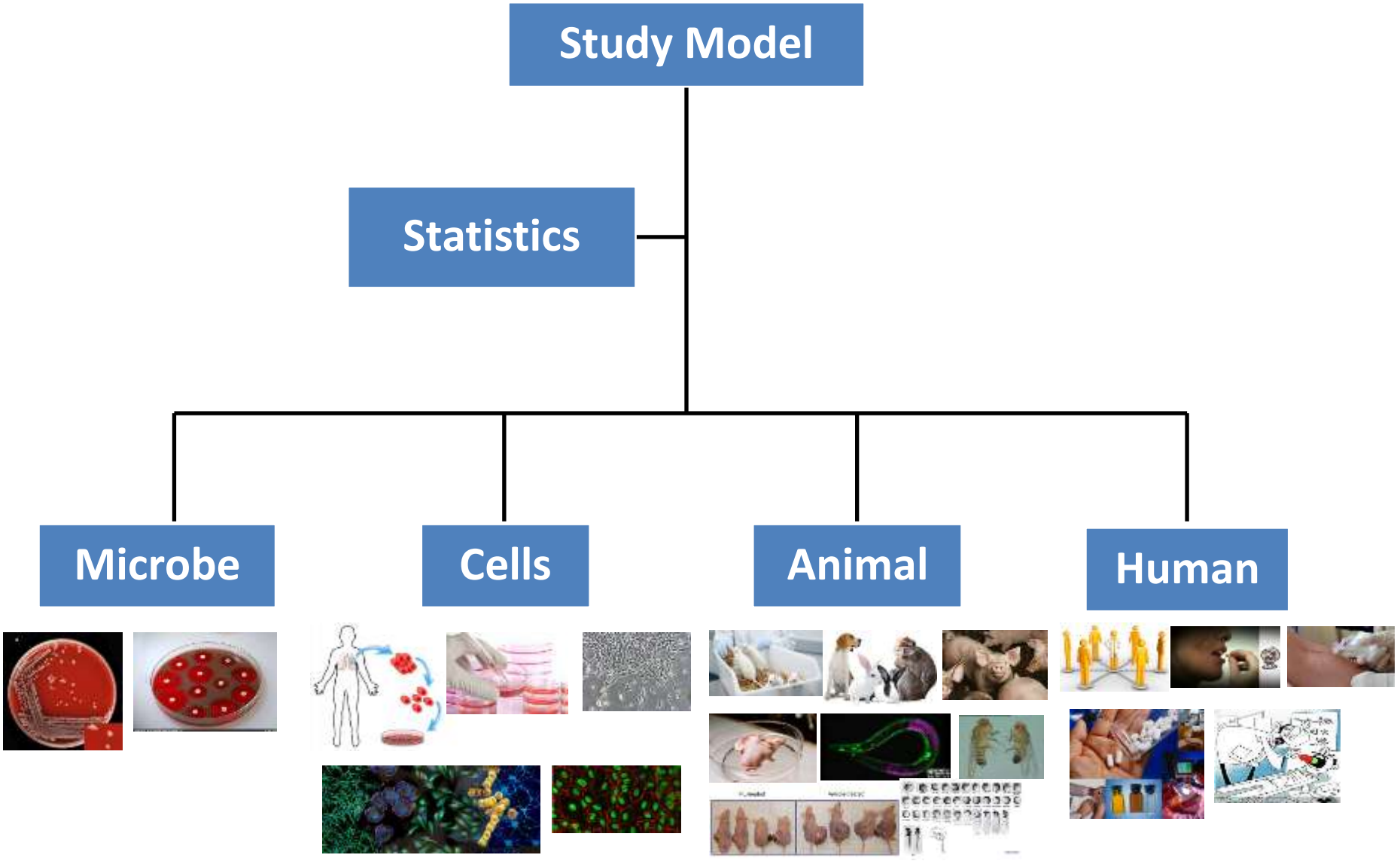
Cell Culture and the Application in the Biomedical Science

轉譯醫學研究中心
李英瑞

2016.06.23

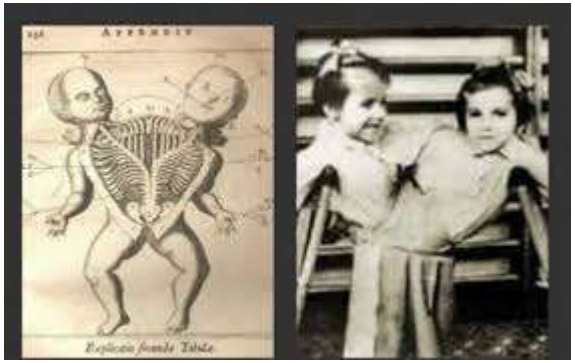
Biomedical Research needs a Suitable Model




funpeer.com


娱乐奇闻网 tule24.com


该图片由 一拂晨风 上传到 club.china.com

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為何生物醫學研究常利用細胞株進行

優勢：

1. 取得容易
2. 操作容易
3. 價格便宜
4. 可選擇性高
5. 來自於人類或其他動物，具同源代表性
6. 不占空間
7. 迴避人道議題

劣勢：

1. 體內外試驗之差異性無法完全排除
2. 細胞長期培養篩選問題無法排除
3. 保存不易

Why is cell culture used for?

Areas where cell culture technology is currently playing a major role.

- Model systems for

  Studying basic cell biology, interactions between disease causing agents and cells, effects of drugs on cells, process and triggering of aging & nutritional studies
- Toxicity testing

  Study the effects of new drugs
- Cancer research

  Study the function of various chemicals, virus & radiation to convert normal cultured cells to cancerous cells

Why is cell culture used for?

- Virology

  Cultivation of virus for vaccine production, also used to study there infectious cycle.

- Genetic Engineering

  Production of commercial proteins, large scale production of viruses for use in vaccine production e.g. polio, rabies, chicken pox, hepatitis B & measles

- Gene therapy

  Cells having a functional gene can be replaced to cells which are having non-functional gene

Basic equipments used in cell culture

- Laminar cabinet are preferable
- Incubation facilities- Temperature of 25-30℃ for insect & 37℃ for mammalian cells, CO_2 2-5% & 95% air at 99% relative humidity. To prevent cell death incubators set to cut out at approx. 38.5℃
- Refrigerators- Liquid media kept at 4℃, enzymes (e.g. trypsin) & media components (e.g. glutamine & serum) at -20℃
- Microscope- An inverted microscope with 10x to 100x magnification
- Tissue culture ware- Culture plastic ware treated by polystyrene





