MULTIPLE MOLECULAR TARGETING ANTICANCER EFFECTS ON MIA-PACA-2 HUMAN PANCREATIC CANCER CELLS BY KOREAN HERBAL RECIPE A2

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- **Pancreatic cancer is one of the most lethal human malignancies and highly resistant to conventional treatment modalities such as systemic chemo therapy and radiation therapy.**

- **Despite continuous efforts to improve the prognosis of patients with pancreatic ductal adenocarcinoma, little progress has been made over the past decades.**

- **A Korean herbal recipe A2 had been found to exert therapeutic effects in pancreatic cancer patients with "Shao Yin (少陰)" constitution according to the oriental Sasang Constitutional Medicine (by Dr. Tae-Young Han). However, little is known about the underlying molecular mechanism.**

- **This study explores the effects of A2 on a poorly differentiated pancreatic cancer cell line MIA PaCa-2 to elucidate the molecular mechanism underlying its clinical effects.**

원장

- 성 명 : 한 태 영
- 면허의 종류 : 한의사
- 경기고등학교 졸업
- 서울대학교 인문대학 동양사학과 졸업
- 경희대학교 한의과대학 졸업

"한의학, 설명할 수 없는 효과 있다" 서울대병원 신희영 교수, 소아암 한방 치료 증례 발표 | 한의학 상식 / 한의학 이야기 2012/02/14 10:25

http://blog.naver.com/naturedam/100150984941

▲신희영 교수가 소아암 환아를 한의학적으로 치료한 증례를 발표하고 있다.

소아암 등 난치성 질환에 한의학적 치료법이 효과가 있다는 주장이 임상의사에 의해 제기됐다.

서울대병원 소아청소년과 신희영 교수는 13일 융합학회 주최로 서울로얄호텔에서 열린 '전통의학, 현대의학 그리고 미래 기술융합' 공청회에서 한의학적 접근으로 치료한 증례를 발표했다.

Clinical Pediatric Hematology-Oncology Volume 18 · Number 2 · October 2011 ORIGINAL ARTICLE

현대의학으로 설명하기 힘들었던 7증례의 난치성 소아혈액종양질환에서 대체의학의 경험

박은실[1] · 한보윤[2] · 김민선[2] · 신희영[2] · 안효섭[2]

[1]경상대학교 의학전문대학원 건강과학연구소, 소아과학교실, [2]서울대학교 의과대학 암연구소, 소아과학교실

Possibility of Alternative Medicine in the Field of Pediatric Hematology-Oncolgy: Analysis of 7 Cases of Unexpected Outcome by Modern Medicine

Eun Sil Park, M.D.[1], Boyun Han, M.D.[2], Min Sun Kim, M.D.[2], Hee Young Shin, M.D.[2] and Hyo Seop Ahn, M.D.[2]

[1]Department of Pediatrics, Institute of Health Science, Gyeongsang National University School of Medicine, Jinju, [2]Department of Pediatrics, Cancer Research Institute, Seoul National University College of Medicine, Seoul, Korea

Table 1. Characteristics, treatment, and outcome of patients

No. of cases	Diagnosis	State when alternative medicine started	Alternative medicine	Outcome
1	Infantile fibrosarcoma	Third relapse with multiple coin lesions in both lungs	Acupuncture twice a week	No progression of tumor for 10 years
2	Recurrent fibromatosis	Progression of fibromatosis after third operation	Herb medication with dietary control	Regression of fibromatosis enabling surgical removal
3	Malignant peripheral nerve sheath tumor	Progression of enhancing lesion of leg on MRI	Herb medication with dietary control	Stable state of enhancing lesion of leg
4	Inflammatory myofibroblastic tumor	Inoperable due to wide gastro-intestinal involvement	Herb medication with dietary control	Regression of tumor and stable for 7 years
5	Hepatic adenoma-multiple nodular hyperplasia	Liver failure waiting for liver transplantation	Dietary control and exercise	Improved liver function and decreased size of focal nodular hyperplasia
6	Renal cell carcinoma	Operation after second relapse	Dietary control	No recurrence of tumor for 8 years
7	Chronic ITP	Unresponsive to treatment waiting for splenectomy	Cepharanthin	Complete recovery of platelet count

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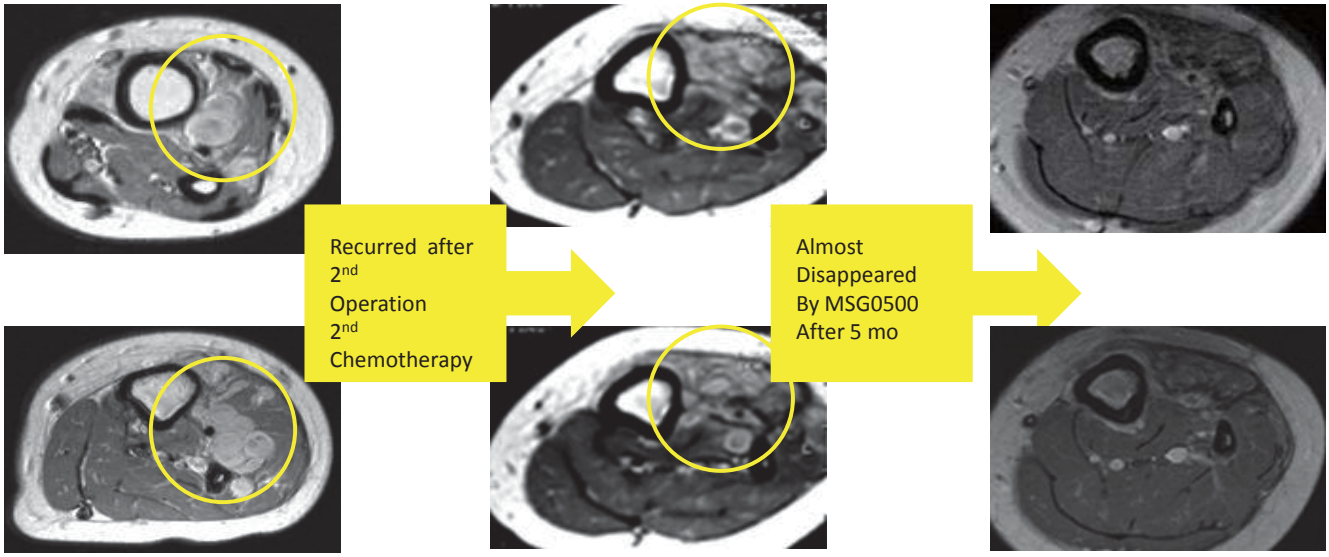
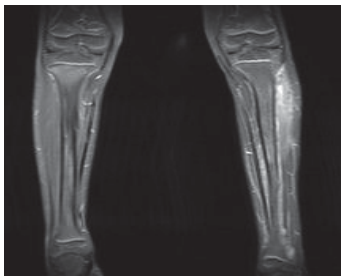
Malignant Peripheral nervesheat tumor. 37

Before /2003.1.15 | 10mo/ 2003.11.15 | 15 mo/ 2004. 5





3 years of herbal medication therapy

No recurrence until 2014. 2. for 12 years.

5

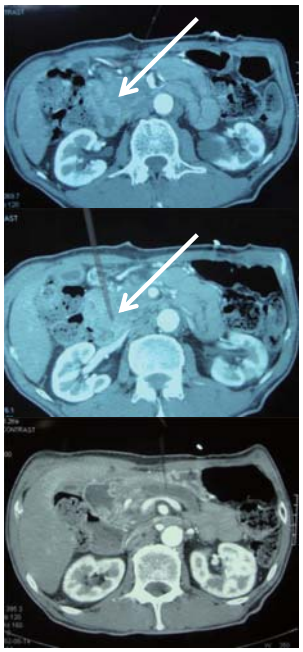
Clinical Case

Case 1. M/72

2002.8 – 2004.1 / total 17 month A2 therapy with futurone (doxyfluridine)

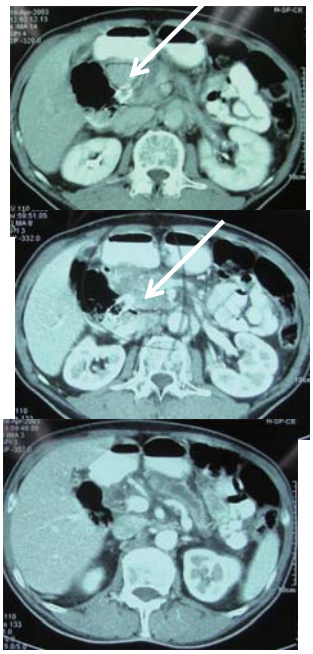
2002. 7. 19	Abdominal pain CT check	**Pancreatic head cancer** **Diameter 4.5 cm** **Lung metastasis** **Duodenal loop invasion** **Stage IV**
2002.7. 23	**Ca 19-9 /768**	
2002.8.4	**Ca 19-9 / 1079** op/pt 81/110 Rgpt 568	First visit on banrong insu herbal clinic **Start A2**
2002.8	NCI ilsan CBD stent insertion	Reject ILSAN NCI treatment
2002.9.7	CT abdomen. 3.1 cm x 3.1 cm x 3.4 cm (WXDXH)	Head mass more regressed
2002.9.26	**Ca 19-9/ 2465**	
2003. 1	(Furtulon / doxyfluridine) start with A2	Local clinic
2003.3.20	**CA 19-9/562**	
2003.5	Head cancer more regressed	
2003.8	Fever. Change stent	
2003.11.26	Abdominal CT Head cancer 35x40x 50 mm	
2004.1	CBD stent obstruction	Stop herbal A2 therapy
2004.4	expire	Total 21 month

first



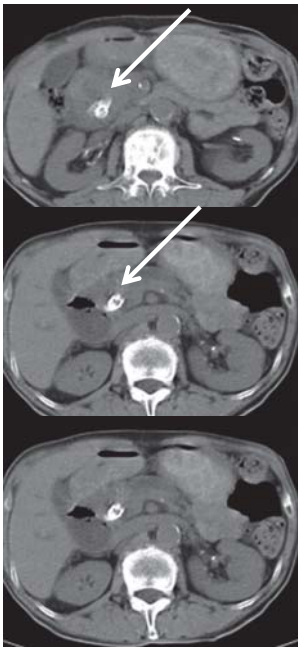
4.5 cm

8 month



4 cm
improve
d

15 month



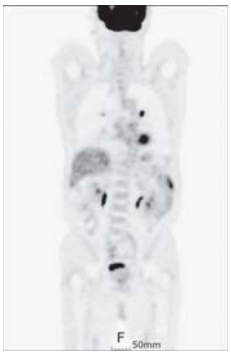
5cm
progress
ed

17 month
Stop A2

20 month
expire

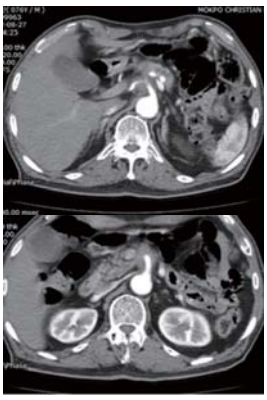
Case 2

		M/77
		A2 #3 gemzar . UFT
2012.8. 27	onset	Pancreatic body mass (4.2 cm x 3.7 cm) retroperitoneal infiltration Encasement of celiac axis, common hepatic artery, splenic artery, SMA and SMV
2012.9. 24	1 mo	PET
2012.10.4	2 mo	CT no interval change
2012.11. 9	3 mo	CT slightly reduced
2013.4.23	8 mo	CT No interval change but Newly developed biliary dilatation
2013.9	13 mo	expire

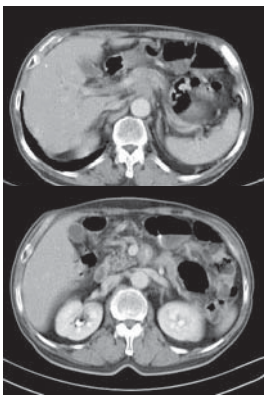


SD?
CA19-9 ?

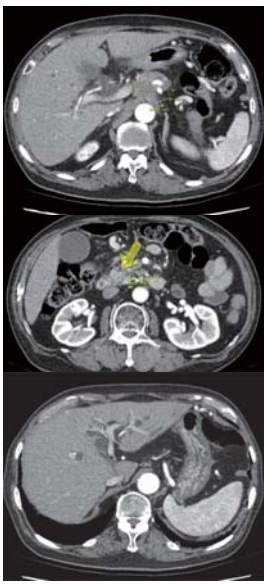
Before



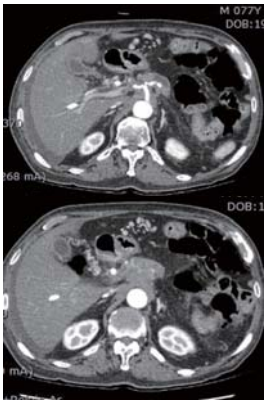
4 month



8 month



12month



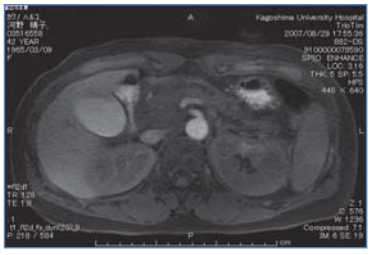
13mo

2012.8. 27	2012.11.9	2013.4.23	2013.8.19	2013. 9
body cancer about 4.2 x 3.7 cm , mass have retroperitoneal infiltration , encasement of celiac axis, common hepatic artery, splenic artery, SMA and SMV .	The body mass was slightly reduced in size and volume	No interval change but newly developed biliary dilatation	Slightly increased body mass. PTBD insertion Small amount of ascites.	Expire

Case. 3 Patient with pancreatic head cancer with liver metastasis failed with TS-1, gemzar therapy

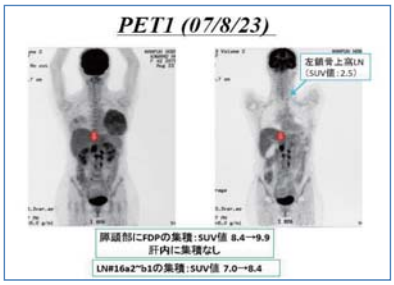
Date		F/42
2007.3	Onset	Diagnosis
2007.8 .	3 mo	Gemzar + TS-1 for 3 months
2007.10 A2 therapy start	5 mo	Multiple liver metastasis, supraclavicular, axillary , mediastinal LNs metastasis
2008.1.29	8 mo	Much reduced liver metastasis, supraclavicular, axillary , mediastinal LNs disappeared
2009. 3	24 mo	Expired - total 24 months
Clinical pediatric hematology-oncology 2011. October volume 18. number 2/ reported By doctor SHIN HEE YOUNG SNUH		현대의학으로 설명하기 힘들었던 7증례의 난치성 소아혈액종양질환에서 대체의학의 경험 신희영 ,안효섭(SNUH) Possibility of alternative medicine in the field of pediatric hematology-oncology: Analysis of 7 cases of Unexpected outcome by modern medicine

Before

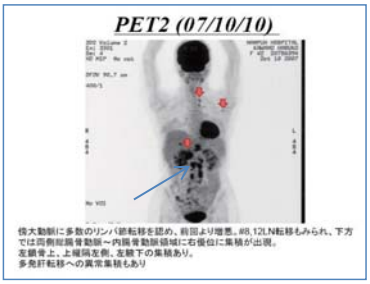


2007.3 onset

4 mo

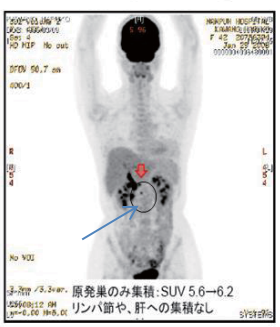


6 mo



TS-1. gemzar More progress # A2 start

9 mo /



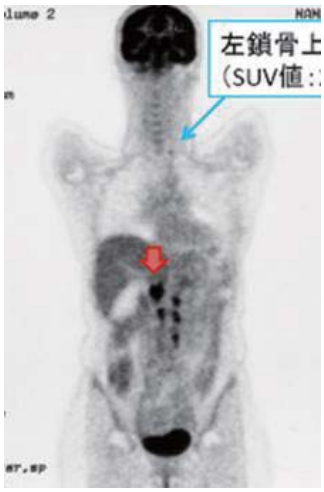
3 mo / A 2 Therapy Much reduced

Good PR? Markers?

