeic acid	71	Theogallin
22	5,7,4'-Trimetoxyflavone	47	6-Gingerol	72	Glabridin
23	5,7-Dimethoxyflavone	48	8-Gingerol	73	Riquiritigenin
24	3,5,7-Trimetoxyflavone	49	10-Gingerol		
25	3,5,7,4'-tetramethoxyflavone	50	6-Shogaol		



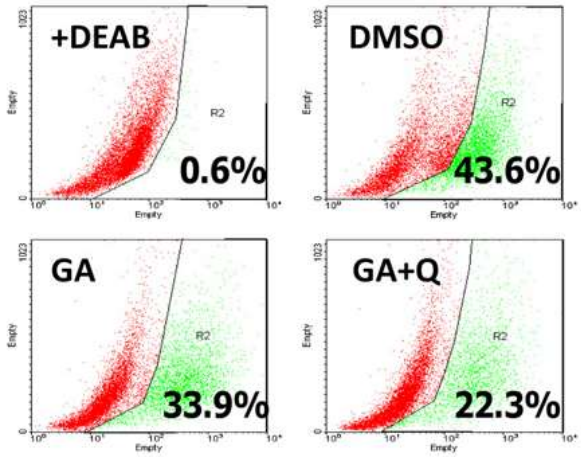


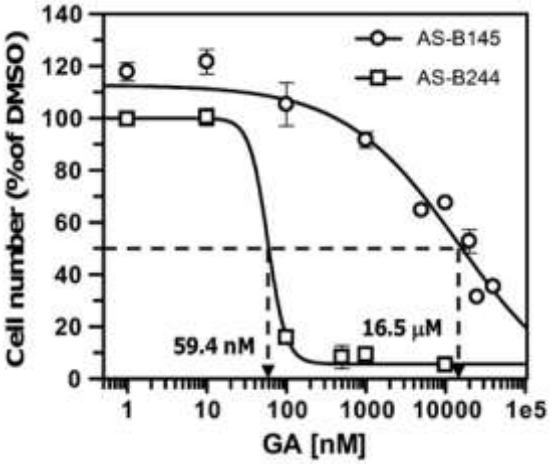


DOI: 10.18632/oncotarget.2356

癌症幹細胞之藥物篩選

候選物對癌細胞株之細胞毒性試驗
（口腔癌、乳癌）



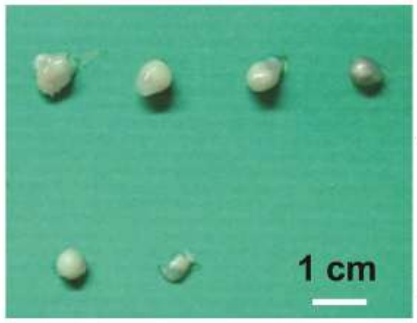


選擇無明顯細胞毒性之濃度
（低於可抑制50%細胞增生之濃度）

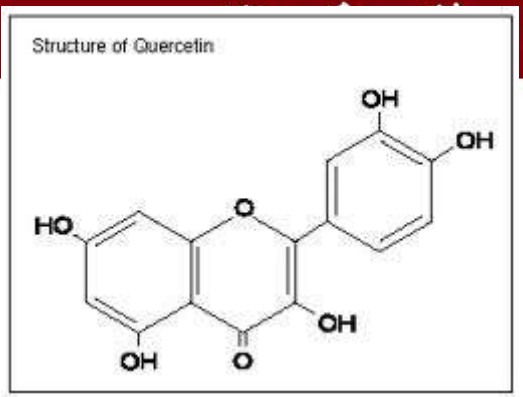
螢光分子標記分析
（流式細胞儀）

癌症球體計數
（懸浮性培養）

活體抗癌生長試驗
（小鼠異體腫瘤模式）



槲皮素 (Quercetin)之抗癌症幹細胞效果



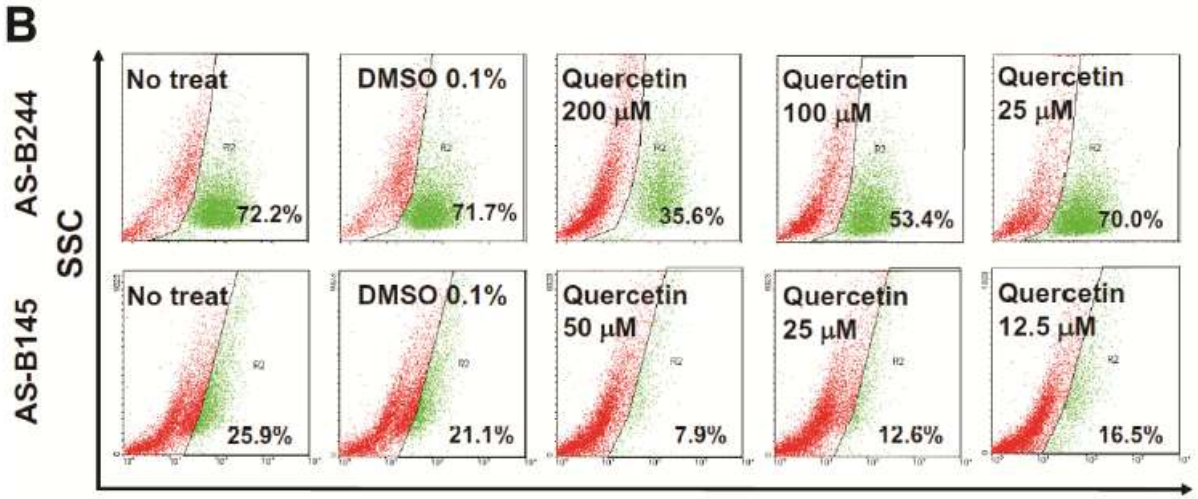
Wei et al. Breast Cancer Research 2011, 13:R101
http://breast-cancer-research.com/content/13/5/R101