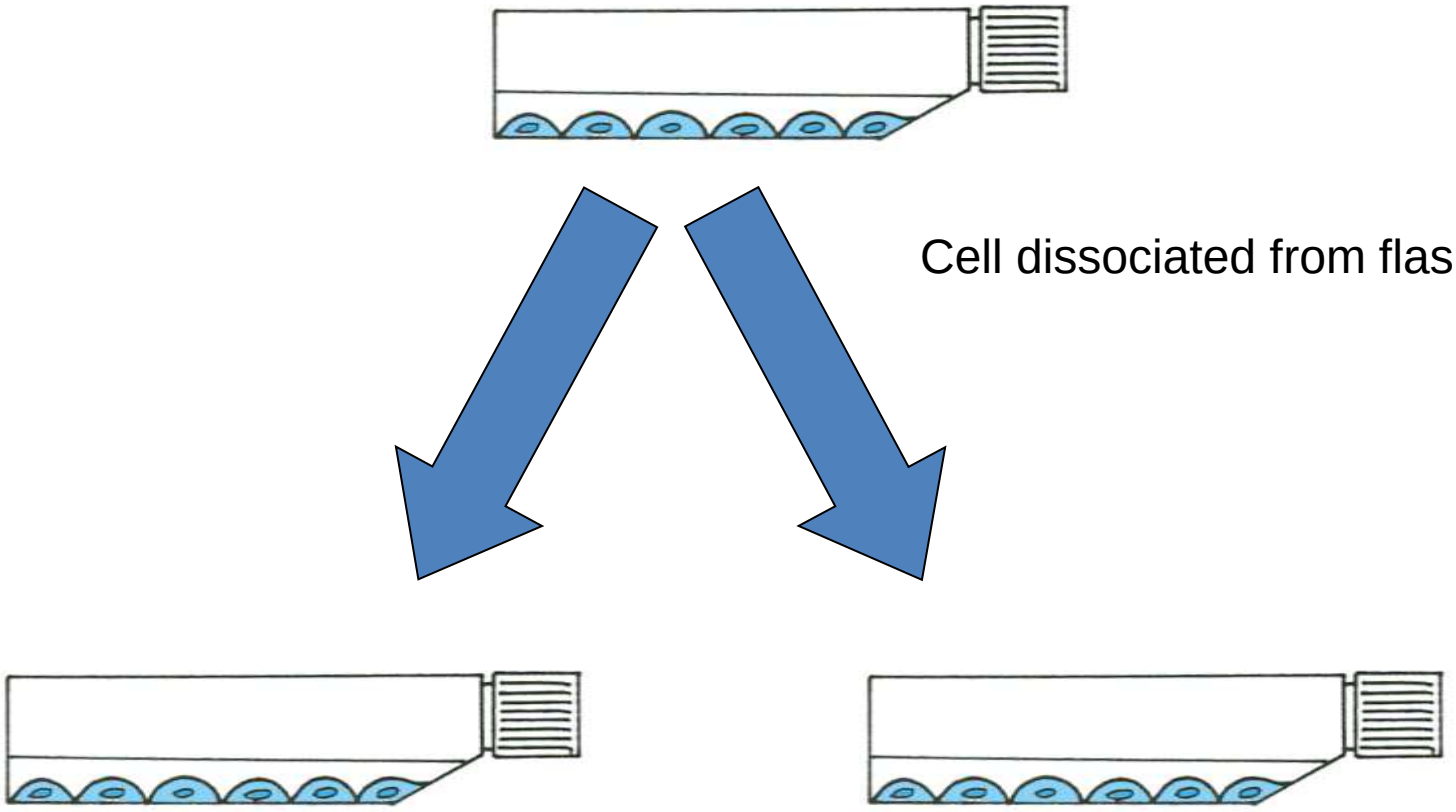
Culture media

- Choice of media depends on the type of cell being cultured
- Commonly used Medium are GMEM, EMEM,DMEM etc.
- Media is supplemented with antibiotics viz. penicillin, streptomycin etc.
- Prepared media is filtered and incubated at 4℃

DMEM

COMPONENTS	Molecular Weight	Concentration (mg/L)	Molarity (mM)
Amino Acids			
Glycine	75	30	0.400
L-Alanyl-Glutamine	217	862	3.97
L-Arginine hydrochloride	211	84	0.398
L-Cystine	313	48	0.153
L-Histidine hydrochloride-H2O	210	42	0.200
L-Isoleucine	131	105	0.802
L-Leucine	131	105	0.802
L-Lysine hydrochloride	183	146	0.798
L-Methionine	149	30	0.201
L-Phenylalanine	165	66	0.400
L-Serine	105	42	0.400
L-Threonine	119	95	0.798
L-Tryptophan	204	16	0.0784
L-Tyrosine disodium salt dihydrate	261	104	0.398
L-Valine	117	94	0.803
Vitamins			
Choline chloride	140	4	0.0286
D-Calcium pantothenate	477	4	0.00839
Folic Acid	441	4	0.00907
i-Inositol	180	7.2	0.0400
Niacinamide	122	4	0.0328
Pyridoxal hydrochloride	204	4	0.0196
Riboflavin	376	0.4	0.00106
Thiamine hydrochloride	337	4	0.0119
Inorganic Salts			
Calcium Chloride (CaCl2) (anhyd.)	111	200	1.80
Ferric Nitrate (Fe(NO3)3"9H2O)	404	0.1	0.000248
Magnesium Sulfate (MgSO4) (anhyd.)	120	97.67	0.814
Potassium Chloride (KCl)	75	400	5.33
Sodium Bicarbonate (NaHCO3)	84	3700	44.05
Sodium Chloride (NaCl)	58	6400	110.34
Sodium Phosphate monobasic (NaH2PO4-H2O)	138	125	0.906
Other Components			
D-Glucose (Dextrose)	180	4500	25.00
Phenol Red	376.4	15	0.0399 ***Note**