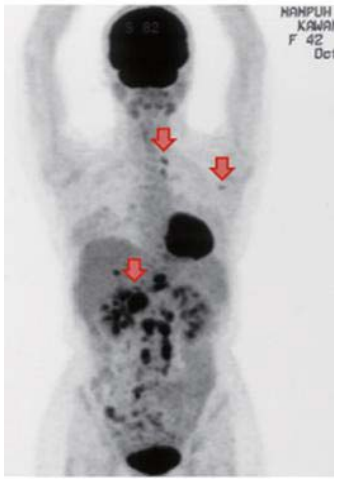
most of time no pain. Usual life

24 mo Expire 2009.3

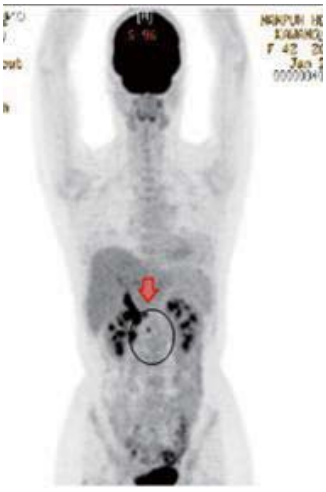
TS-1.gemzar failed/ A2 - 3 mo



2007.8.23
before



2007.10.10
TS+gemzar
progress



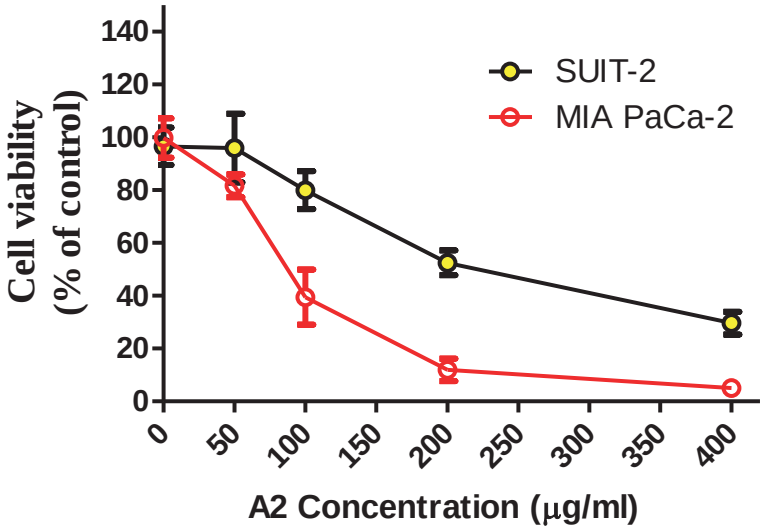
2008.1.29
3 month
A2 therapy
With TS-1.
gemzar

13

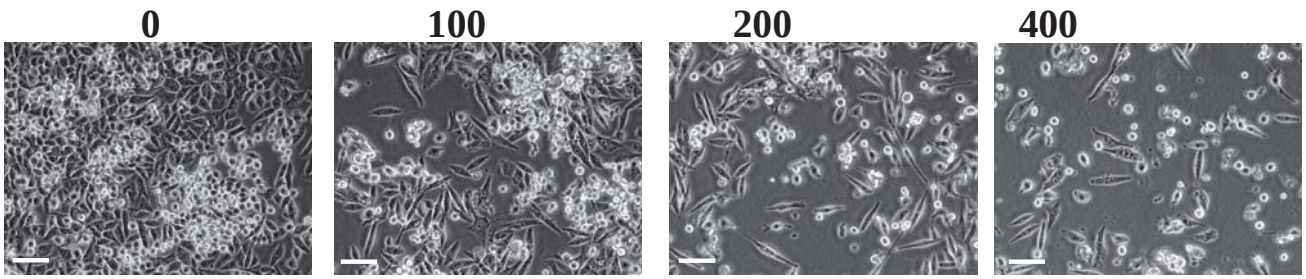
Materials and Methods

- The **herbal recipe A2** was provided by Dr. Han, Banronginsu Oriental Medicine Clinic, Korea.
- Moderately differentiated (Suit-2) and **poorly**-differentiated (MIA PaCa-2) human pancreatic cancer cells were chosen for this study.
- **Cell viability** was determined by SRB assay.
- The **cell-cycle distribution** was analyzed by flow cytometry.
- Western blot and antibody against specific phosphorylated protein was employed to investigate the affected **signaling pathways**.
- RT-PCR was used to evaluate the **mRNA expression**.
- Aldefluor® assays were used to analyze the **cancer stem-like** ALDH+ population.

Effects of A2 on the proliferation of moderately differentiated (Suit-2) and poorly-differentiated (MIA PaCa-2) human pancreatic cancer cells

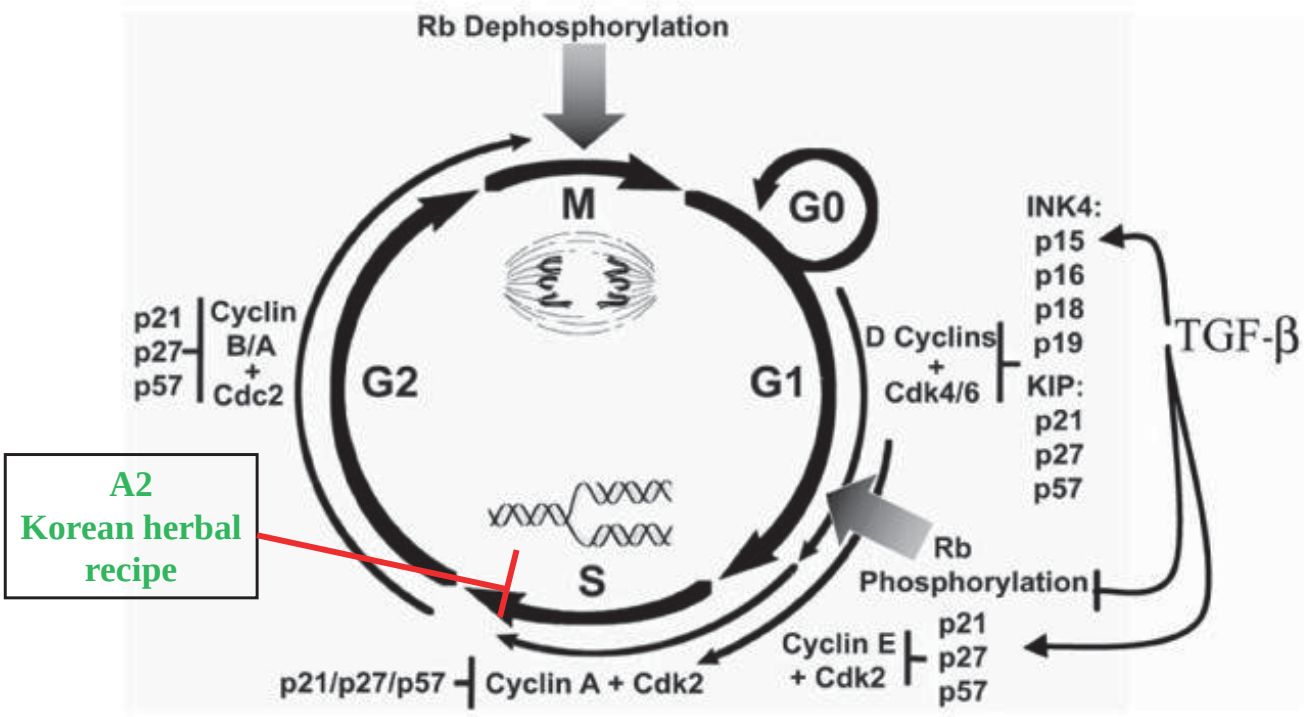


Phase contrast images of A2-treated MIA PaCa-2 pancreatic cancer cells



Scale bar= 50μm, 72 h of treatment

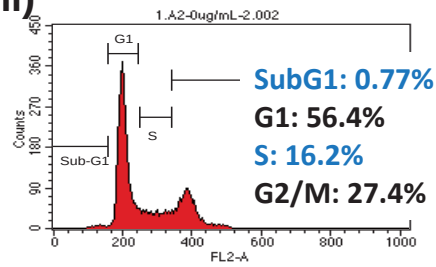
A2 arrests the cell cycle of pancreatic cancer cells in S phase



A2 increases S phase percentage and induces apoptosis in SUIT-2 pancreatic cancer cells (72 h of treatment)

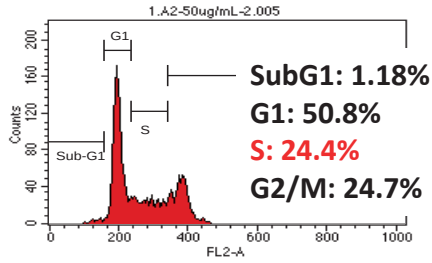
A2 (μg/ml)

0



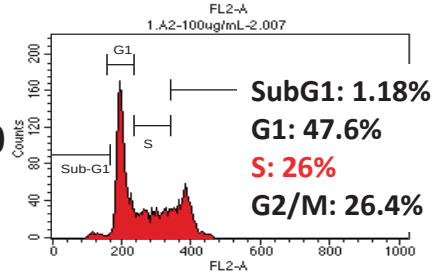
SubG1: 0.77%
G1: 56.4%
S: 16.2%
G2/M: 27.4%

50



SubG1: 1.18%
G1: 50.8%
S: 24.4%
G2/M: 24.7%

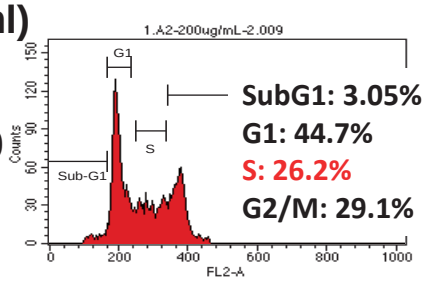
100



SubG1: 1.18%
G1: 47.6%
S: 26%
G2/M: 26.4%

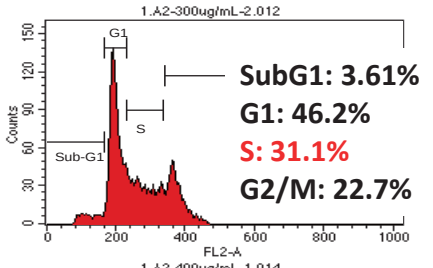
A2 (μg/ml)

200



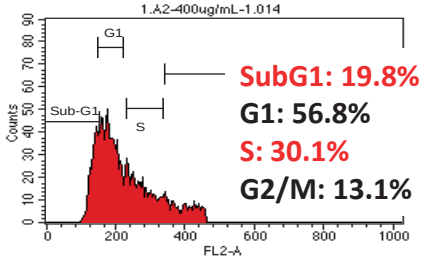
SubG1: 3.05%
G1: 44.7%
S: 26.2%
G2/M: 29.1%

300



SubG1: 3.61%
G1: 46.2%
S: 31.1%
G2/M: 22.7%

400



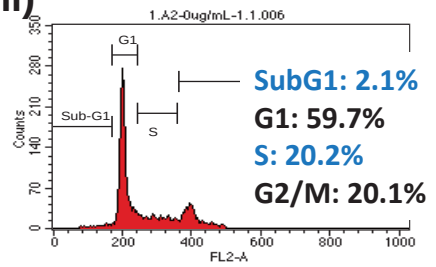
SubG1: 19.8%
G1: 56.8%
S: 30.1%
G2/M: 13.1%

102.06.26

A2 increases S phase percentage and induces apoptosis in MIA PaCa-2 pancreatic cancer cells (72 h of treatment)

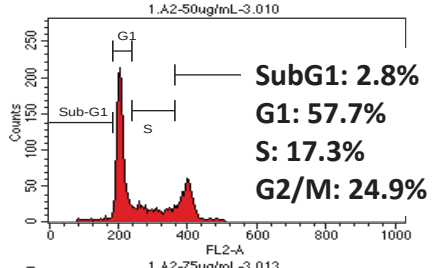
A2 (μg/ml)

0



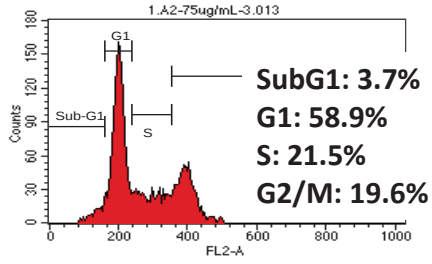
SubG1: 2.1%
G1: 59.7%
S: 20.2%
G2/M: 20.1%