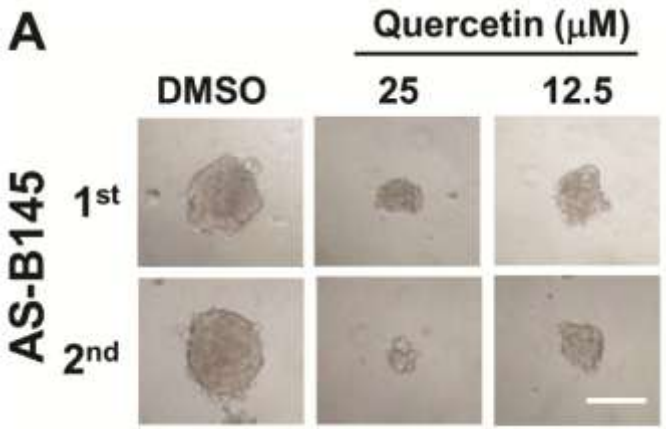
Breast Cancer RESEARCH

RESEARCH ARTICLE Open Access

Hsp27 participates in the maintenance of breast cancer stem cells through regulation of epithelial-mesenchymal transition and nuclear factor-κB

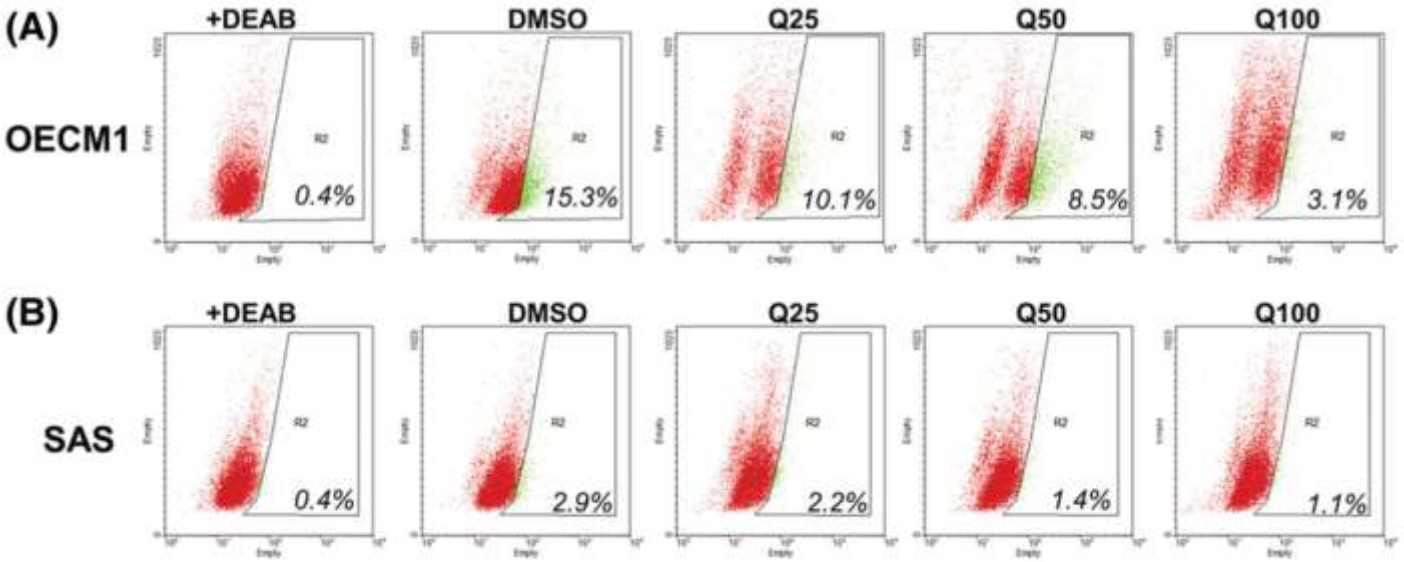
Li Wei[1,2], Tsung-Ta Liu[3], Hsiu-Huan Wang[4], Hui-Mei Hong[4,5], Alice L Yu[6], Hsiang-Pu Feng[4] and Wen-Wei Chang[4,5*]

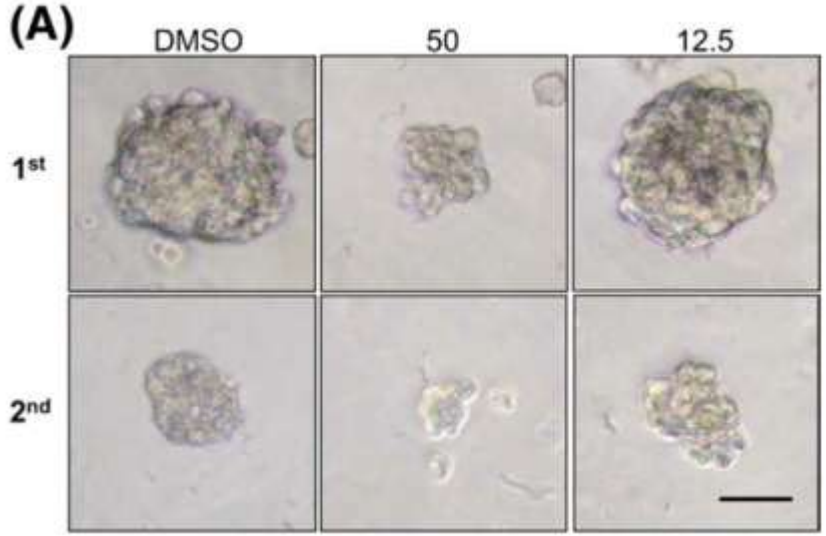




Quercetin in elimination of tumor initiating stem-like and mesenchymal transformation property in head and neck cancer