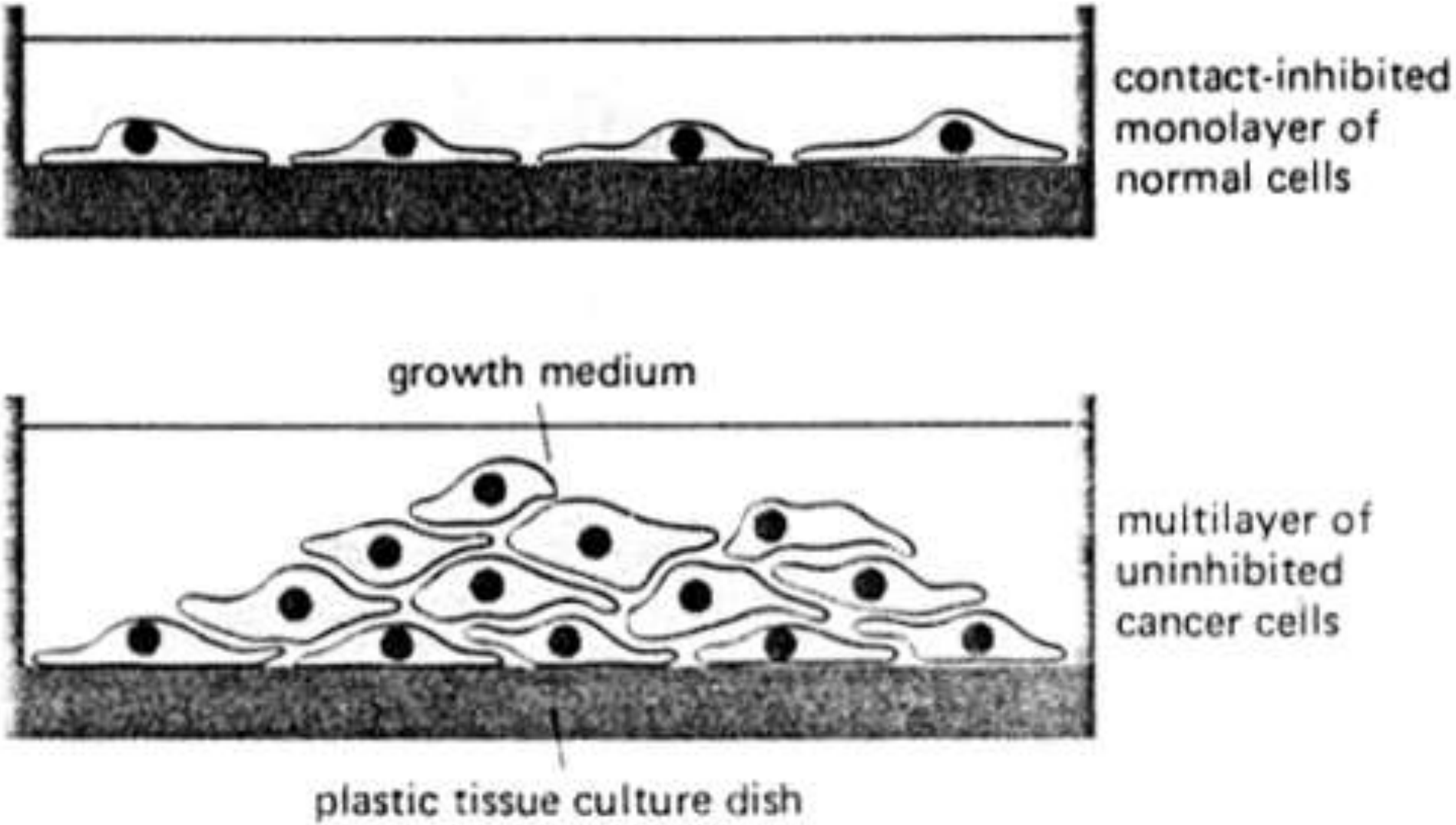
Passaging or sub-culture



Cell dissociated from flask

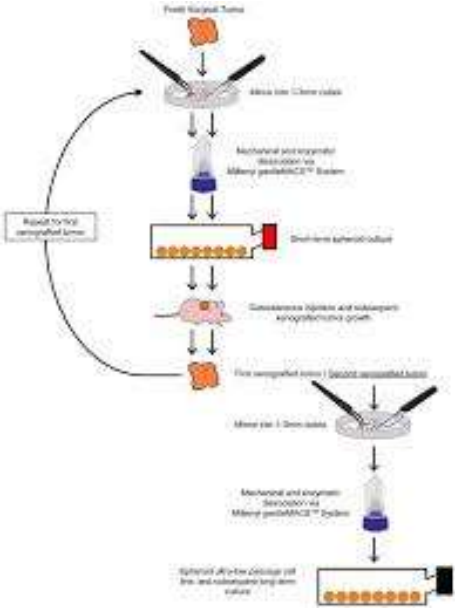
Split 1 in 2

Contact inhibition



Primary culture

- Cells when surgically or enzymatically removed from an organism and placed in suitable culture environment will attach and grow are called as primary culture
- Primary cells have a finite life span
- Primary culture contains a very heterogeneous population of cells
- Sub culturing of primary cells leads to the generation of cell lines
- Cells such as macrophages and neurons do not divide in vitro so can be used as primary cultures



Continous cell lines

- Most cell lines grow for a limited number of generations after which they ceases
- Cell lines which either occur spontaneously or induced virally or chemically transformed into Continous cell lines
- Characteristics of continous cell lines

  -smaller, more rounded, less adherent with a higher nucleus /cytoplasm ratio

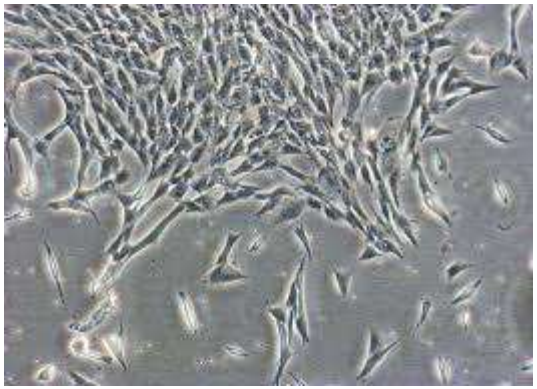
  -Fast growth and have aneuploid chromosome number

  -reduced serum and anchorage dependence and grow more in suspension conditions

  -ability to grow upto higher cell density

  -different in phenotypes from donor tissue

  -stop expressing tissue specific genes



Freezing cells for storage

- Remove the growth medium, wash the cells by PBS and remove the PBS by aspiration
- Dislodge the cells by trypsin-versene
- Dilute the cells with growth medium
- Transfer the cell suspension to a 15 ml conical tube, centrifuge at 200g for 5 mts at RT and remove the growth medium by aspiration
- Resuspend the cells in 1-2ml of freezing medium
- Transfer the cells to cryovials, incubate the cryovials at -80℃ overnight
- Next day transfer the cryovials to Liquid nitrogen

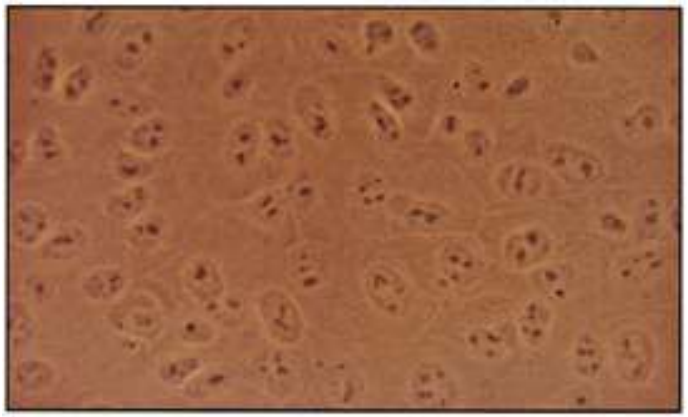
Sources of Contamination

- Bacteria
- Fungi
- Mould
- Yeast
- Mycoplasma
- Other cell types

Contaminant’s of cell culture

Cell culture contaminants of two types

- Chemical-difficult to detect caused by endotoxins, plasticizers, metal ions or traces of disinfectants that are invisible
- Biological-cause visible effects on the culture they are mycoplasma, yeast, bacteria or fungus or also from cross-contamination of cells from other cell lines