50



SubG1: 2.8%
G1: 57.7%
S: 17.3%
G2/M: 24.9%

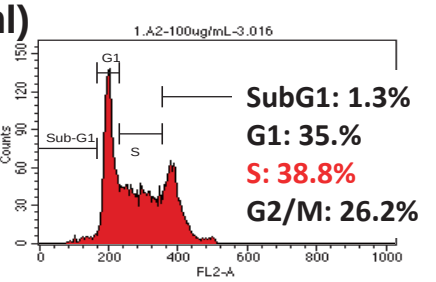
75



SubG1: 3.7%
G1: 58.9%
S: 21.5%
G2/M: 19.6%

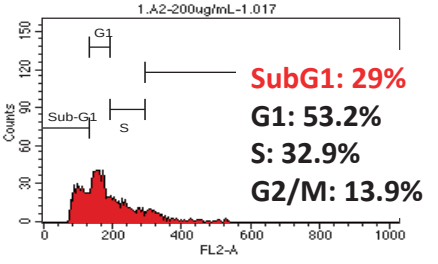
A2 (μg/ml)

100



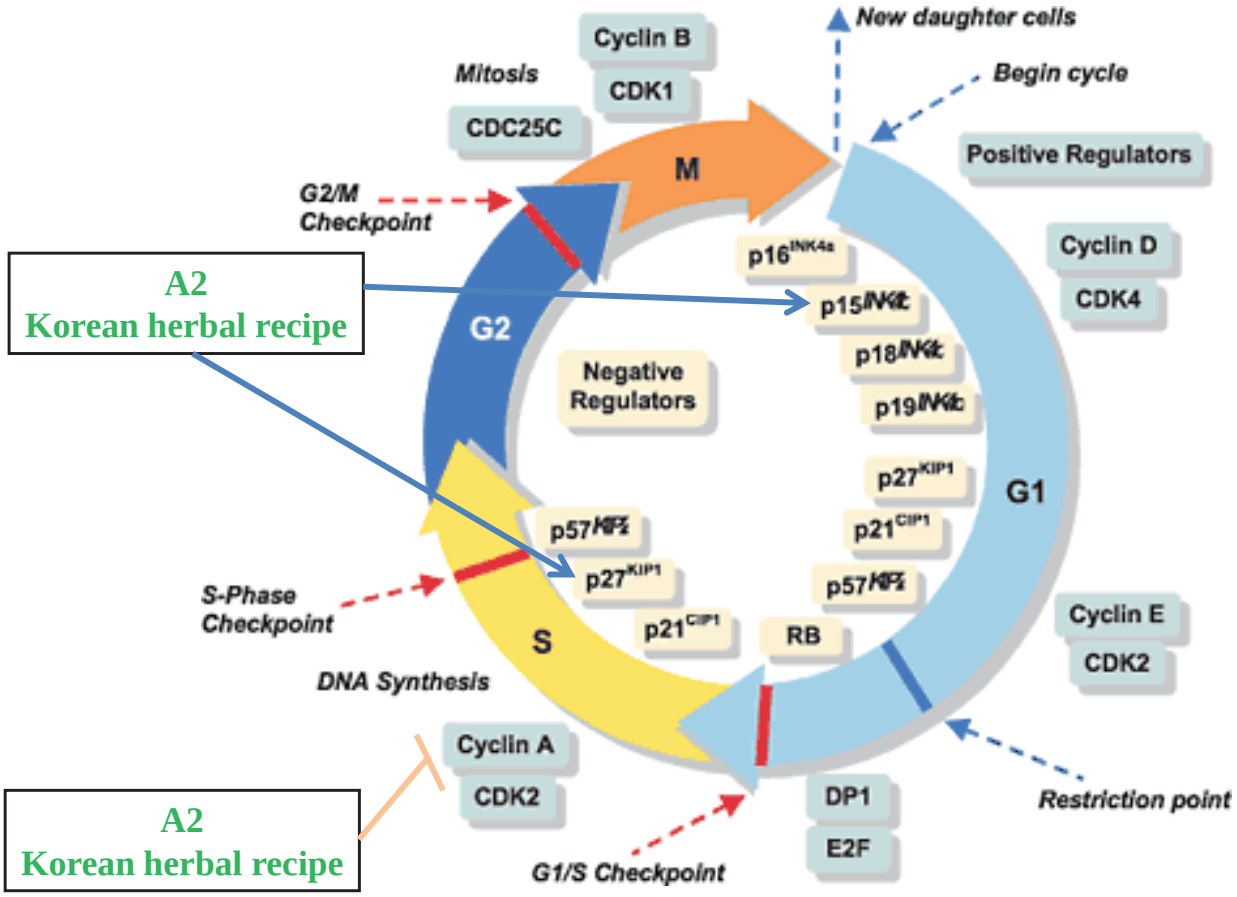
SubG1: 1.3%
G1: 35.%
S: 38.8%
G2/M: 26.2%

200



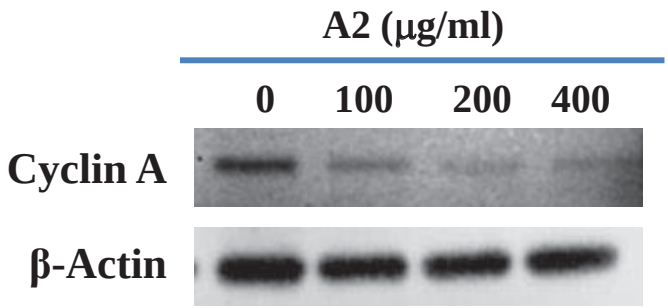
SubG1: 29%
G1: 53.2%
S: 32.9%
G2/M: 13.9%

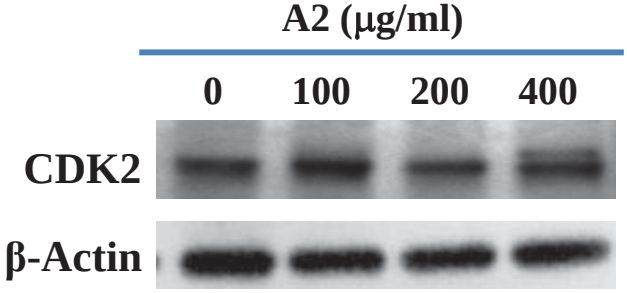
102.06.26

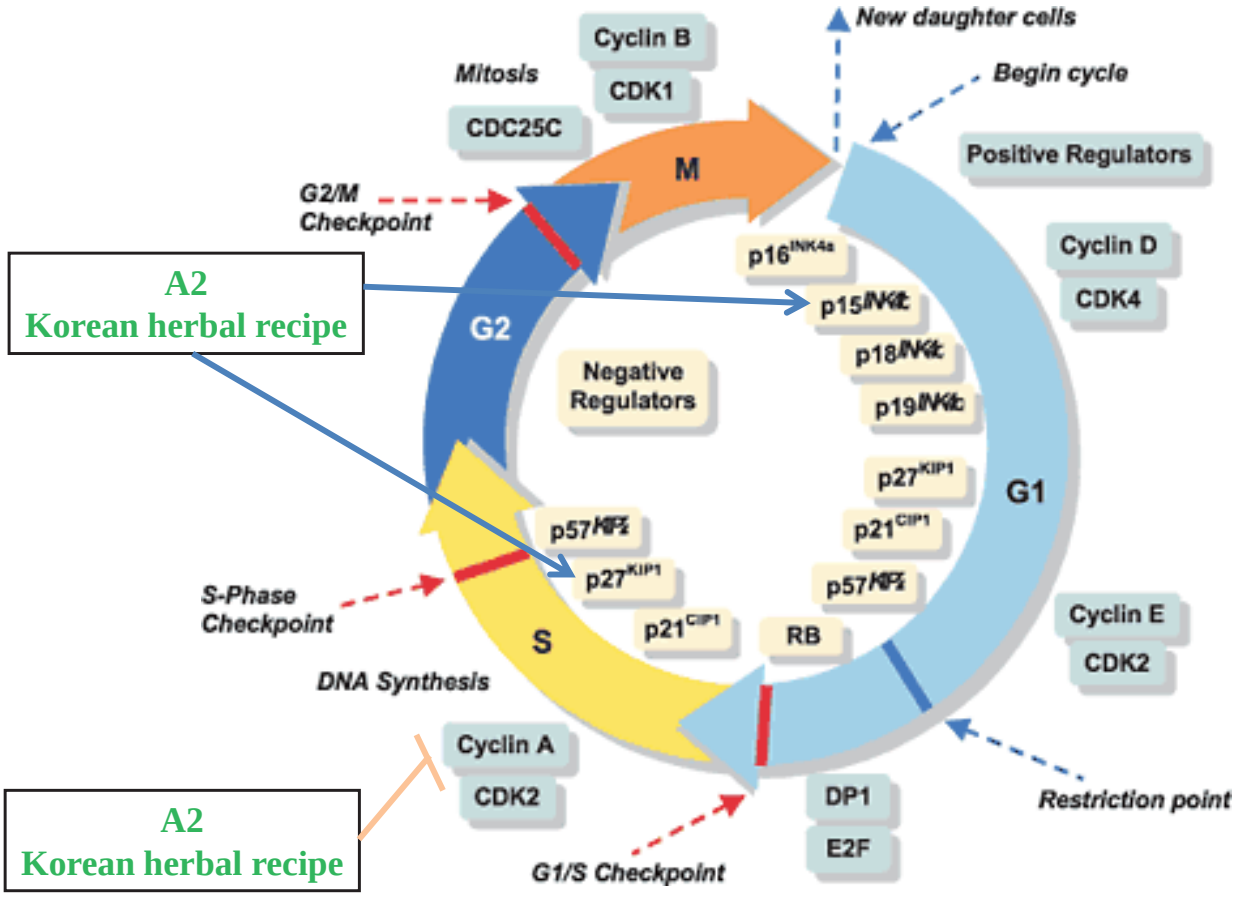


Drugs Fut 2003, 28(9): 881

A2 decreases the cyclin A in MIA PaCa-2 pancreatic cancer cells (72 h of treatment)

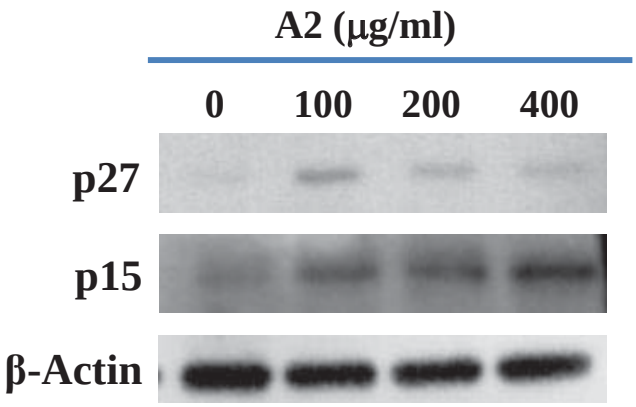




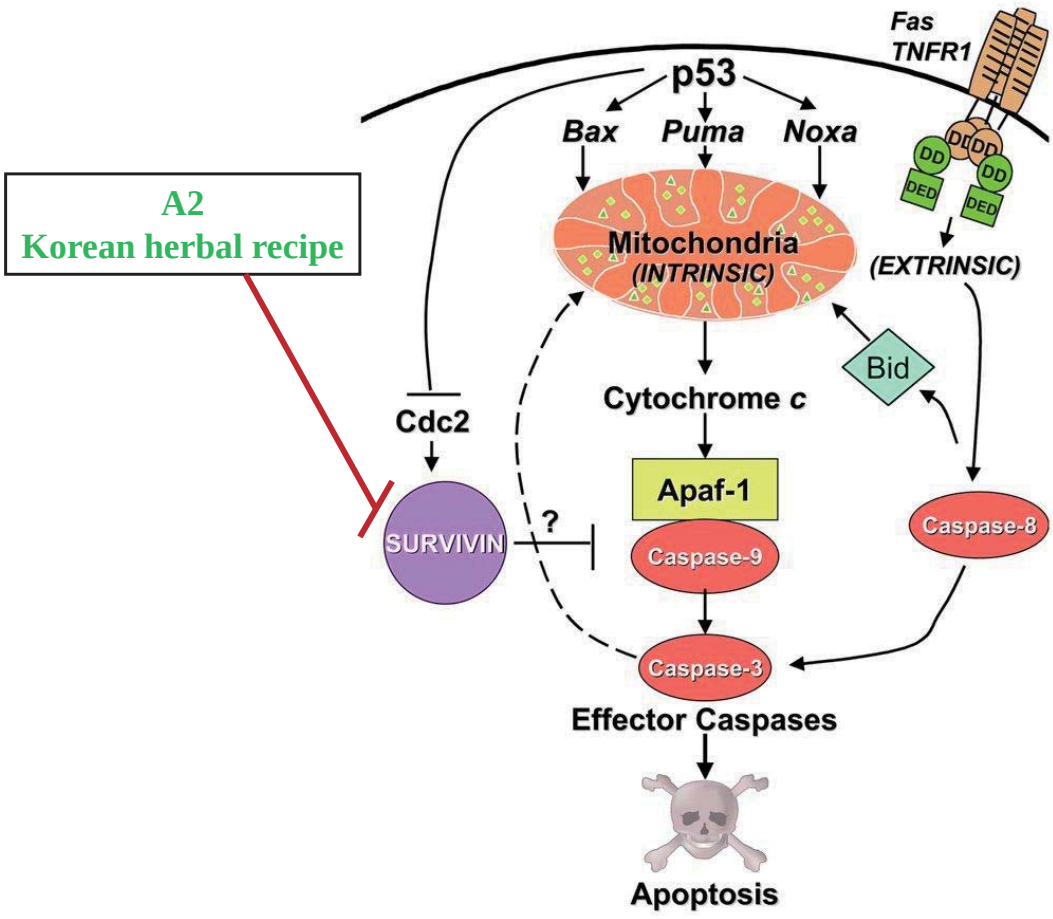


Drugs Fut 2003, 28(9): 881

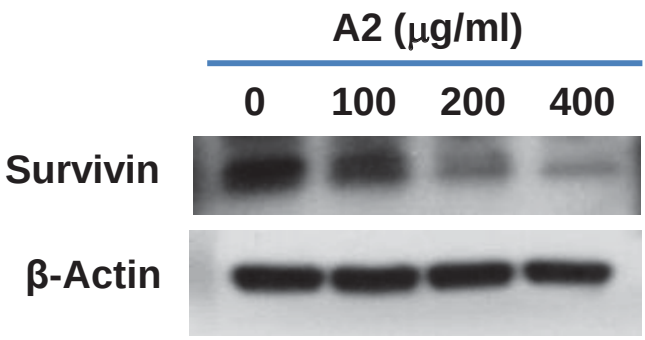
A2 increases the CDK inhibitors (p15 and p27) in MIA PaCa-2 pancreatic cancer cells (72 h of treatment)