Wen–Wei Chang, PhD,[1,2] Fang–Wei Hu, DDS, PhD,[3,4†] Cheng–Chia Yu, PhD,[3,4,5*] Hsiu–Huan Wang, BS,[1] Hsiang–Pu Feng, BS,[1] Chih Lan, BS,[1] Lo–Lin Tsai, DDS, PhD,[3,4] Yu–Chao Chang, DDS, PhD[3,4*]





牛樟芝三萜類萃取物 Methyl Antcinate A 之抗乳癌幹細胞效果

Molecules **2013**, *18*, 2539-2548; doi:10.3390/molecules18032539

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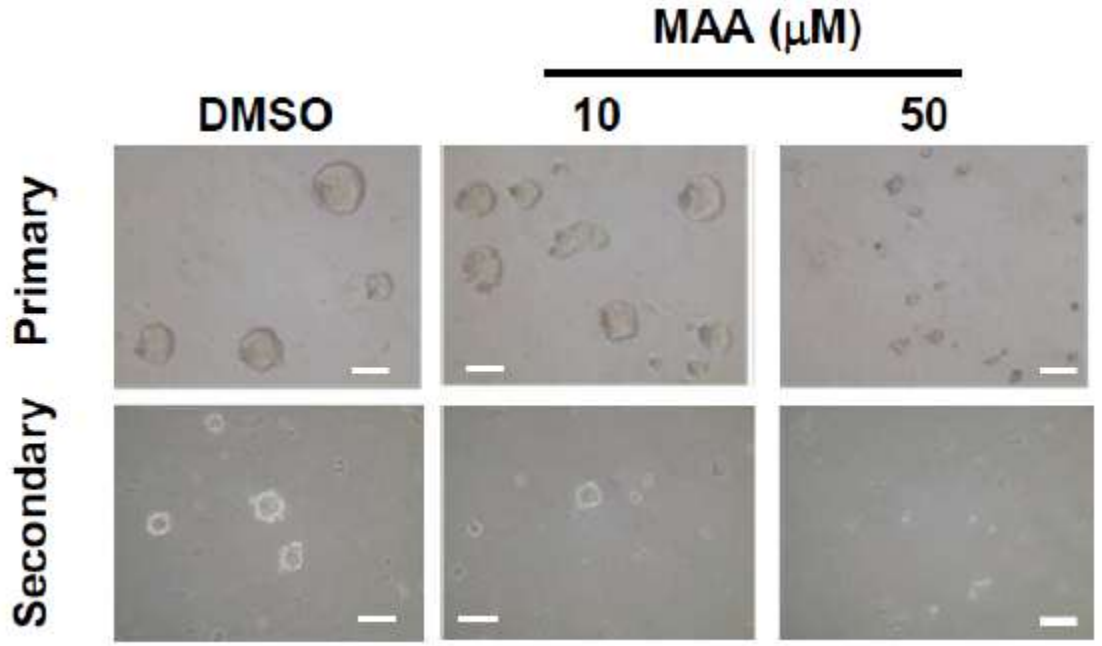
ISSN 1420-3049

www.mdpi.com/journal/molecules

Article

Methyl Antcinate A Suppresses the Population of Cancer Stem-Like Cells in MCF7 Human Breast Cancer Cell Line

Chih-Yu Peng [1,2], Pin-Chung Fong [3], Cheng-Chia Yu [1,2,4], Wan-Chi Tsai [5,6], Yew-Min Tzeng [7] and Wen-Wei Chang [3,8,*]