Cell Purchase

https://catalog.bcrc.firdi.org.tw/BSAS_cart/controller?event=WELCOME

JCRB細胞バンク

- 分譲申し込み方法
- ユーザー入口
- 研究者の皆様へ
- 代理店の皆様へ
- よくあるご質問（FAQ）
- ご質問・お問い合わせ
- 細胞認証データベース
- JCRBメールマガジン
- 個人情報について
- JCRB生物資源バンク資源紹介

お知らせ&新着情報

2016.06.01 研究に使用されている細胞、間違いありませんか？
2016.04.25 日本人由来初代培養細胞を分譲しています（ヒト組織バンク）
2015.12.03 年末年始の細胞分譲受付・発送につきまして
2015.11.19 サーバーメンテナンスにともなうサービス一時停止のお知らせ
2015.10.16 細胞バンクJCRBによる「細胞培養の参考書」が出来ました。
2015.08.26 ホームページURL変更いたしました
2015.08.06 ホームページURL変更のお知らせ
2015.08.06 【終了致しました】サーバーメンテナンスに伴うサービス一時停止のお知らせ

»お知らせ一覧

関連サイト・リンク

公開中の細胞数

JCRB	IFO	合計

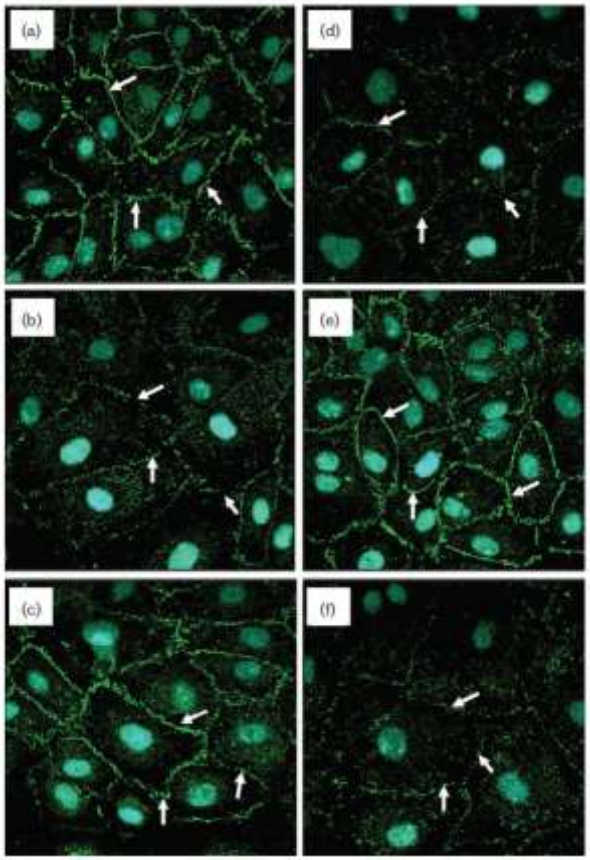
Primary application of animal cell culture in the investigation of:

- The mechanisms of cell cycle control
- The production of cells for biochemical analysis
- The characteristics of cancer cells
- The detection of stem cells
- The detection, production and function of growth factors and hormones
- The detection and production of viruses
- The study of differentiation processes
- The study of specialised cell function
- The study of cell-cell and cell-matrix interactions

Journal of General Virology (2006), 87, 3623–3630 DOI 10.1099/vir.0.82093-0

MCP-1, a highly expressed chemokine in dengue haemorrhagic fever/dengue shock syndrome patients, may cause permeability change, possibly through reduced tight junctions of vascular endothelium cells

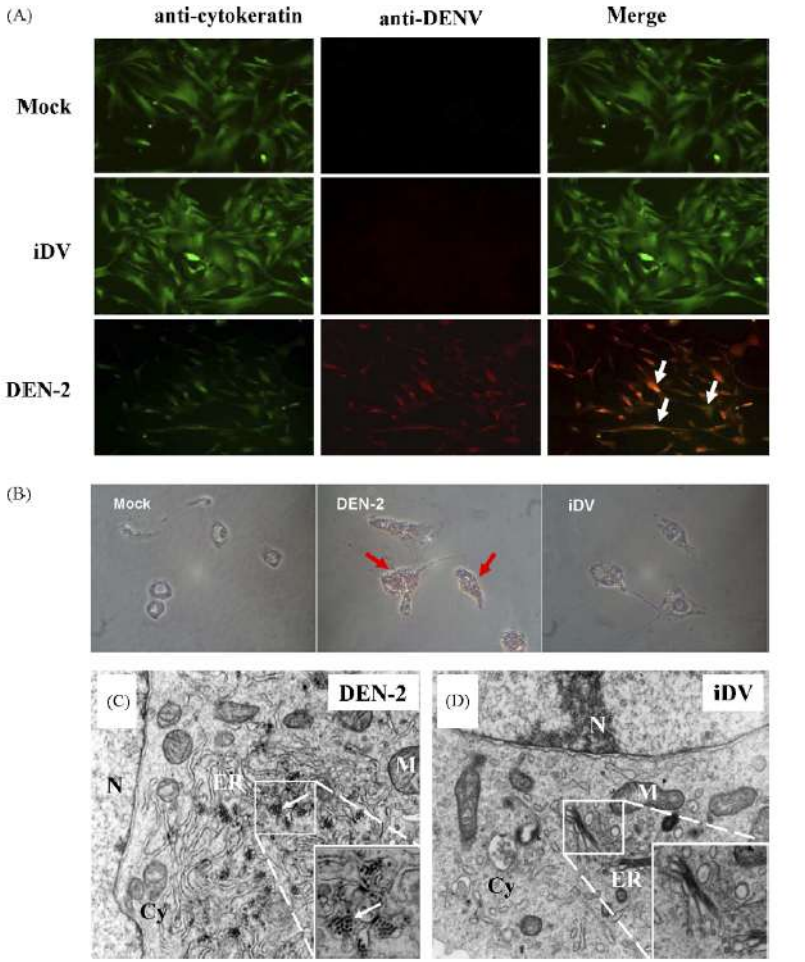
Ying-Ray Lee,[1] Ming-Tao Liu,[2] Huan-Yao Lei,[1,3] Ching-Chuan Liu,[4] Jing-Ming Wu,[4] Yi-Ching Tung,[5] Yee-Shin Lin,[3] Trai-Ming Yeh,[3] Shun-Hua Chen[3] and Hsiao-Sheng Liu[3]



ELSEVIER
Available online at www.sciencedirect.com
ScienceDirect
Virus Research 126 (2007) 216–225
Virus Research
www.elsevier.com/locate/virusres