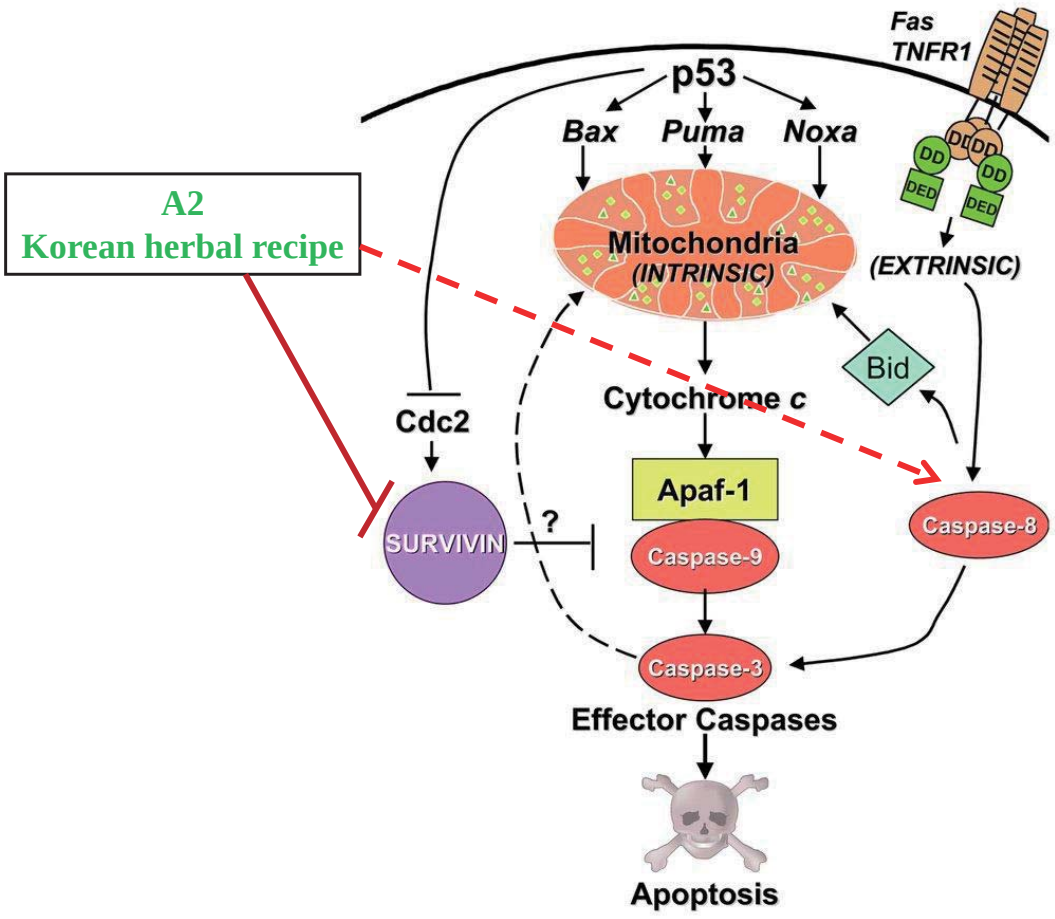
A2 promotes intrinsic and extrinsic apoptotic pathways in pancreatic cancer cells



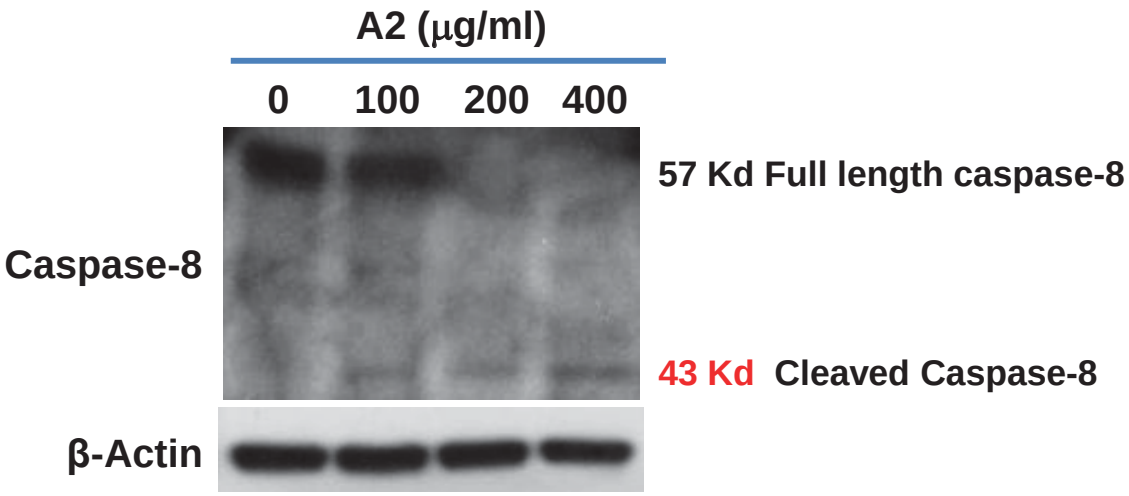
A2 decreases the survivin and increases of MIA PaCa-2 pancreatic cancer cells (72 h of treatment)



102.12.03

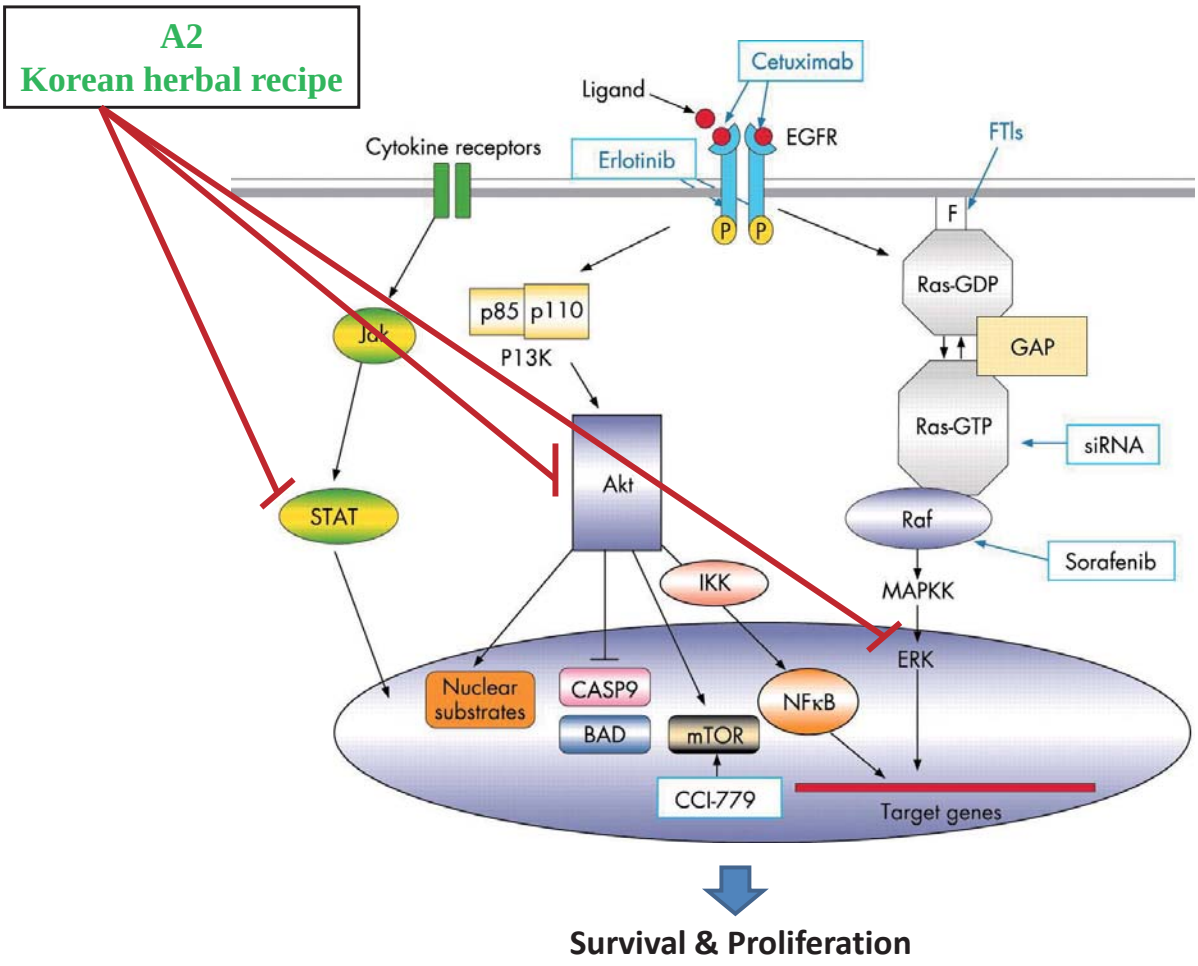


A2 decreases the full length caspase-8 and increases the cleaved caspase-8 in MIA PaCa-2 pancreatic cancer cells (72 h of treatment)

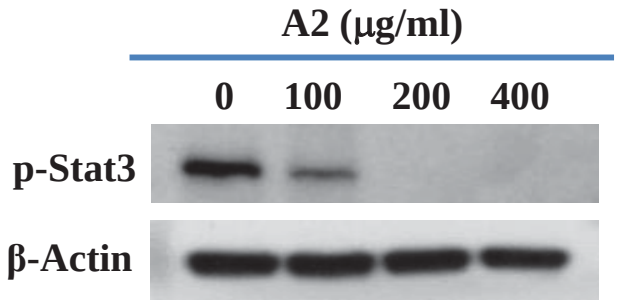


102.11.28

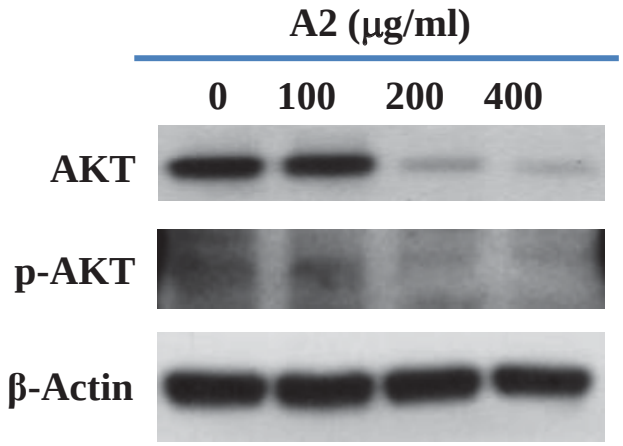
A2 inhibits the oncogenic signaling pathways in pancreatic cancer cells



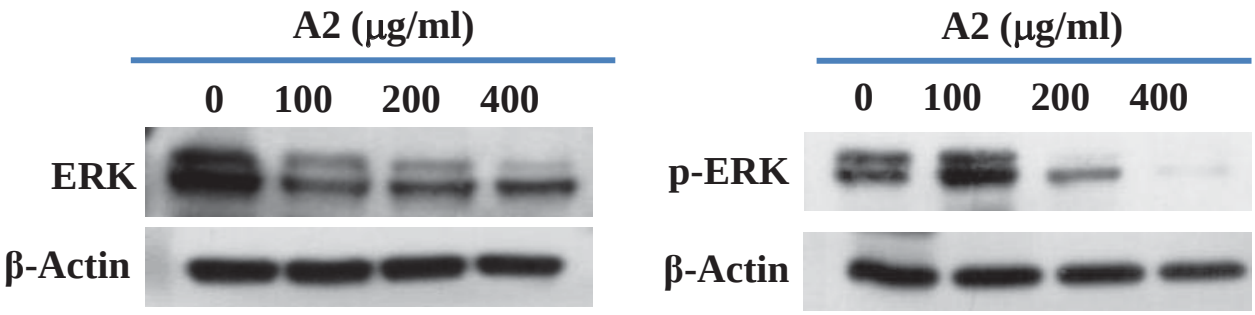
A2 decreases the activities of Stat3 signaling in MIA PaCa-2 pancreatic cancer cells (72 h of treatment)



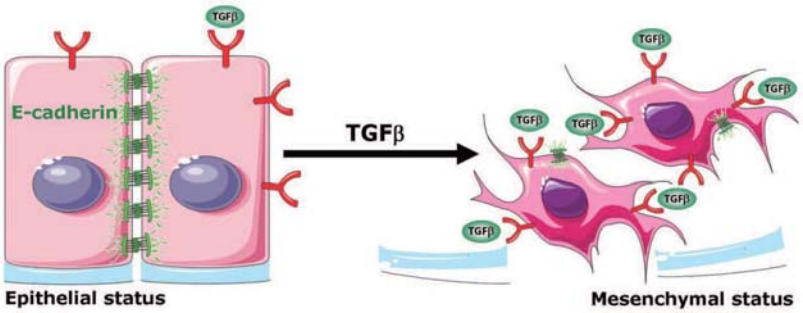
A2 decreases the activities of AKT signaling in MIA PaCa-2 pancreatic cancer cells (72 h of treatment)



A2 decreases the activities of ERK signaling in MIA PaCa-2 pancreatic cancer cells (72 h of treatment)



A2 inhibits EMT and cancer stem cell traits in pancreatic cancer cells



The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell* 2008, **133**(4):704-715.