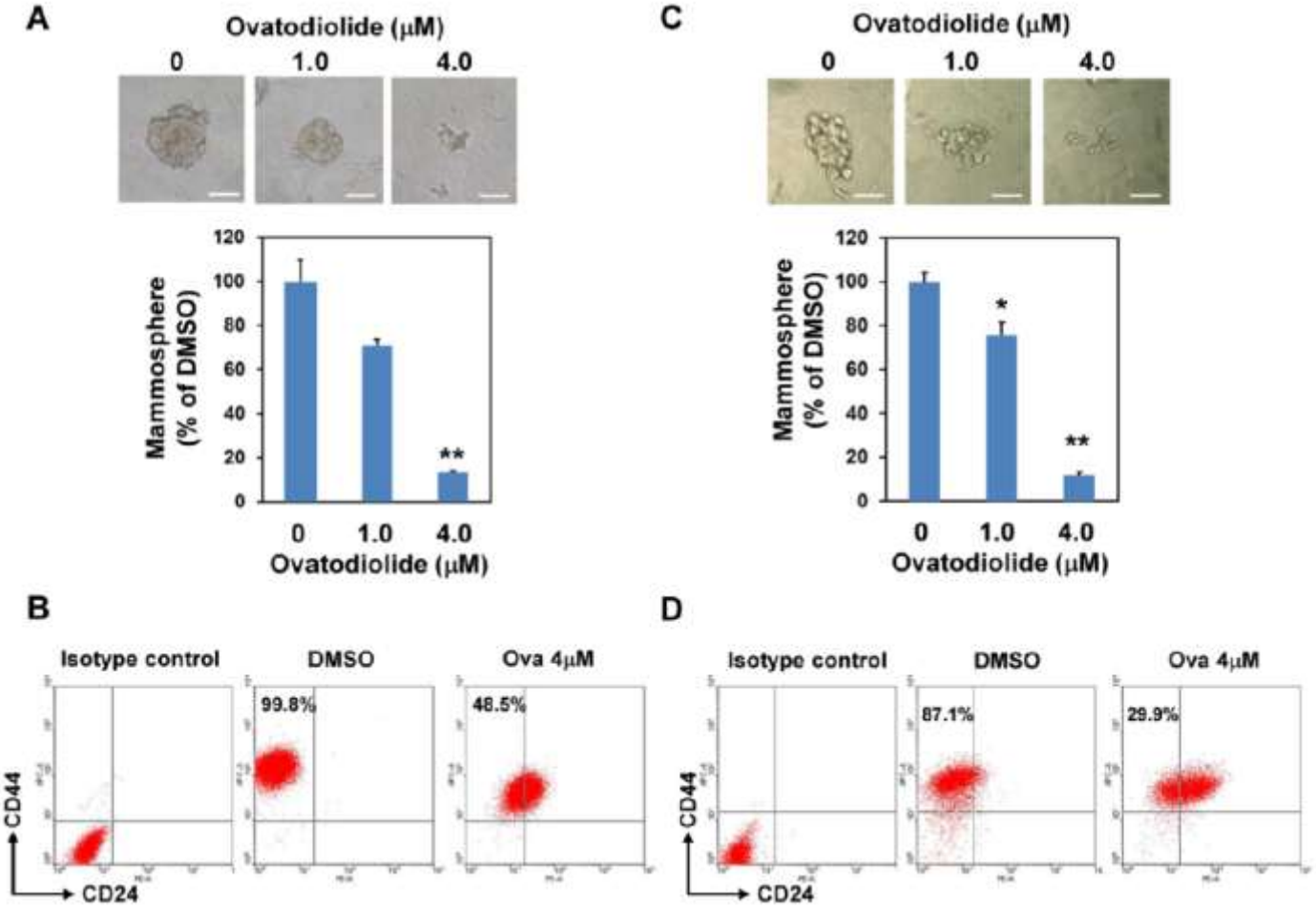
魚針草內酯Ovatodiolide 之抗乳癌幹細胞效果

toxins

MDPI

Article

Ovatodiolide Inhibits Breast Cancer Stem/Progenitor Cells through SMURF2-Mediated Downregulation of Hsp27



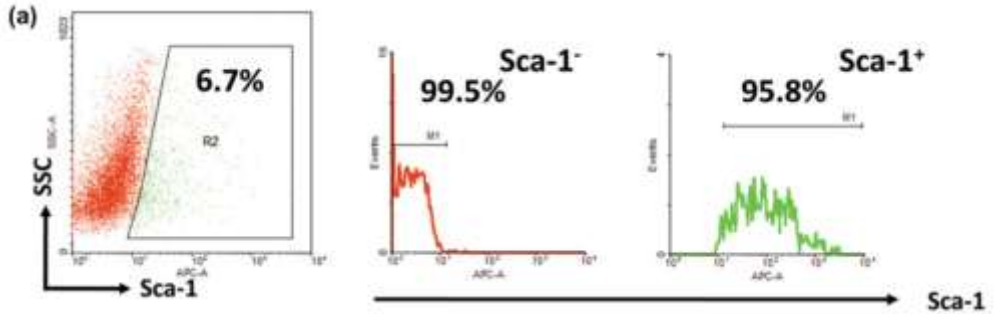
Tracking of mouse breast cancer stem-like cells with *Salmonella*

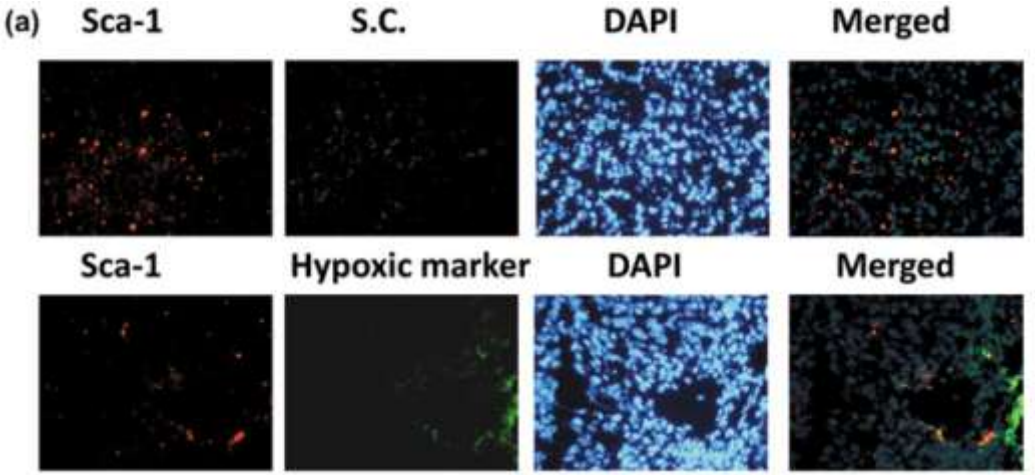
誠 ◦ 愛 ◦ 精 EBM

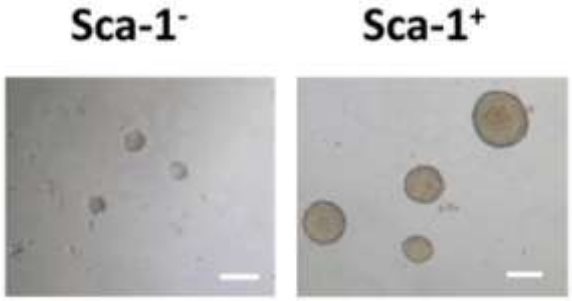
Wen-Wei Chang[1,2], Yu-Diao Kuan[3], Man-Chin Chen[4], Song-Tao Lin[4] and Che-Hsin Lee[3,4]