Dengue viruses can infect human primary lung epithelia as well as lung carcinoma cells, and can also induce the secretion of IL-6 and RANTES

Ying-Ray Lee[a,1], Ching-Yao Su[b,1], Nan-Haw Chow[c], Wu-Wei Lai[d], Huan-Yao Lei[e], Chia-Lun Chang[e], Tsuey-Yu Chang[f], Shun-Hua Chen[e], Yee-Shin Lin[e], Trai-Ming Yeh[g], Hsiao-Sheng Liu[e,*]

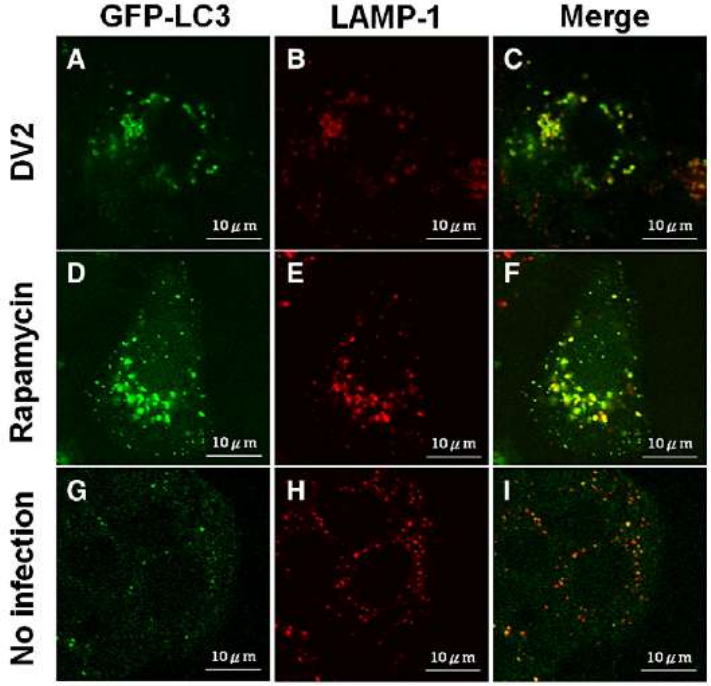


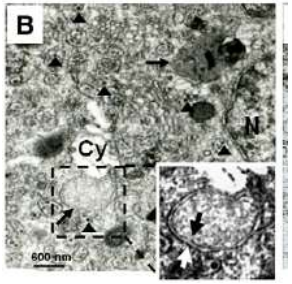
Available online at www.sciencedirect.com
ScienceDirect
Virology 374 (2008) 240–248
VIROLOGY
www.elsevier.com/locate/yviro

Rapid Communication

Autophagic machinery activated by dengue virus enhances virus replication

Ying-Ray Lee [a], Huan-Yao Lei [a,b], Ming-Tao Liu [c], Jen-Ren Wang [d], Shun-Hua Chen [b], Ya-Fen Jiang-Shieh [c], Yee-Shin Lin [b], Trai-Ming Yeh [d], Ching-Chuan Liu [f], Hsiao-Sheng Liu [b,*]



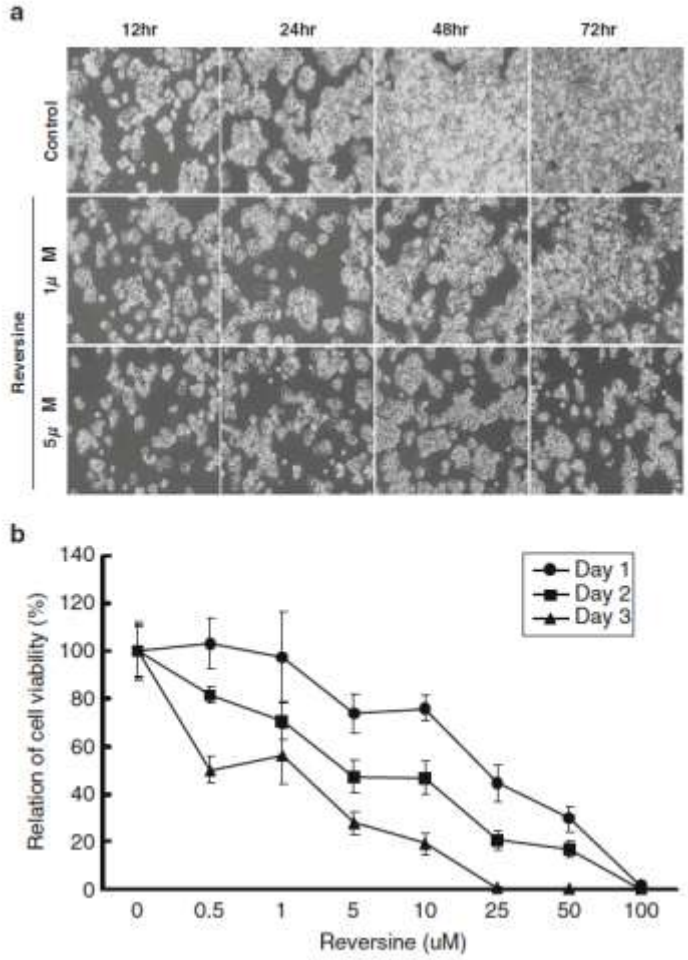


Pharm Res (2012) 29:1990–2005
DOI 10.1007/s11095-012-0727-3

RESEARCH PAPER

Reversine, a 2,6-disubstituted Purine, as an Anti-cancer Agent in Differentiated and Undifferentiated Thyroid Cancer Cells

Shih-Che Hua • Tien-Chun Chang • Hau-Ren Chen • Chieh-Hsiang Lu • Yi-Wen Liu • Shu-Hsin Chen • Hui-I Yu • Yi-Ping Chang • Ying-Ray Lee

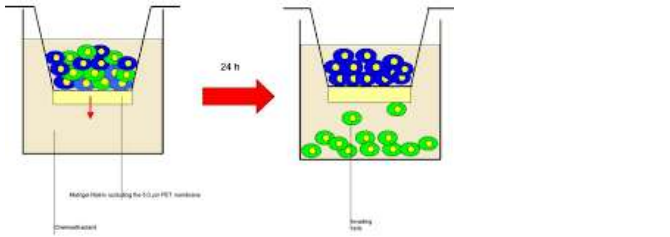


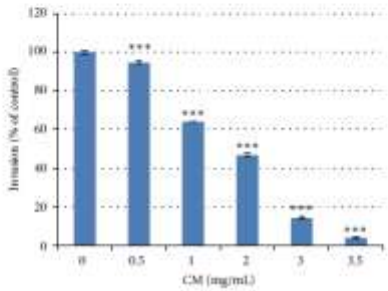
Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2013, Article ID 207279, 8 pages
http://dx.doi.org/10.1155/2013/207279