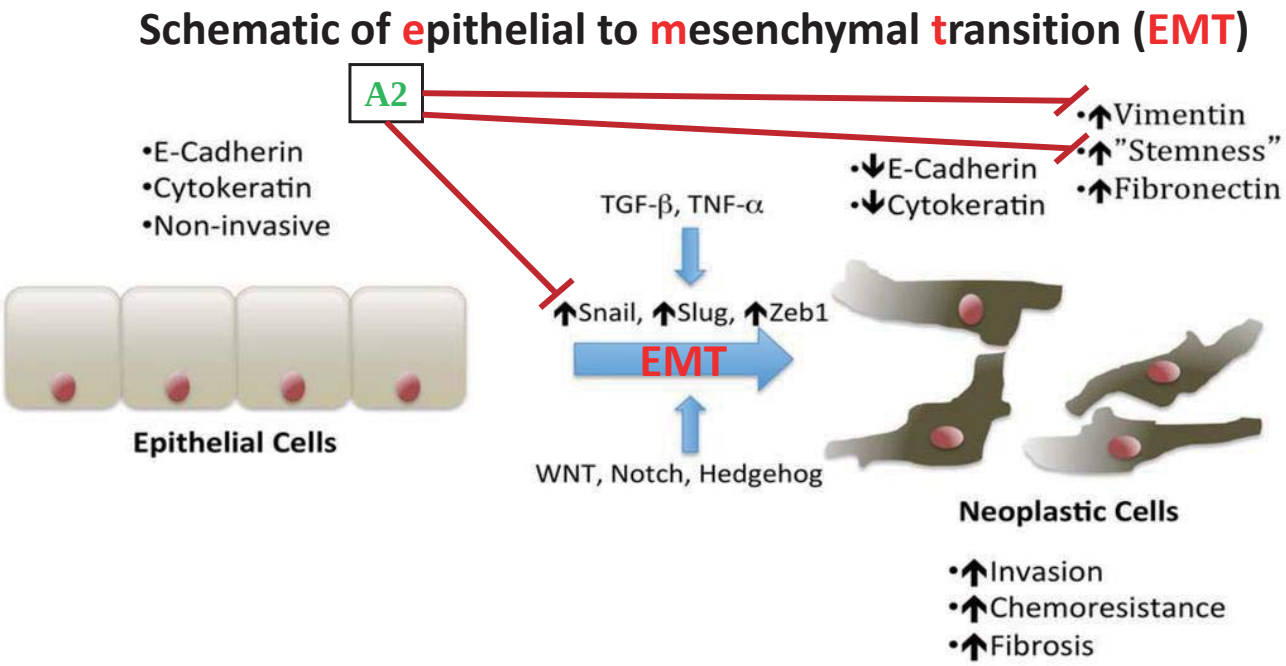
Contribution of epithelial-to-mesenchymal transition and cancer stem cells to pancreatic cancer progression. --- J Surg Res. 2012; 173: 105-12.

Pancreatic cancer cells surviving gemcitabine treatment express markers of stem cell differentiation and epithelial-mesenchymal transition. --- Int J Oncol. 2012; 41: 2093-102.

Chemoresistance is associated with cancer stem cell-like properties and epithelial-to-mesenchymal transition inpancreatic cancer cells. --- Anticancer Res. 2012; 32: 3847-3853.

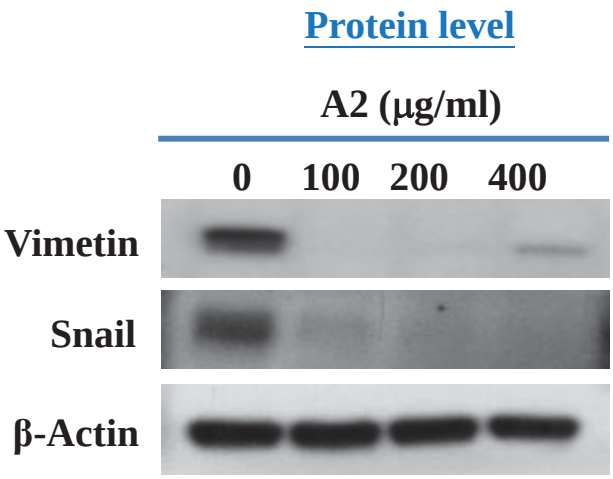
Pancreatic cancer stem cells: emerging target for designing novel therapy.
--- Cancer Lett. 2013; 338: 94-100.

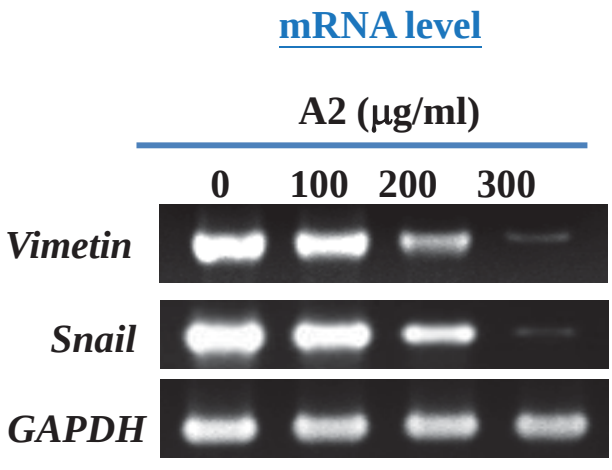
Cancers **2010**, *2*, 2084-2097



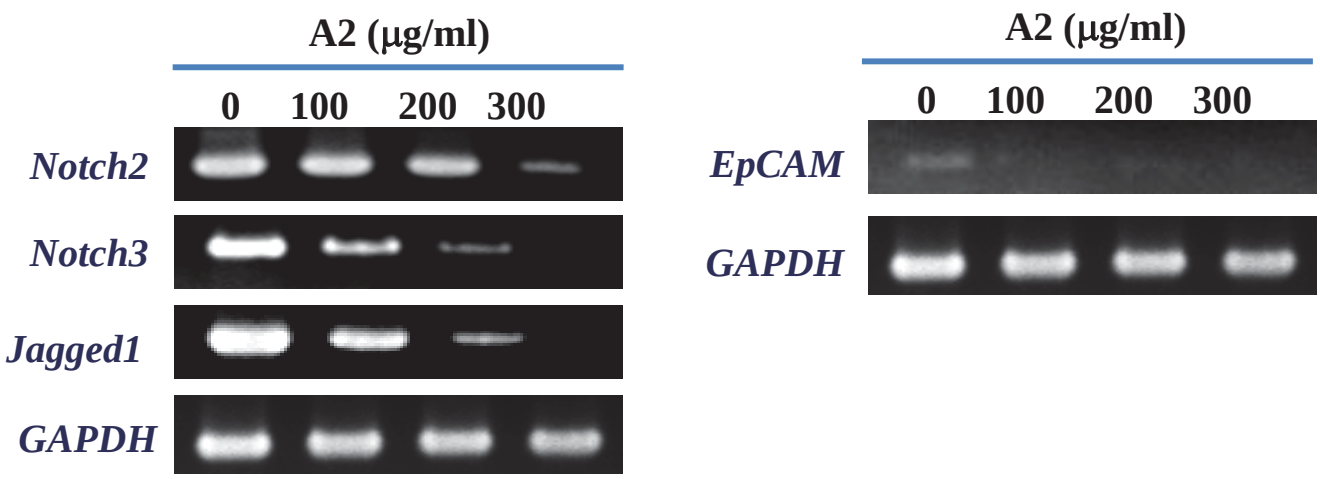
The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell* 2008, **133**(4):704-715.

A2 decreases the mesenchymal markers in MIA PaCa-2 pancreatic cancer cells (72 h of treatment)





A2 decreases the stemness genes (*Notch3*, *Jagged1* and *EpCAM*) in MIA PaCa-2 pancreatic cancer cells (72 h of treatment)

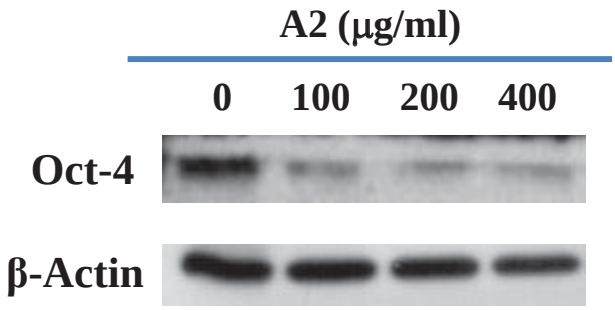


Pancreas. 2010 Jul;39(5):622-6. doi: 10.1097/MPA.0b013e3181c75f5e.
Oct4 and Nanog expression is associated with early stages of pancreatic carcinogenesis.

Knockdown of Oct4 and Nanog expression inhibits the stemness of pancreatic cancer cells

Cancer Letters Volume 340, Issue 1, 28 October 2013, Pages 113–123

A2 decreases the Oct-4 protein in MIA PaCa-2 pancreatic cancer cells (72 h of treatment)



Pancreatic cancer stem cells: emerging target for designing novel therapy.

--- Cancer Lett. 2013; 338: 94-100.

PLoS ONE 8(10): e78130.

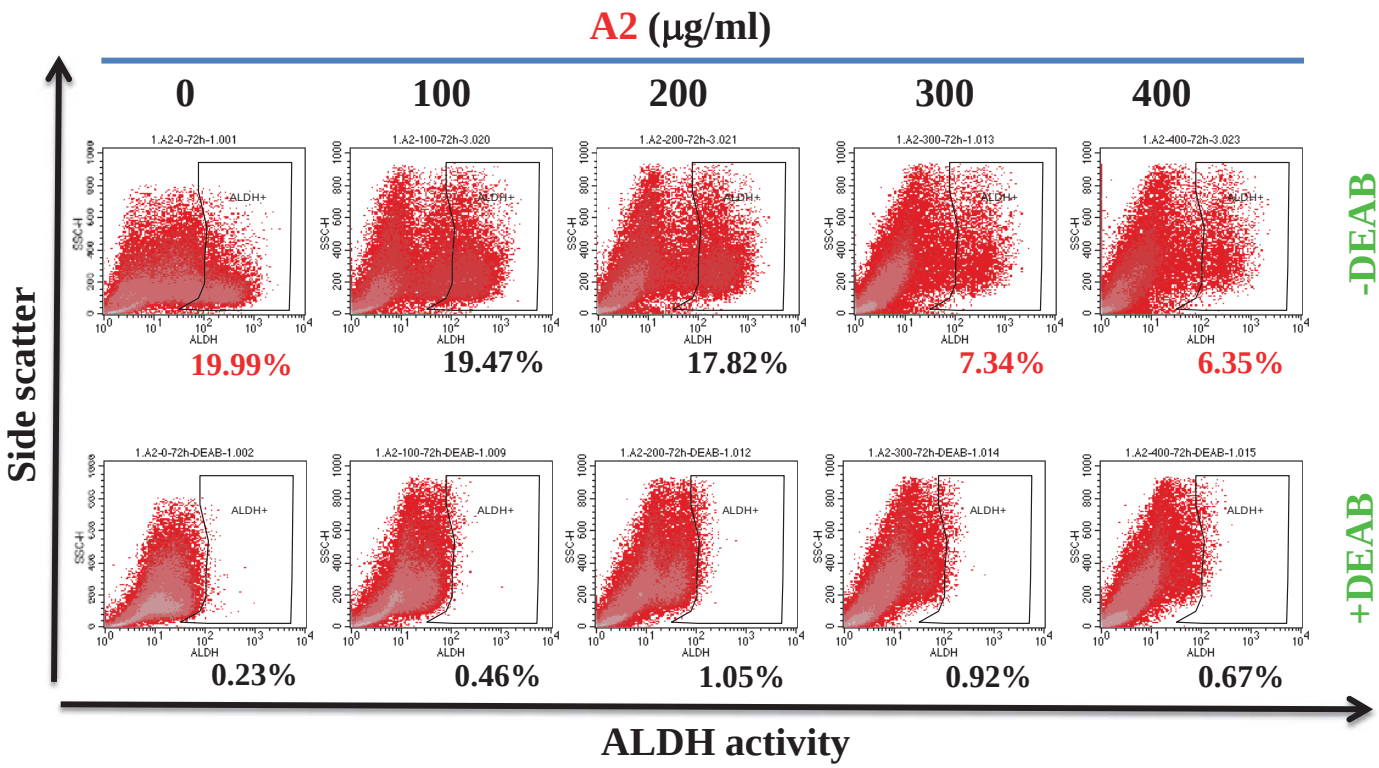
Reversing the Intractable Nature of Pancreatic Cancer by Selectively Targeting ALDH-High, Therapy-Resistant Cancer Cells

Sang Kyum Kim1*¤, Honsoul Kim2, Da-hye Lee3, Tae-shin Kim3, Tackhoon Kim3, Chaeuk Chung1, Gou Young Koh1, Hoguen Kim4, Dae-Sik Lim3*

Yonsei University, Seoul, Korea

We identified a subpopulation of cells in **pancreatic** ductal adenocarcinoma with cancer stem cell features that were enriched for aldehyde dehydrogenase (**ALDH**), a **marker** expressed in certain stem/progenitor cells.

A2 decreases the ALDH-positive cancer-stem-like cells in MIA PaCa-2 pancreatic cancer cells (72 h of treatment)

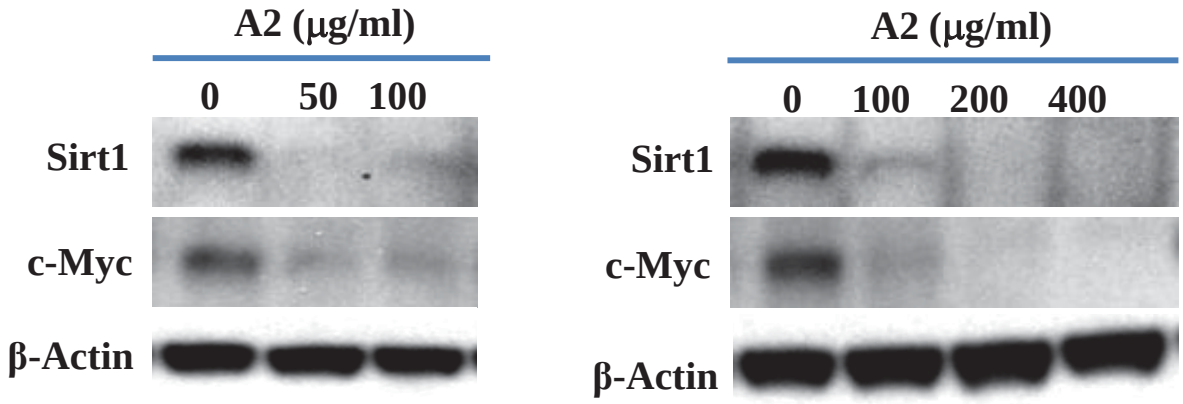


The percentage of ALDH+ population in each group is indicated below the dot plot without DEAB (Diethylaminobenzaldehyde).