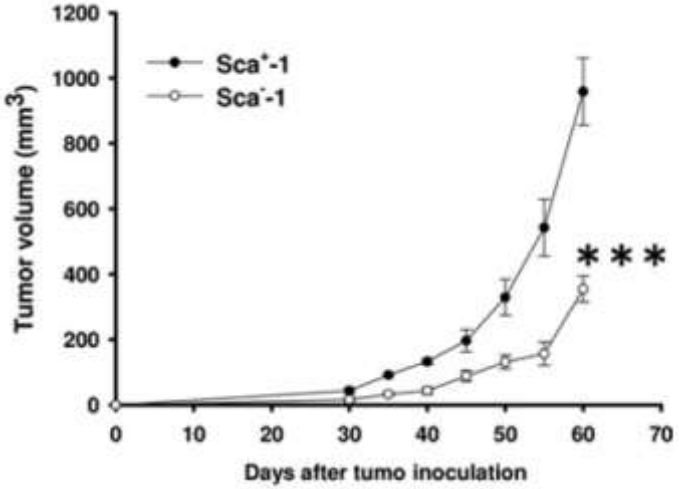
[1]Department of Biomedical Sciences, College of Medical Science and Technology; [2]Department of Medical Research, Chung Shan Medical University Hospital, Taichung 40401; [3]Department of Microbiology; [4]Graduate Institute of Basic Medical Science, School of Medicine, China Medical University, 91 Hsueh-Shih Road, Taichung 40402, Taiwan

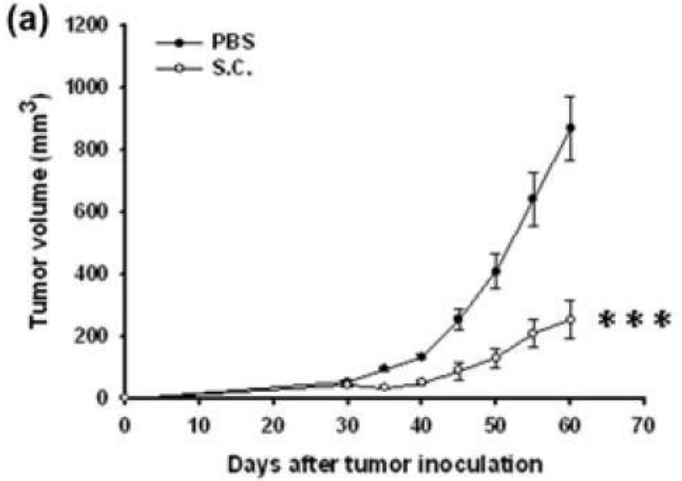
Corresponding author: Dr Che-Hsin Lee. Email: chlee@mail.cmu.edu.tw