Research Article

Cortex Moutan Induces Bladder Cancer Cell Death via Apoptosis and Retards Tumor Growth in Mouse Bladders

Mei-Yi Lin,[1,2] Ying-Ray Lee,[3,4] Su-Yin Chiang,[1] Yi-Zhen Li,[5] Yueh-Sheng Chen,[1,6] Cheng-Da Hsu,[3] and Yi-Wen Liu[5]



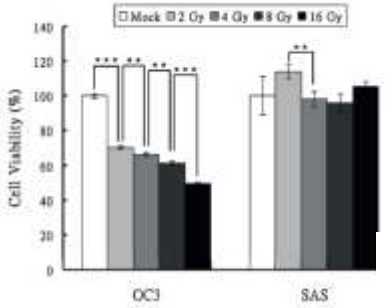


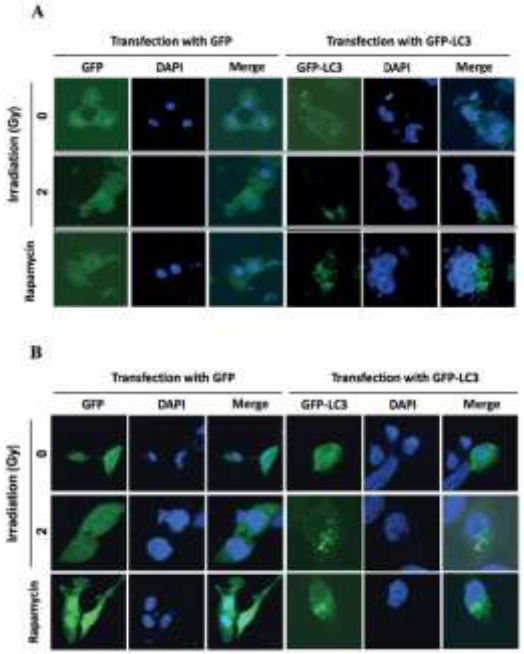
JBUON 2014; 19(1): 137-144
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

ORIGINAL ARTICLE

Ionizing radiation induces autophagy in human oral squamous cell carcinoma

Szu-Yuan Wu[1-4], Yi-Wen Liu[5], Yang-Kao Wang[6], Tsung-Hsing Lin[7], Yi-Zhen Li[8], Shu-Hsin Chen[9], Ying-Ray Lee[8,9]





OPEN

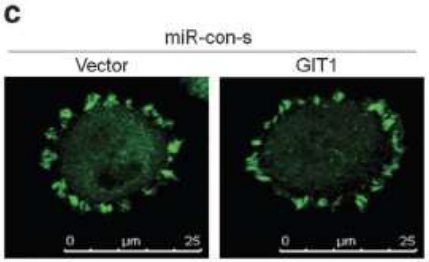
Oncogene (2014), 1–12

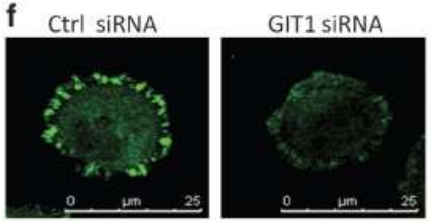
www.nature.com/onc

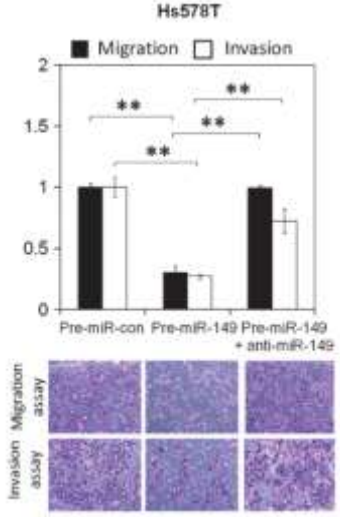
ORIGINAL ARTICLE

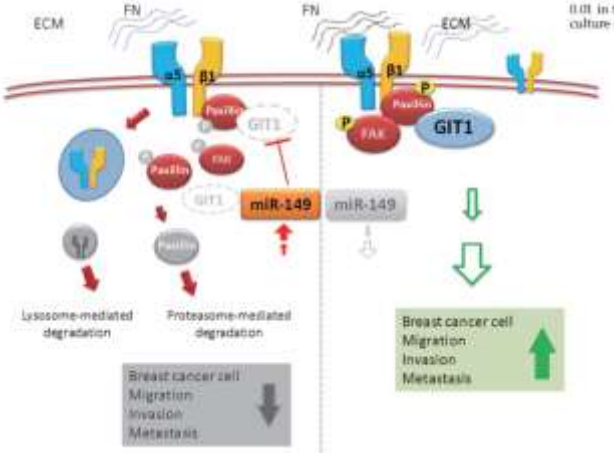
MicroRNA-149 targets GIT1 to suppress integrin signaling and breast cancer metastasis

S-H Chan[1,11], W-C Huang[1,2,11], J-W Chang[1], K-J Chang[3], W-H Kuo[3], M-Y Wang[3], K-Y Lin[4], Y-H Uen[4], M-F Hou[5], C-M Lin[1], T-H Jang[1], C-W Tu[6], Y-R Lee[7], Y-H Lee[8], M-T Tien[9] and L-H Wang[1,2,10]









MONOCLONAL ANTIBODIES IN IMMUNODIAGNOSIS AND IMMUNOTHERAPY
Volume 32, Number 6, 2013

DOI: 10.1089/mab.2013.0033

Monoclonal Antibodies for Diagnosis of Enterovirus 71

Li Xu,[1] Kao-Jean Huang,[2] Tzong-Shiann Ho,[3] Chia-Chyi Liu,[4] Ying-Ray Lee,[5] Ching-Yen Lin,[2] David Shiuan,[2] and Xing-Hong Jiang[1]

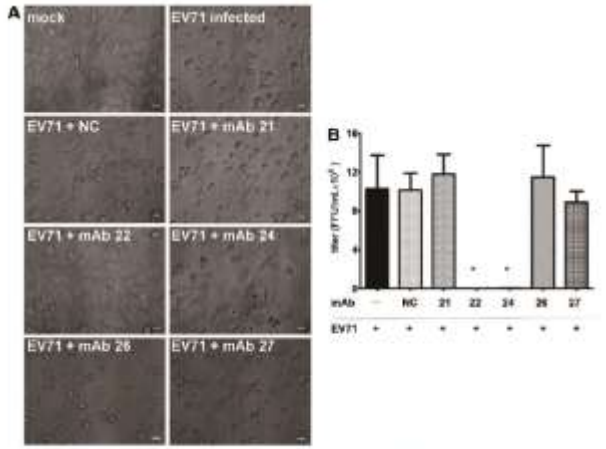


FIG. 5. Potent neutralization effects on EV71 mediated by selected MAbs. RD cells were infected with EV71 at the m.o.i. of 0.01 in the presence of indicated hybridoma culture supernatants. Cell images were acquired (A) at 12 h post-infection, and culture supernatants were collected at 24 h post-infection and titered for infectious EV71 by fluorescence focusing assay (B).