- Effects of A2 on novel potential therapeutic targets of pancreatic cancer

- High SIRT1 expression is a negative prognosticator in pancreatic ductal adenocarcinoma. BMC Cancer. 2013 Oct 2;13:450. doi: 10.1186/1471-2407-13-450.
- The c-Myc plays a significant role in the progression and maintenance of pancreatic ductal adenocarcinoma. *Cancer Res*; **73**; 1821–30. 2012.

A2 decreases the potential targets for pancreatic cancer therapy such as c-Myc and Sirt1 (72 h of treatment)

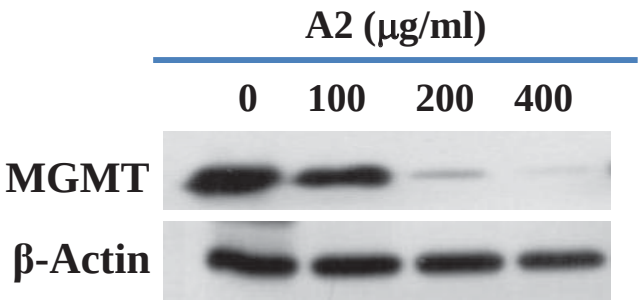


O^6 methyl guanine DNA methyl transferase (**MGMT**) is overexpressed in **pancreatic** cancer, and overexpression of MGMT is known to cause **drug-related resistance** to chemotherapeutic agents. --- Cancer Res. 2009 January 1; 15(1): 338–345.

***O*6-Methylguanine DNA Methyltransferase (MGMT) Deficiency and Response to Temozolomide-Based Therapy in Patients with Neuroendocrine Tumors**
--- Cancer Res. 2009 January 1; 15(1): 338–345.

Blockade of MGMT Expression by O^6 Benzyl Guanine Leads to Inhibition of Pancreatic Cancer **Growth** and Induction of **Apoptosis** --- Clin Cancer Res 2009;15(19):6087–95

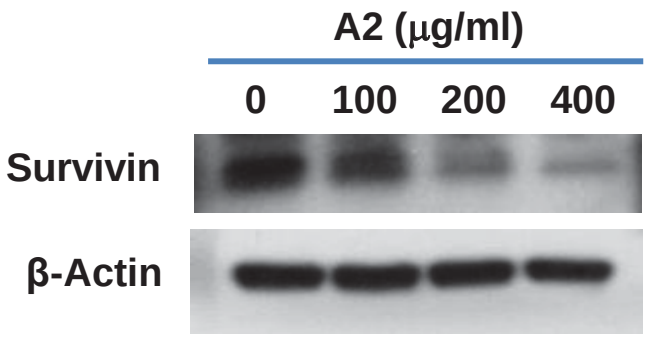
A2 decreases the MGMT in MIA PaCa-2 pancreatic cancer cells (72 h of treatment)



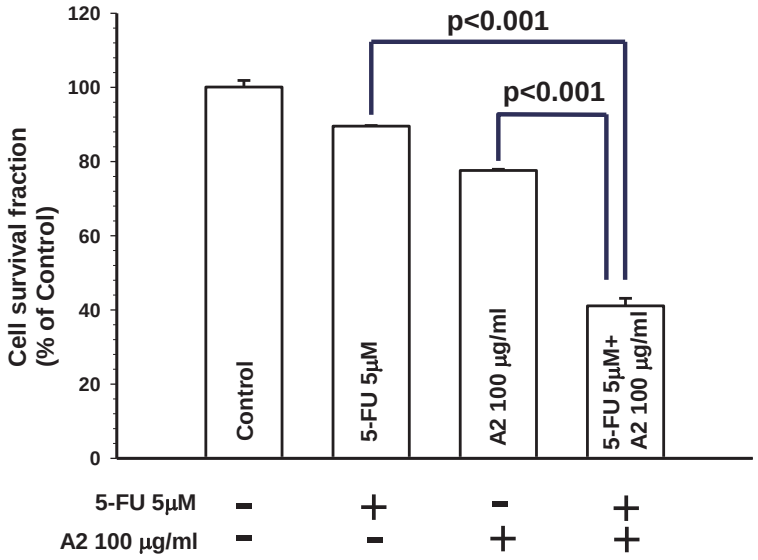
MGMT: O^6-Methylguanine DNA Methyltransferase

MGMT inhibition decreases survivin expression in pancreatic cancer.-- Mol Cancer Ther , November 2013 12; A251 Abstract A251:

A2 decreases the survivin and increases of MIA PaCa-2 pancreatic cancer cells (72 h of treatment)



MIA PaCa-2 pancreatic cancer cells / 72 h of treatment



Conclusions

- **A2 arrests the cell cycle of pancreatic cancer cells in S phase.**
- **A2 promotes intrinsic and extrinsic apoptotic pathway in pancreatic cancer cells.**
- **A2 inhibited oncogenic signaling pathways (AKT, ERK, Stat3).**
- **A2 inhibits EMT & cancer stem cell traits in pancreatic cancer cells.**
- **A2 decreases the novel potential therapeutic targets of pancreatic cancer (Sirt1, Myc and MGMT).**
- **These multiple targeting effects might be responsible for the clinical benefits observed in A2-treated pancreatic patients.**

Thank You

Molecular Cancer Therapeutics
mct.aacrjournals.org

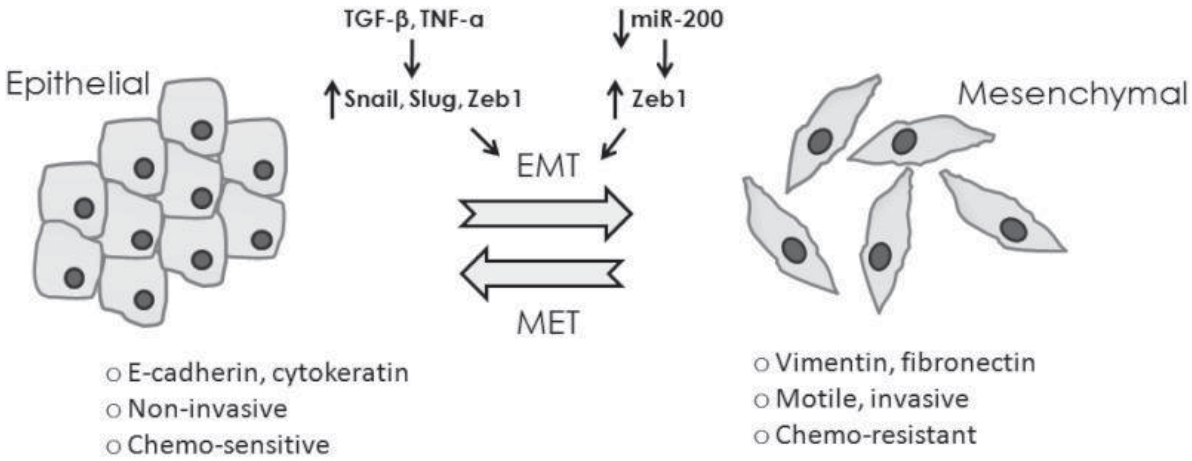
doi: 10.1158/1535-7163.TARG-13-A251
Mol Cancer Ther November 2013 12; A251

Abstract A251: MGMT inhibition decreases survivin expression in pancreatic cancer.

George C. Bobustuc[1], Anand Patel[2], Michael Thompson[1], Srivenugopal S. Kalkunte[3], Jacob Frick[1], James Weese[1], and Santhi D. Konduri[1]

Author Affiliations

Chemo / Radio - sensitization?



EMT regulation in pancreatic cancer. Some of the main drivers of EMT in pancreatic cancer are transcription factors Snail, Slug and Zeb1, which are in turn regulated by cytokines (NF-α) and growth factors (TGF-β) as well as microRNAs. These signaling molecules transform non-invasive and chemo-sensitive cells into motile and invasive chemo-resistant cells that have stem-cell-like properties.

Pancreatic Cancer and Tumor Microenvironment.
Grippo PJ, Munshi HG, editors.
Trivandrum (India): Transworld Research Network; 2012.

MULTIPLE MOLECULAR TARGETING ANTICANCER EFFECTS ON MIA-PACA-2 HUMAN PANCREATIC CANCER CELLS BY KOREAN HERBAL RECIPE A2

Chih-Jung Yao[1, 2], Tae-Young Han[3], Wan-Ju Chao[4], Gi-Ming Lai[1, 2, 4]

[1]Division of Hematology and Medical Oncology, Wan Fang Hospital, Taipei Medical University, Taiwan
[2]Comprehensive Cancer Center of Taipei Medical University, Taiwan
[3]Banronginsu Oriental Medicine Clinic, Korea
[4]National Institute of Cancer Research, National Health Research Institutes, Taiwan

ABSTRACT

Purpose: A Korean herbal recipe A2 had been found to exert therapeutic effects in pancreatic cancer patients with "Shao Yin" constitution according to the oriental Sasang Constitutional Medicine. However, little is known about the underlying molecular mechanism. This study explores the effects of A2 on a poorly differentiated pancreatic cancer cell line MIA PaCa-2 to elucidate the molecular mechanism underlying its clinical effects.

Methods: The A2 was provided by Dr. Han, Banronginsu Oriental Medicine Clinic. Cell viability was determined by SRB assay. The cell-cycle distribution was analyzed by flow cytometry. Aldefluor® assays were used to analyze the cancer stem-like ALDH+ population. RT-PCR was used to evaluate the mRNA expression. Western blot and antibody against specific phosphorylated protein was employed to investigate the affected signaling pathways.

Results: After 72 h of treatment, A2 increased S phase percentage and induced apoptosis of MIA PaCa-2 cells accompanied by caspase-3 and -8 activation and Survivin inhibition. A2 decreased not only the signaling proteins like p-Stat3, p-AKT and p-ERK but also the potential targets for pancreatic cancer therapy such as c-Myc and Sirt1. Moreover, consistent with the decrease of ALDH+ population, repression of stemness genes (*Notch3, Jagged1* and *EpCAM*) and Vimentin and Snail that related to epithelial-mesenchymal transition (EMT) were observed in A2-treated cells.

Conclusions: During apoptosis induction in pancreatic cancer cells, A2 exerted effects on cell-cycle regulation, signaling inhibition as well as suppression of EMT and stemness markers. These multiple targeting effects might be responsible for the clinical benefits observed in A2-treated pancreatic patients.

Pancreatic ductal adenocarcinoma is a highly aggressive disease that invariably evades early diagnosis [2]. The mean survival time for patients with metastatic disease is only 3–6 months, and only 20–30% of pancreatic cancer cases are alive after 12 months.

Pathway Central: Cellular Apoptosis Pathway - SABiosciences
www.sabiosciences.com › Resources › Pathway Central
翻譯這個網頁
In the first pathway Caspase8 cleaves BID (Bcl2 Interacting Protein) and its ... The Intrinsic Apoptosis pathway begins when an injury occurs within the cell.

- Nicotinamide prohibits proliferation and enhances chemosensitivity of pancreatic cancer cells through deregulating SIRT1 and Ras/Akt pathways. *Pancreatology*, **13**, 140-146, 2013.
- High SIRT1 expression is a negative prognosticator in pancreatic ductal adenocarcinoma. BMC Cancer. 2013 Oct 2;13:450. doi: 10.1186/1471-2407-13-450.
- The c-Myc plays a significant role in the progression and maintenance of pancreatic ductal adenocarcinoma. *Cancer Res*; **73**; 1821–30. 2012.

J Surg Res. 2012 Mar;173(1):105-12. doi: 10.1016/j.jss.2011.09.020. Epub 2011 Oct 8.

Contribution of epithelial-to-mesenchymal transition and cancer stem cells to pancreatic cancer progression.

Krantz SB[1], Shields MA, Dangi-Garimella S, Munshi HG, Bentrem DJ.

Abstract

Pancreatic adenocarcinoma remains among the most lethal of human malignancies. Overall 5-y survival is less than 5%, and only 20% of patients presenting with localized disease amenable to surgical resection. Even in patients who undergo resection, long-term survival remains extremely poor. A major contributor to the aggressiveness of multiple cancers, and pancreatic cancer in particular, is the process of epithelial-to-mesenchymaltransition (EMT). This review highlights the growing evidence of EMT in pancreatic cancer progression, focusing on the contribution of EMT to the development of cancer stem cells and on interaction of EMT with other pathways central to cancer progression, such as Hedgehog signaling, the K-ras oncogene, and transforming growth factor-beta (TGF-β). We will also discuss EMT-targeting agents currently in development and in clinical trials that may help to reduce the morbidity and mortality associated with pancreatic cancer.

Int J Oncol. 2012 Dec;41(6):2093-102. doi: 10.3892/ijo.2012.1648. Epub 2012 Oct 1.

Pancreatic cancer cells surviving gemcitabine treatment express markers of stem cell differentiation and epithelial-mesenchymal transition.

Objective response rates to standard chemotherapeutic regimens remain low in pancreatic cancer. Subpopulations of cells have been identified in various solid tumors which express stem cell-associated markers and are associated with increased resistance against radiochemotherapy. We investigated the expression of stem cell genes and markers of epithelial-mesenchymal transition in pancreatic cancer cells that survived high concentrations of gemcitabine treatment. Capan-1 and Panc-1 cells were continuously incubated with 1 and 10 μM gemcitabine. Surviving cells were collected after 1, 3 and 6 days. Expression of PDX-1, SHH, CD24, CD44, CD133, EpCAM, CBX7, OCT4, SNAIL, SLUG, TWIST, Ki-67, E-cadherin, β-catenin and vimentin were quantified by qPCR or immunocytochemistry. Migration was assessed by wound-healing assay. SHH was knocked down using RNA interference. Five primary pancreatic cancer cell lines were used to validate the qPCR results. All investigated genes were upregulated after 6 days of gemcitabine incubation. Highest relative expression levels were observed for OCT4 (13.4-fold), CD24 (47.3-fold) and EpCAM (15.9-fold) in Capan-1 and PDX-1 (13.3-fold), SHH (24.1-fold), CD44 (17.4-fold), CD133 (20.2-fold) and SLUG (15.2-fold) in Panc-1 cells. Distinct upregulation patterns were observed in the primary cells. Migration was increased in Panc-1 cells and changes in the expression of E-cadherin and β-catenin were typical of epithelial-mesenchymal transition in both cell lines. SHH knockdown reduced IC(50) from 30.1 to 27.6 nM in Capan-1 while it strongly inhibited proli-feration in Panc-1 cells. Cells surviving high-dose gemcitabine treatment express increased levels of stem cell genes, show characteristics associated with epithelial-mesenchymal transition and retain their proliferative capacity.