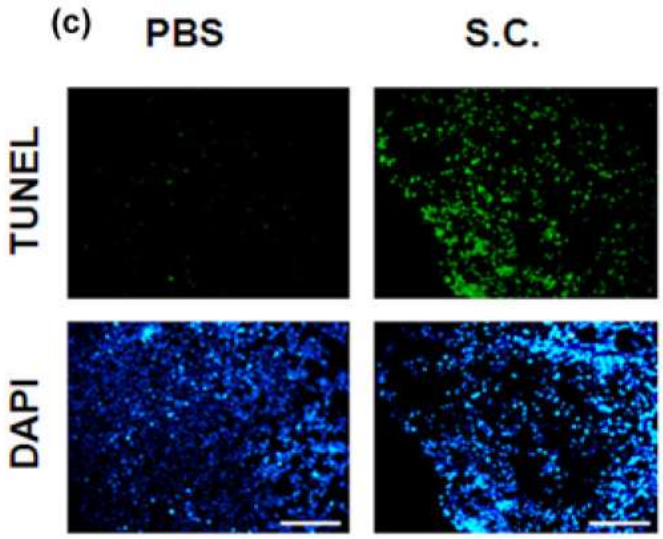




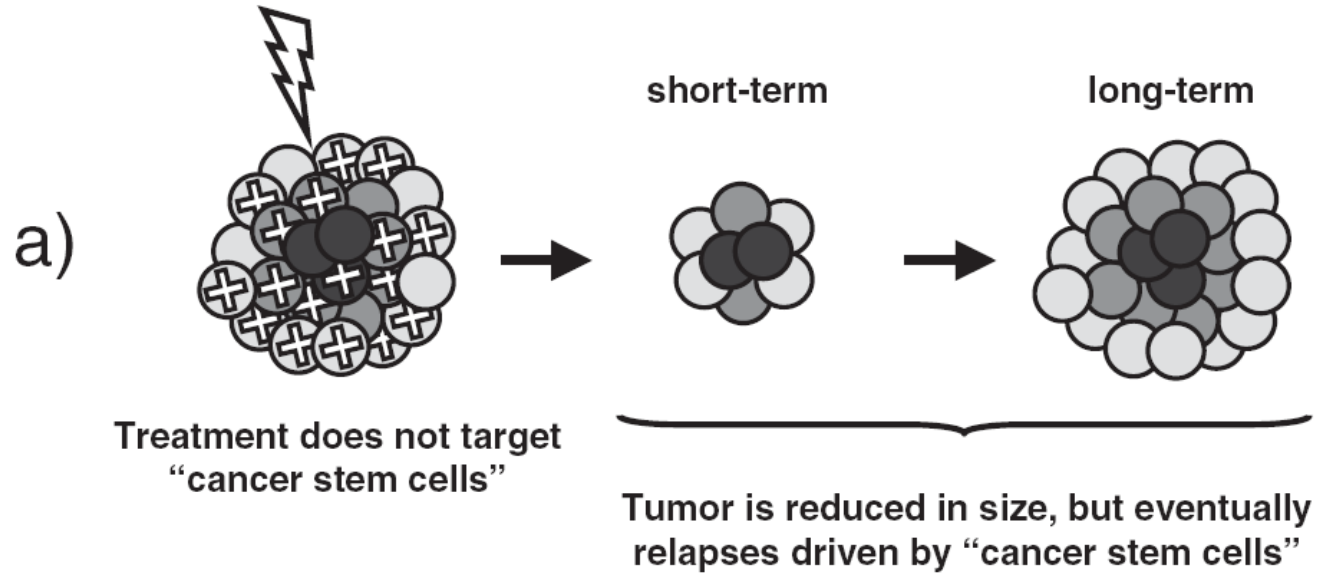




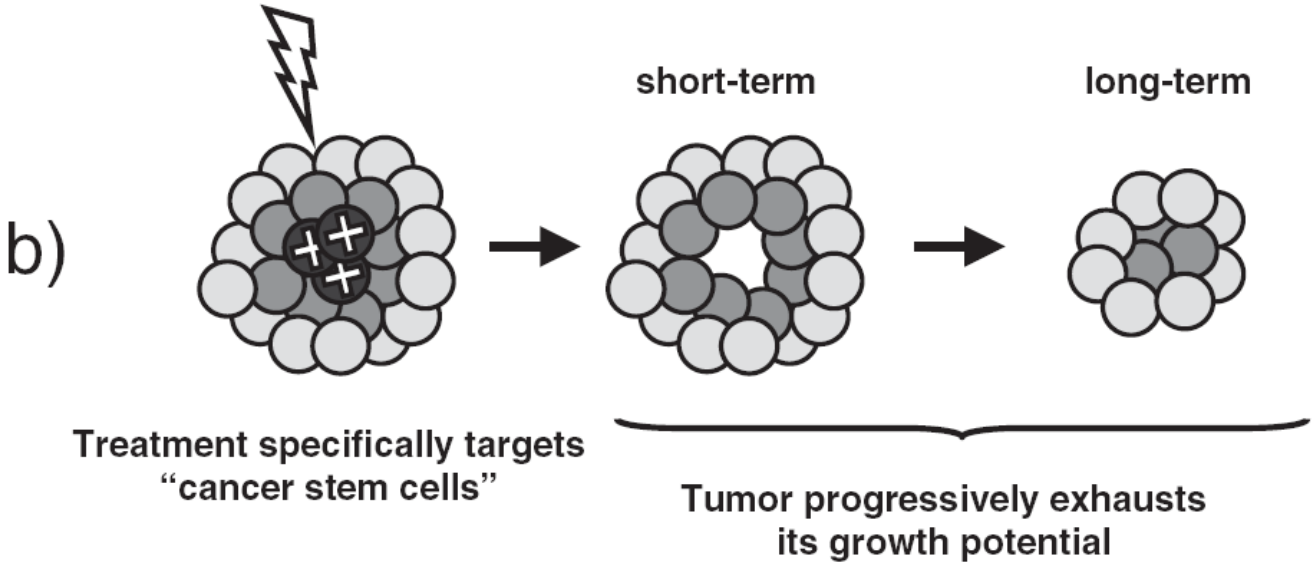




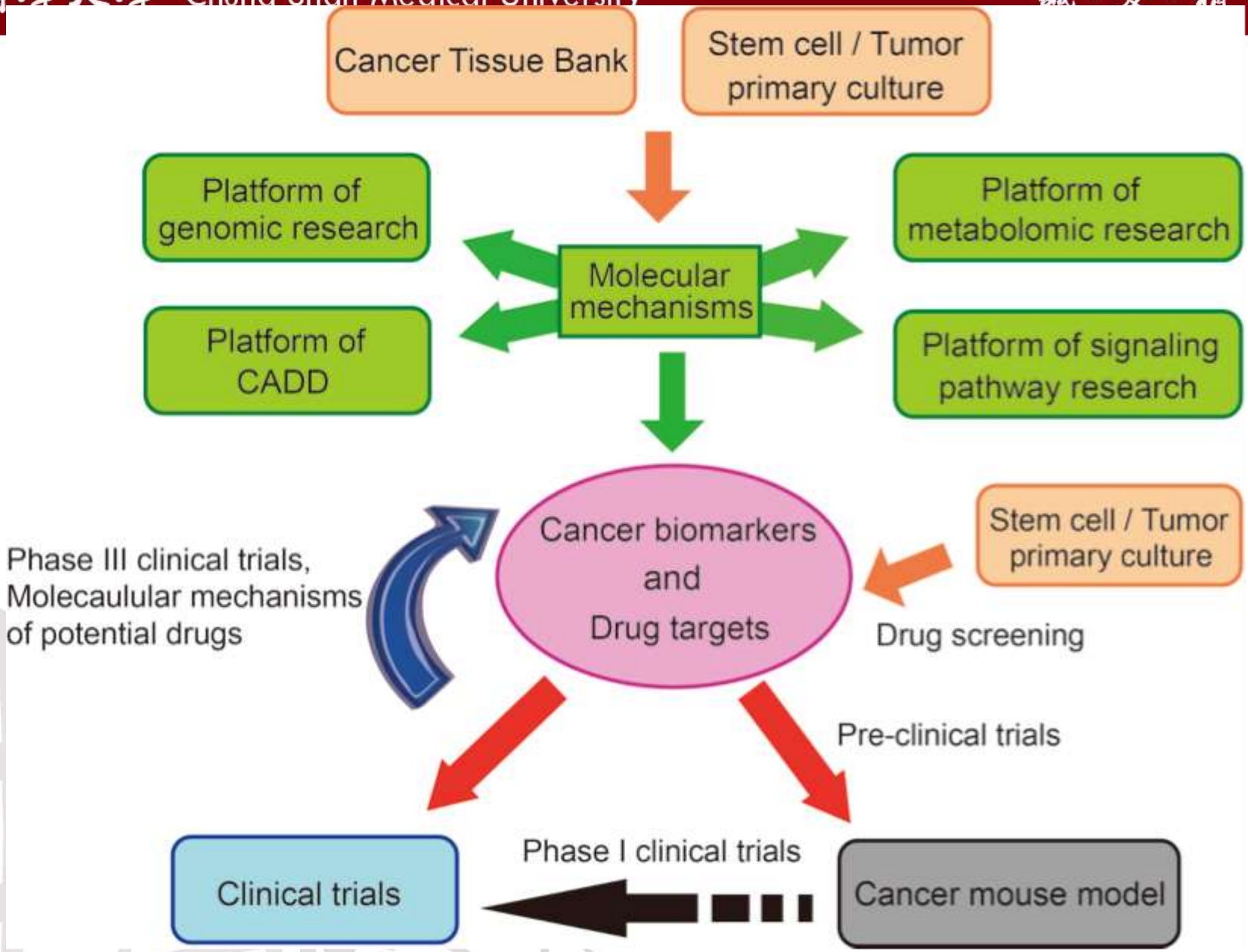
Therapeutic potential of targeting CSCs



斬草不除根
春風吹又生