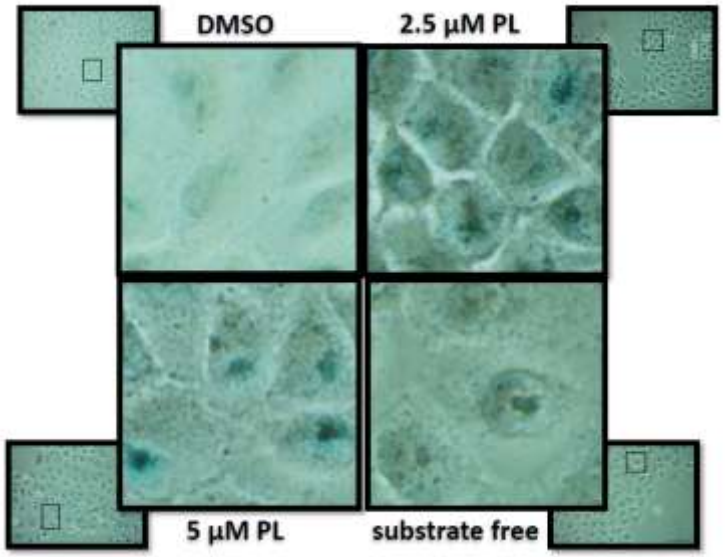
International Journal of
Molecular Sciences

MDPI

Article

Piperlongumine Suppresses Proliferation of Human Oral Squamous Cell Carcinoma through Cell Cycle Arrest, Apoptosis and Senescence

San-Yuan Chen [1,2,†], Geng-Hung Liu [2,†], Wen-Ying Chao [3], Chung-Sheng Shi [4], Ching-Yen Lin [5], Yun-Ping Lim [6], Chieh-Hsiang Lu [7], Peng-Yeh Lai [2], Hau-Ren Chen [2,*] and Ying-Ray Lee [3,5,*]



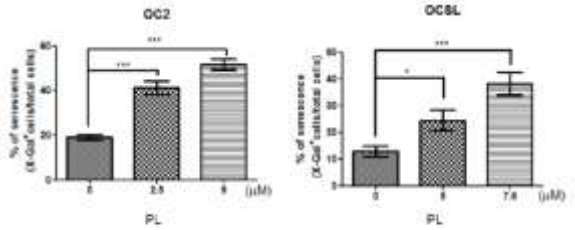


Figure 4. Piperlongumine (PL) treatment induces cellular senescence in human OSCC cells. Senescent cell assays were conducted in OC2 and OCSL cells treated with various concentrations of piperlongumine for 24 h by senescence-associated β-galactosidase (SA-β-Gal) staining, and the percentage of β-Gal staining positive cells was statistically analyzed after staining. Data are presented as the mean ± SD. * $p < 0.05$ and *** $p < 0.001$ as compared to the control (0 μM).

European Journal of Pharmacology 762 (2015) 449–458

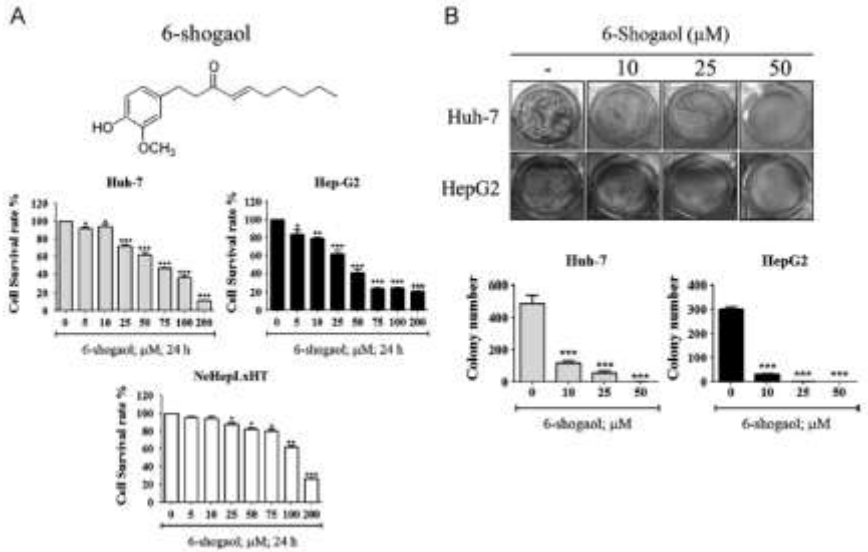
Contents lists available at ScienceDirect

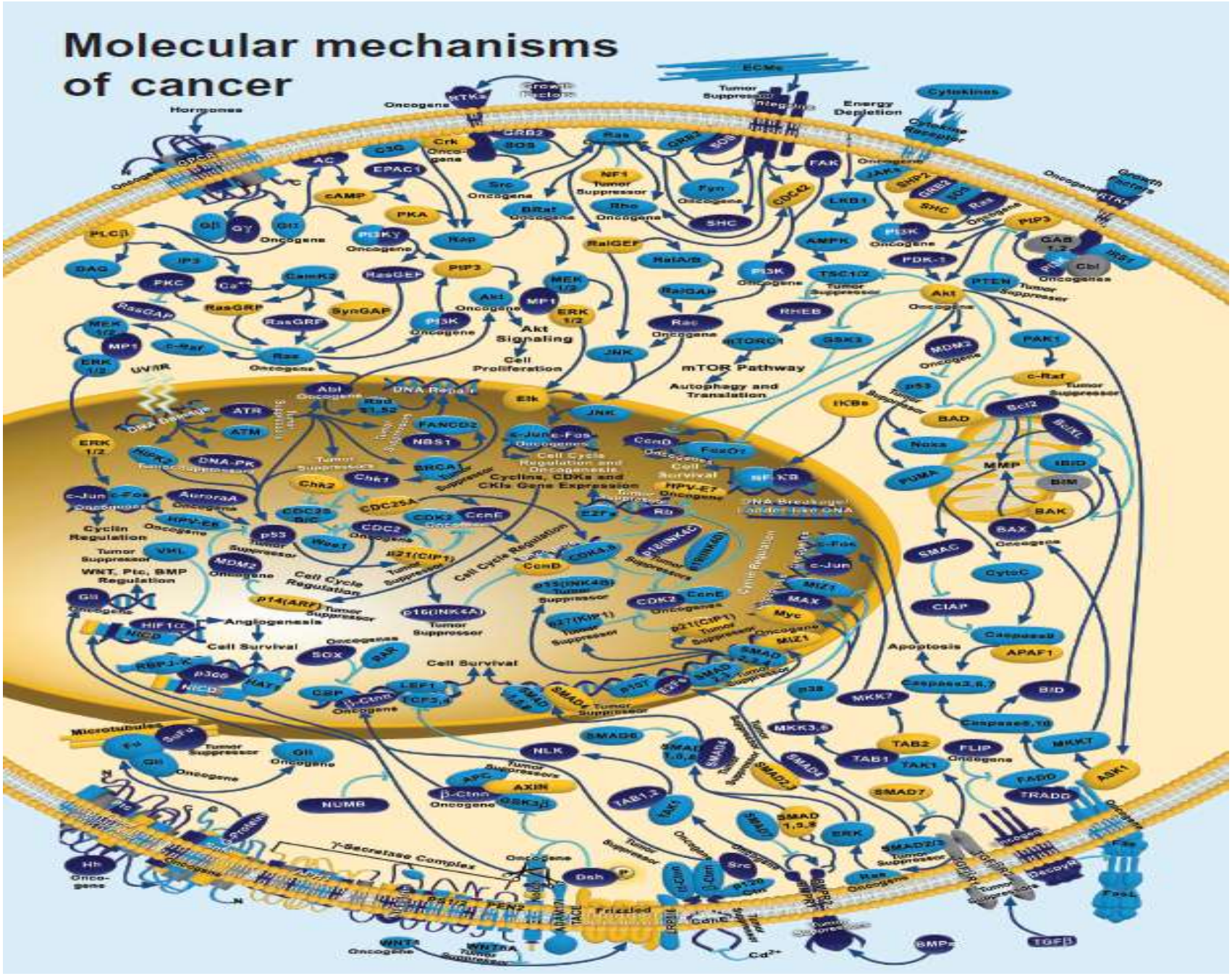
European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