Anticancer Res. 2012 Sep;32(9):3847-53.

Chemoresistance is associated with cancer stem cell-like properties and epithelial-to-mesenchymal transition inpancreatic cancer cells.

Izumiya M[1], Kabashima A, Higuchi H, Igarashi T, Sakai G, Iizuka H, Nakamura S, Adachi M, Hamamoto Y, Funakoshi S, Takaishi H, Hibi T.

Author information

Abstract

BACKGROUND:

The aim of this study was to evaluate whether apoptosis-resistant cancer cells have cancer stem cell (CSC)-like properties.

MATERIALS AND METHODS:

Panc-1 pancreatic cancer cells were incubated in the presence of 5-fluorouracil (5-FU) for 24 h, and further incubated without 5-FU for 28 days. To assess the capacity of self-renewal, surviving cells were planted for sphere-forming assay. Epithelial-to-mesenchymal transition (EMT) was induced with TGF-β, then mRNA expression was evaluated by real-time PCR for E-cadherin, SNAIL, and vimentin. The E-Cadherin protein levels were also examined by immunoblot analysis. The Local invasion ability was analyzed by Matrigel invasion assay.

RESULTS:

The frequency of cells that were capable of initiating spheres was higher in 5-FU-pre treated cells, which also overexpressed stem cell marker genes, OCT4 and NANOG. Matrigel invasion activity of apoptosis-resistant Panc-1 cells was greater than that of control Panc-1 cells.

CONCLUSION:

Apoptosis-resistant cancer cells have CSC-like properties, i.e., able to initiate sphere formation, express stem cell genes, and

Cancer Lett. 2013 Sep 10;338(1):94-100. doi: 10.1016/j.canlet.2012.03.018. Epub 2012 Mar 20.

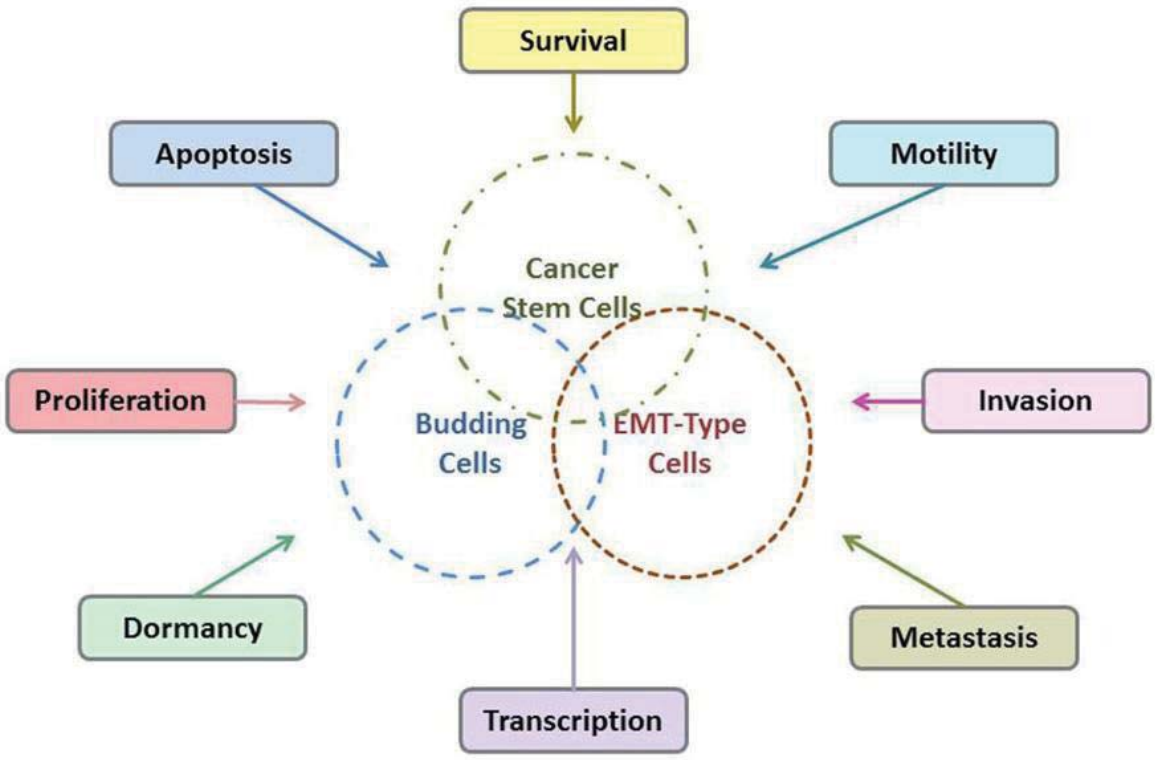
Pancreatic cancer stem cells: emerging target for designing novel therapy.

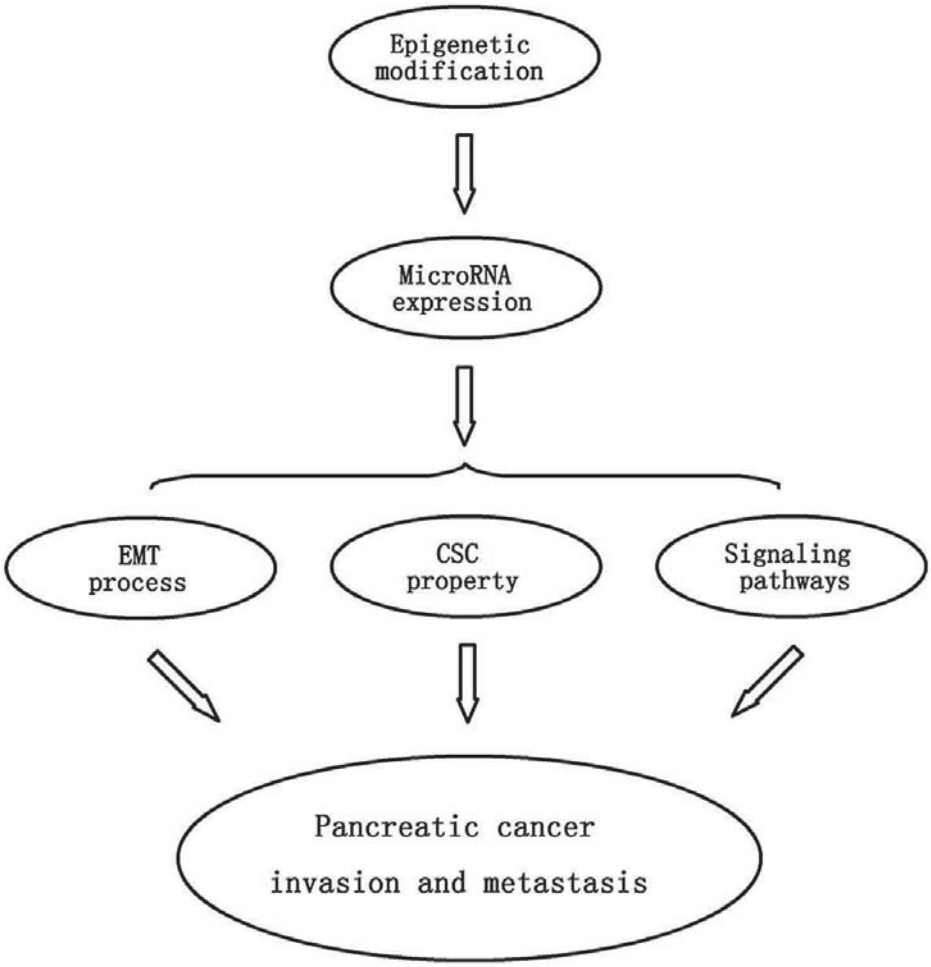
Li Y[1], Kong D, Ahmad A, Bao B, Sarkar FH.

Author information

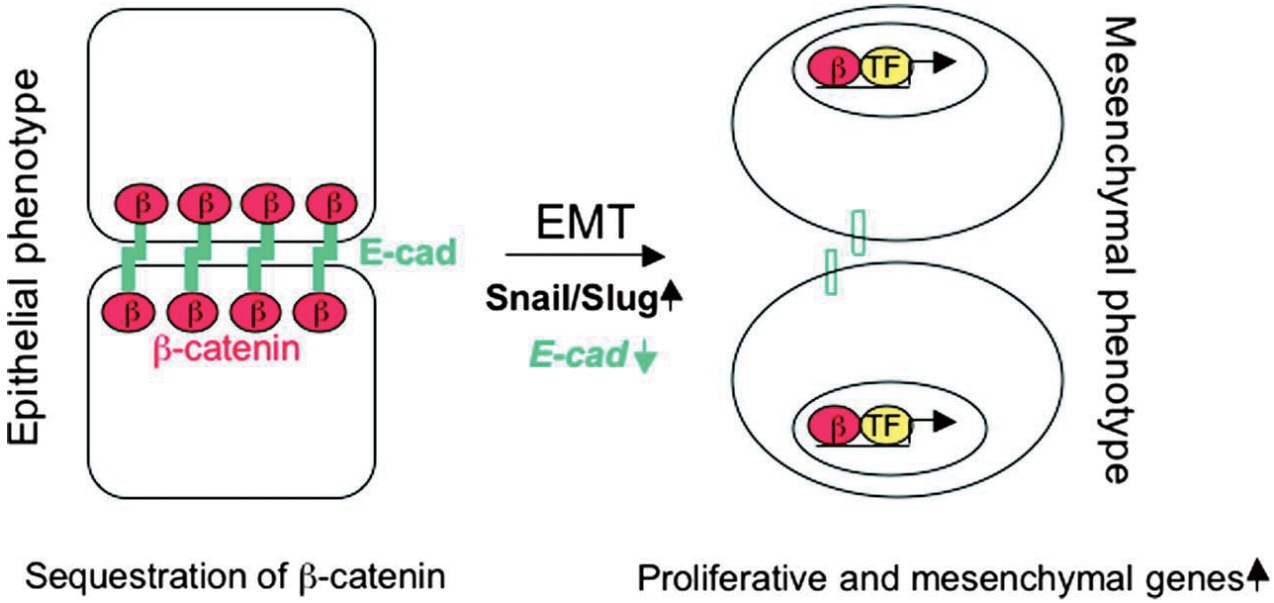
Abstract

In the past few years, there have been significant advances in the research on cancer stem cells (CSCs). The emerging evidences have demonstrated that CSCs and epithelial-mesenchymal transition (EMT)-type cells, which share molecular characteristics with CSCs, play critical roles in drug resistance, invasion, and metastasis. Pancreatic cancer (PC) has a high mortality due to both intrinsic (de novo) and extrinsic (acquired) drug resistance, leading to increased invasive and metastatic potential of PC cells. Therefore, targeting pancreatic CSCs and EMT-type cells could be a novel therapeutic strategy for the treatment of PC. In this article, we will review the current state of our knowledge on the role of pancreatic CSCs and EMT-type cells, and summarize the novel therapeutic strategies that could target pancreatic CSCs and EMT-type cells, leading to the reversal of EMT phenotype, the induction of drug sensitivity, and the inhibition of invasion and metastasis of PC, which is expected to yield better treatment outcome.





EXPERIMENTAL AND THERAPEUTIC MEDICINE 4: 181-187, 2012



Pancreas. 2010 Jul;39(5):622-6. doi: 10.1097/MPA.0b013e3181c75f5e.

Oct4 and Nanog expression is associated with early stages of pancreatic carcinogenesis.

Wen J[1], Park JY, Park KH, Chung HW, Bang S, Park SW, Song SY.

Author information

Abstract

OBJECTIVE:

To characterize the role of Oct4 and Nanog, two important homeobox transcription factors of embryonic development, in pancreaticcarcinogenesis.

METHODS:

Using a tissue microarray of human pancreatic carcinoma and adjacent noncancerous tissues as well as the N-nitrosobis(2-oxopropyl)amine-induced Syrian golden hamster pancreatic cancer model, we characterized the expression of Oct4 and Nanog. The presence of K-ras mutation with the time course of carcinogenesis in hamster model was also evaluated.

RESULTS:

Oct4 expression in metaplastic ducts was significantly stronger than in normal acini and pancreatic carcinoma ($P < 0.05$). Of 24 cases, 19 (79.2%) showed a strong Oct4 expression in metaplastic ducts. In contrast, only 6 (19.4%) of 31 cancer tissues and 3 (16.7%) of 18 noncancer tissues showed a strong Oct4 expression. Nanog also showed similar patterns as Oct4. Restriction fragment length polymorphism-polymerase chain reaction showed the overt K-ras mutation after the expression of Oct4 in the hamster model.

CONCLUSIONS:

The strong expression of Oct4 and Nanog in metaplastic ducts and Oct4 expression preceding Ras mutation suggests that these homeobox transcription factors are associated with the early stage of pancreatic cancer carcinogenesis and may play an important role in that process.

Knockdown of Oct4 and Nanog expression inhibits the stemness of pancreatic cancer cells

Cancer Letters Volume 340, Issue 1, 28 October 2013, Pages 113–123

Pancreatic cancer is notorious for its difficult diagnosis at early stage and poor recurrence-free prognosis. This study aimed to investigate the possible involvement of Oct4 and Nanog in pancreatic cancer. The high expressions of Oct4 and Nanog in human pancreatic cancer tissues were found to indicate a worse prognostic value of patients. The pancreatic cancer stem cells (PCSCs) that isolated from PANC-1 cell line by flow cytometry exhibited high expressions of Oct4 and Nanog. To investigate whether Oct4 and Nanog play crucial role in maintaining the stemness of PCSCs, double knockdown of Oct4 and Nanog demonstrated that Oct4 and Nanog significantly reduced proliferation, migration, invasion, chemoresistance, and tumorigenesis of PCSCs in vitro and in vivo. The altered expression of the genes related to pancreatic carcinogenesis, metastasis, drug resistance and epithelial–mesenchymal transdifferentiation (EMT) might affect the biological characteristics of PCSCs. Our results suggest that Oct4 and Nanog may serve as a potential marker of prognosis and a novel target of therapy for pancreatic cancer.