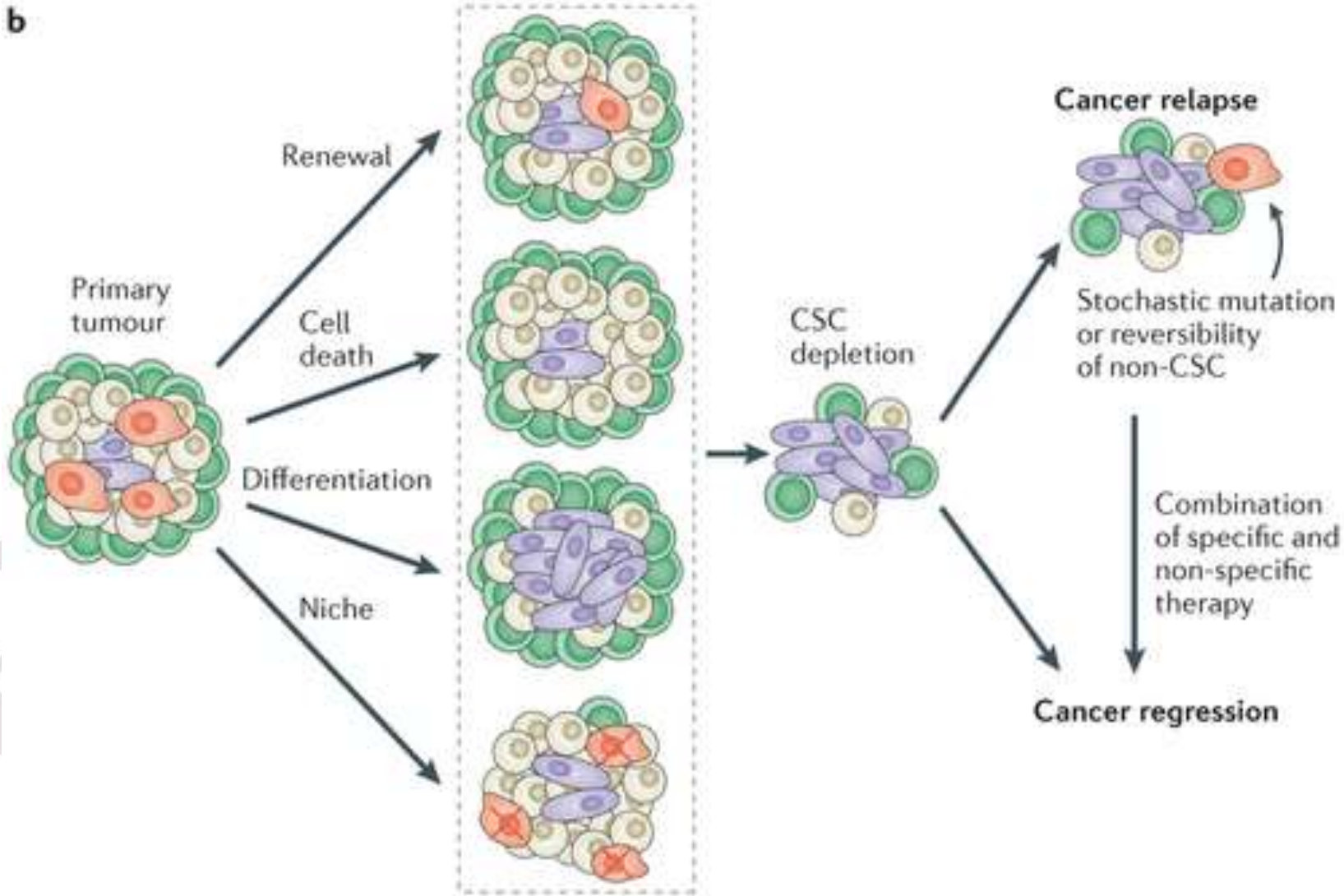


擒賊先擒王



http://irice-ca.mc.ntu.edu.tw/_eng/research.php

Combination therapy may be the best choice in next generation cancer therapy



癌症球體分析用於癌症藥物臨床試驗

Table 2: Application of tumorsphere assay in clinical trials[1]