6-Shogaol induces cell cycle arrest and apoptosis in human hepatoma cells through pleiotropic mechanisms

Jung-Ju Wu [a,1], Hany A. Omar [b,c,1], Ying-Ray Lee [d,1], Yen-Ni Teng [e], Pin-Shern Chen [f], Yu-Chung Chen [f], Hsiao-Shan Huang [a], Kuan-Han Lee [g,h], Jui-Hsiang Hung [f,h,*]




Molecular mechanisms of cancer
Hormones
GPCR
Oncogene
RTK
Oncogene
Growth Factors
ECMs
Tumor Suppressor Integrins
Energy Depletion
Cytokines
Cytokine Receptor
Oncogene
Growth Factors
Oncogene RTKs
AC
C3G
Crk Onco-gene
GRB2
SOS
Ras
GRB2
SOS
EPAC1
cAMP
PKA
Src Oncogene
NF1 Tumor Suppressor
Fyn Oncogene
FAK
JAK2
SHP2
GRB2
SOS
SHC
Ras Oncogene
PI3K Oncogene
PIP3
GAB 1,2
SHP2
Cbl
Oncogenes
PDK1
PTEN Tumor Suppressor
Akt Oncogene
PAK1
c-Raf Tumor Suppressor
BAD
Bcl2
BclXL
Noxa
PUMA
MMP
tBID
BIM
BAK
BAX
Oncogene
SMAC
CytoC
CIAP
Caspase9
APAF1
Apoptosis
Caspase3,6,7
BID
Caspase8,10
MKK7
MKK3,6
TAB2
TAB1
TAK1
FLIP Oncogene
MKK4
FADD
TRADD
ASK1
Oncogene
p38
JNK
ERK 1/2
MEK 1/2
MP1
c-Raf
Ras Oncogene
UVR
RasGAP
RasGRP
SynGAP
RasGEF
PIP3
PI3K Oncogene
Akt Oncogene
MP1
ERK 1/2
Akt Signaling
Cell Proliferation
JNK
RalGEF
RalA/B
RalGAP
Rac Oncogene
PI3K Oncogene
TSC1/2 Tumor Suppressor
RHEB
mTORC1
mTOR Pathway
Autophagy and Translation
GSK3
IKBs
MDM2 Oncogene
p53 Tumor Suppressor
Abl Oncogene
DNA Repair
Rad 51,52
FANCD2
NBS1
ATR
ATM
DNA Damage
Tumor Suppressor
BRCA1 Tumor Suppressor
Chk2
Chk1
DNA-PK
ERK 1/2
MEKK1 Tumor Suppressor
c-Jun-Fos-Oncogenes
Cell Cycle Regulation and Oncogenesis
Cyclins, CDKs and CKIs Gene Expression
CcnD Oncogene
FoxO1 Cell Survival
HPV-E7 Oncogene
NF-KB
DNA Breakage Ladder Low DNA
E2F1
Rb
Tumor Suppressor
CDC25A
CDC25 B/C
CDK2
CcnE
CDC2 Oncogene
Wee1
p21(CIP1) Tumor Suppressor
Cell Cycle Regulation
p14(ARF) Tumor Suppressor
p16(INK4A) Tumor Suppressor
p15(INK4B) Tumor Suppressor
CcnD
CDK4,6
p18(INK4C) Tumor Suppressor
p27(KIP1) Tumor Suppressor
p53(INK4B) Tumor Suppressor
CDK2
CcnE Oncogenes
p21(CIP1) Tumor Suppressor
SMAD 2,3 Tumor Suppressor
SMAD4 Tumor Suppressor
p15
E2F1
Cytostatic Regulation
c-Fos
c-Jun
MIZ1
MAX
Myc Oncogene
MIZ1
AuroraA Oncogene
HPV-E6 Oncogene
Cyclin Regulation
VHL Tumor Suppressor
MDM2 Oncogene
WNT, Ptc, BMP Regulation
Gli Oncogene
HIF1α
HIF1β
Angiogenesis
Cell Survival
SOX Oncogenes
SAR
RBPJ-K
p300
HAT
NICD
CBP
β-Catn Oncogene
LEF1
TCF3,4
SMAD4 Tumor Suppressor
SMAD 1,5,8
SMAD6
SMAD7
Cell Survival
Microtubules
Su(f) Tumor Suppressor
Fu
Gli
Gli Oncogene
NLK
APC Tumor Suppressor
AXIN
β-Catn Oncogene
GSK3β
NUMB
TAB1,2
TAK1
SMAD 1,3,5
ERK
SMAD2/3 Tumor Suppressor
Ras Oncogene
TGFβR1 Tumor Suppressor
TGFβR2
Decorin
FasL
TGFβ
BMP2
γ-Secretase Complex
Oncogene
Dsh
P
Frizzled
DKK1
WNT5A Tumor Suppressor
WNT4 Oncogene
Cd2+
Hh Onco-gene
PEN2
PS1/2
APH1
NCT
PEN2
N
Src
p120 Ctn
E-Cad
β-Catn
Tumor Suppressor
Oncogene

Thank you for your attention!

QIAGEN

Molecular Mechanism of Cancer