Tumor type	Status	Main application	ClinicalTrials.gov identifier
Recurrent Glioblastoma	No specified	Tertiary outcome measures	NCT02133183
Lung cancer Colorectal Cancer Breast Cancer	Phase II	Generation of orthotopic xenograft models that recapitulate the parental tumor behavior	NCT01483001
Prostate Carcinoma	No specified	Primary outcome measures	NCT02425800
Recurrent High-Grade Glioma	No specified	Secondary outcome measures	NCT02101905
Esophageal Squamous Cell Carcinoma	Phase II	Efficacy examination in pre-clinical evaluation	NCT02423811

[1] The information of listed clinical trials were obtained from the website of ClinicalTirals.gov.

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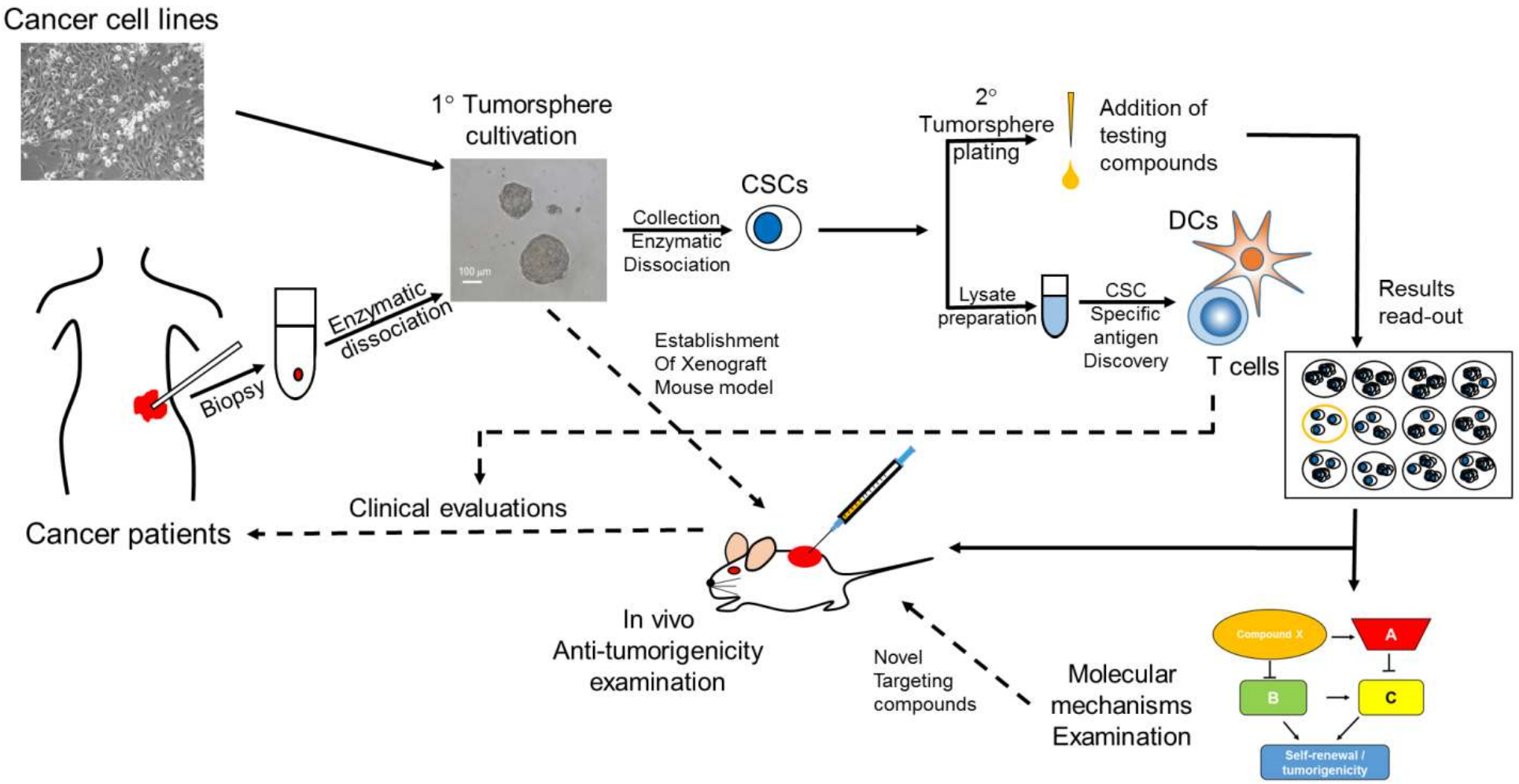
Tumorsphere as an effective *in vitro* platform for screening anti-cancer stem cell drugs

Che-Hsin Lee[1,2], Cheng-Chia Yu[3,4,5], Bing-Yen Wang[6,7,8] and Wen-Wei Chang[9,10]



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