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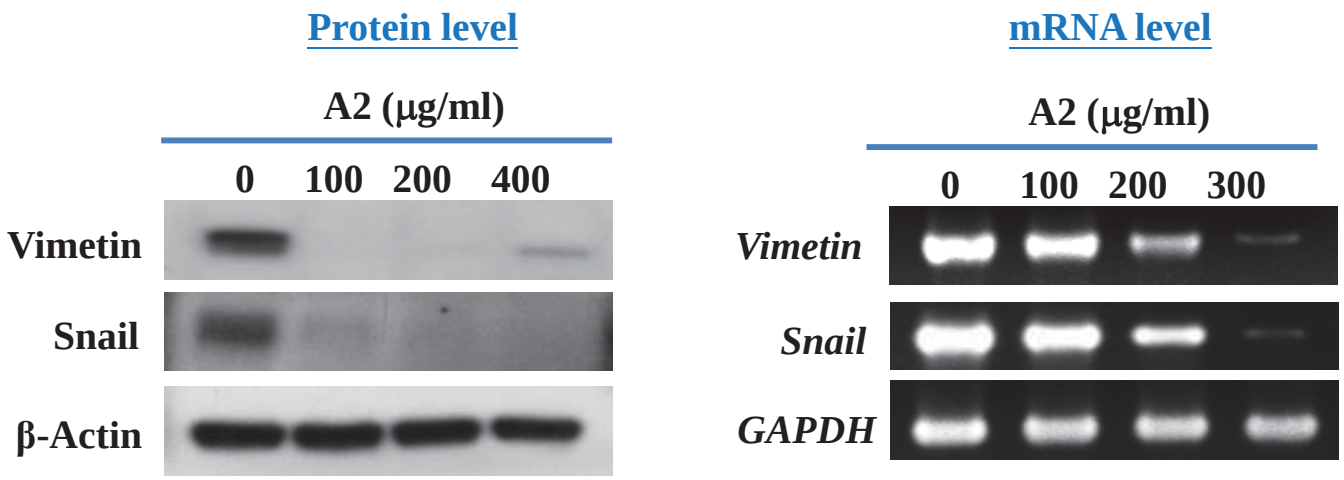
Knockdown of Oct4 and Nanog expression inhibits the stemness of pancreatic cancer cells

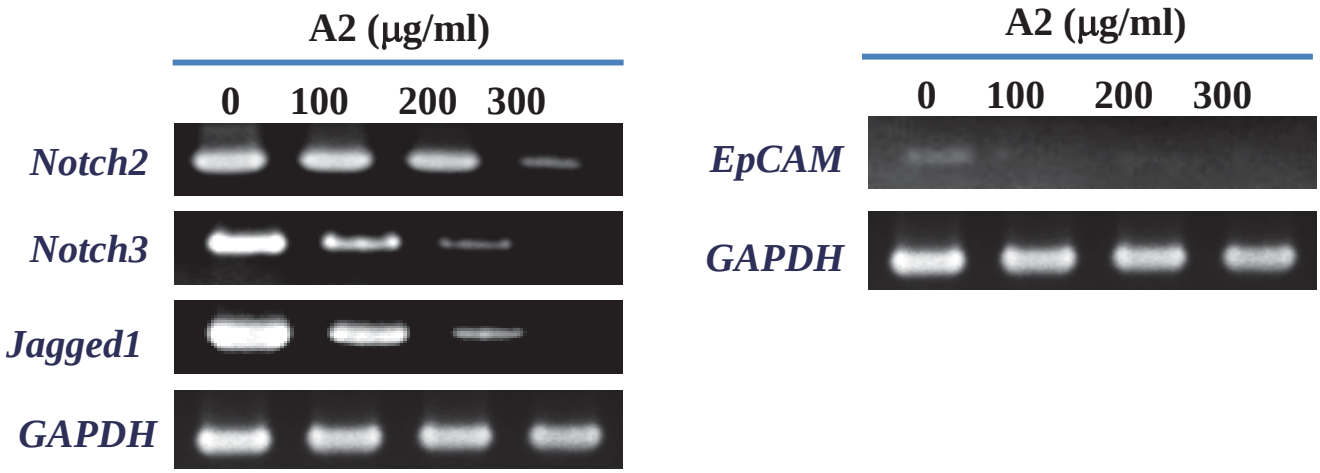
Cancer Letters Volume 340, Issue 1, 28 October 2013, Pages 113–123

EMT (epithelial to mesenchymal transition) **inhibition?**
Cancer stem cell elimination?

A2 decreases the mesenchymal markers in MIA PaCa-2 pancreatic cancer cells (72 h of treatment)



A2 decreases the stemness genes (*Notch3*, *Jagged1* and *EpCAM*) in MIA PaCa-2 pancreatic cancer cells (72 h of treatment)

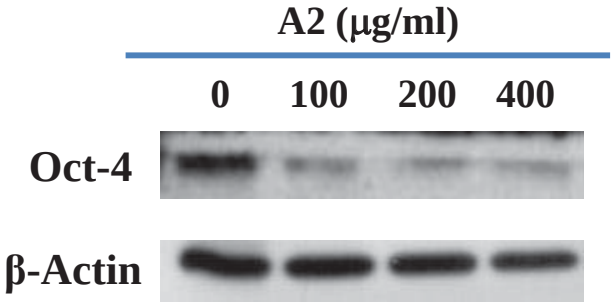


Pancreas. 2010 Jul;39(5):622-6. doi: 10.1097/MPA.0b013e3181c75f5e.
Oct4 and Nanog expression is associated with early stages of pancreatic carcinogenesis.

Knockdown of Oct4 and Nanog expression inhibits the stemness of pancreatic cancer cells

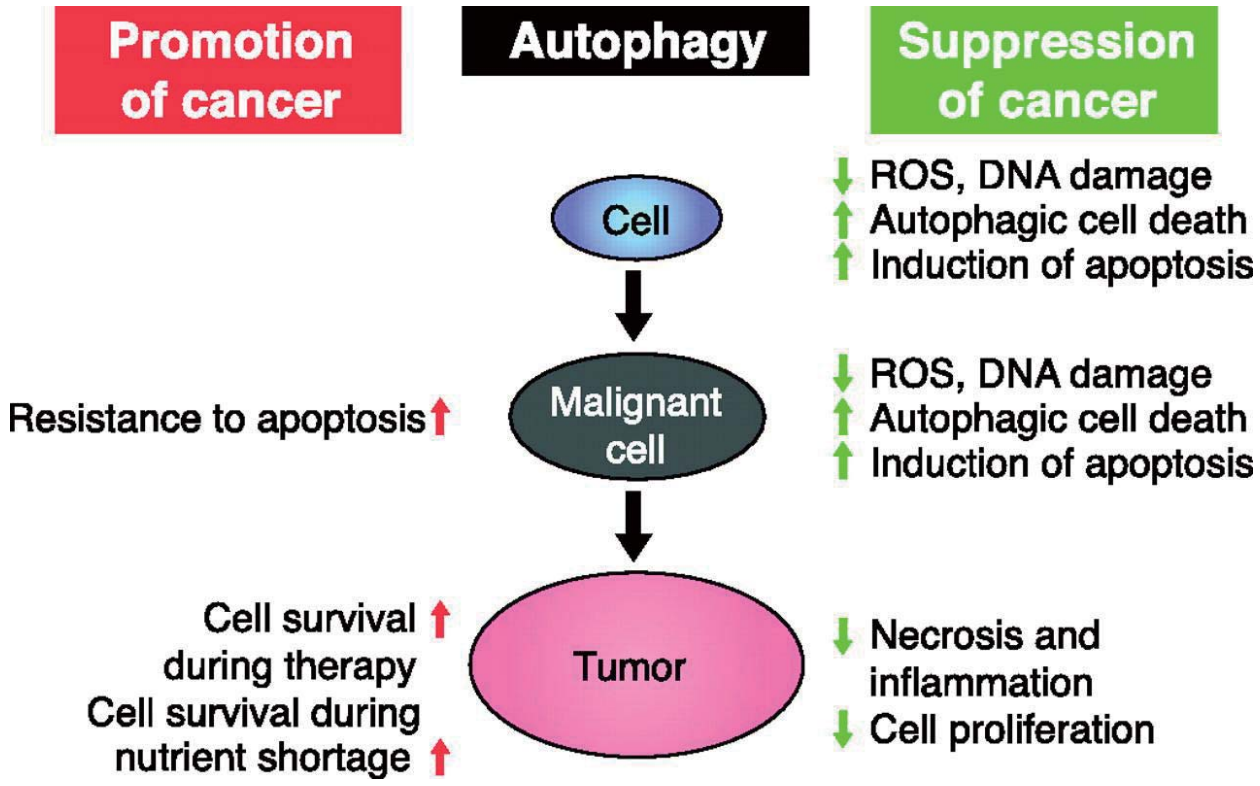
Cancer Letters Volume 340, Issue 1, 28 October 2013, Pages 113–123

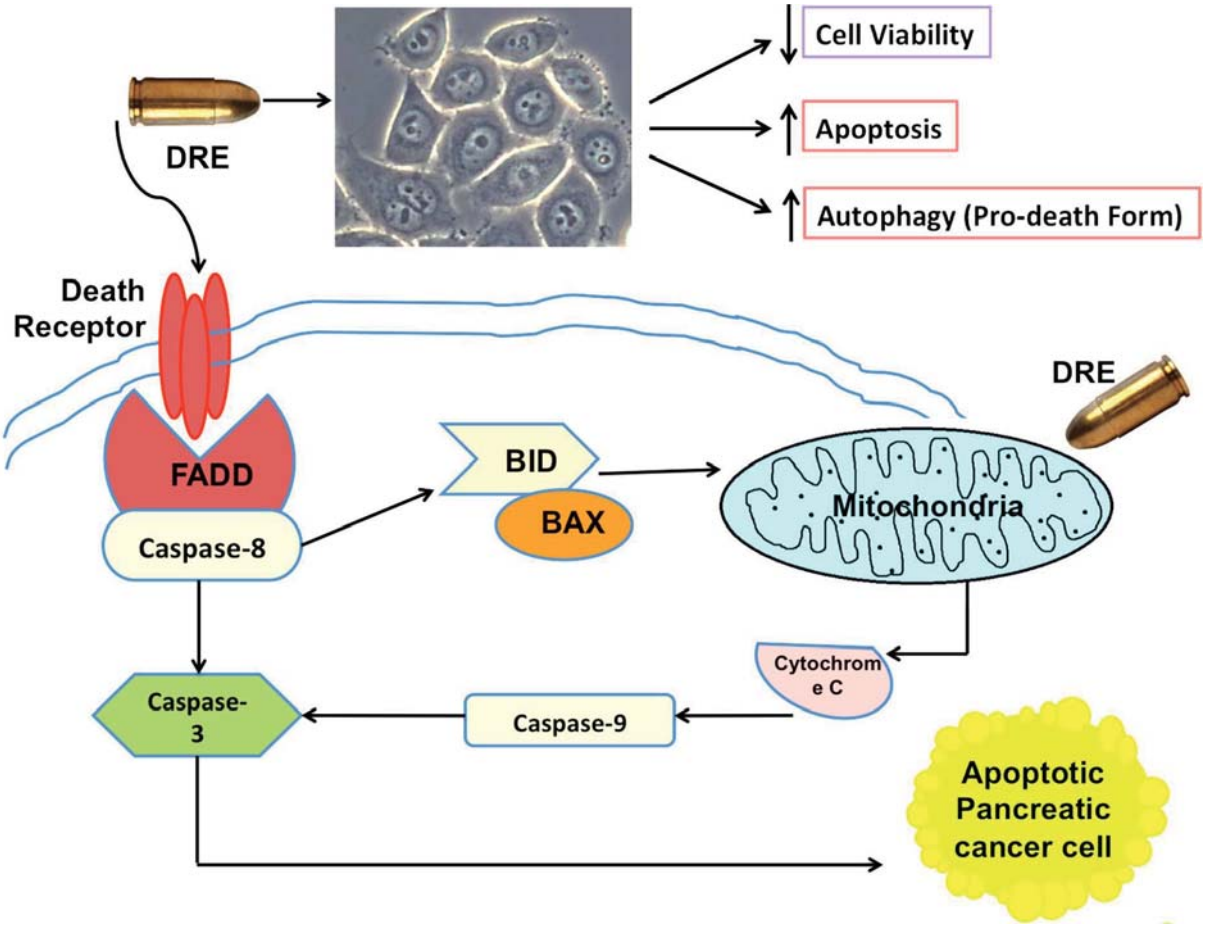
A2 decreases the Oct-4 protein in MIA PaCa-2 pancreatic cancer cells (72 h of treatment)



Blockade of **MGMT** Expression by O6 Benzyl Guanine Leads to Inhibition of **Pancreatic Cancer** Growth and Induction of Apoptosis ---Clin Cancer Res 2009;15(19):6087–95.

Purpose: We sought to determine whether administration of a MGMT blocker, O6-benzyl guanine (O6BG), at an optimal biological dose alone or in combination with gemcitabine inhibits human pancreatic cancer cell growth.
Experimental Design: Human pancreatic cancer L3.6pl and PANC1 cells were treated with O6BG, either alone or in combination with gemcitabine, and the therapeutic efficacy and biological activity of these drug combinations were investigated.
Results: **O6BG sensitized pancreatic cancer cells to gemcitabine**. Protein and mRNA expression of MGMT, cyclin B1, cyclin B2, cyclin A, and ki-67 were significantly decreased in the presence of O6BG. In sharp contrast, protein expression and mRNA message of p21cip1 were significantly increased. Interestingly, O6BG increases p53-mediated p21cip1 transcriptional activity and suppresses cyclin B1. In addition, our results indicate that p53 is recruited to p21 promoter. Furthermore, an increase in p21cip1 and a decrease in cyclin transcription are p53 dependent. The volume of pancreatic tumors was reduced by 27% in mice treated with gemcitabine alone, by 47% in those treated with O6BG alone, and by 65% in those mice given combination. Immunohistochemical analysis showed that O6BG inhibited expression of MGMT and cyclins, and increased expression of p21cip1. Furthermore, there was a significant decrease in tumor cell proliferation and an increase in tumor cell apoptosis. Conclusions: Collectively, our results show that decreased MGMT expression is correlated with p53 activation, and significantly reduced primary pancreatic tumor growth. These findings suggest that O6BG either alone or in combination with gemcitabine may provide a novel and effective approach for the treatment of human pancreatic cancer.





Pancreas. 2012 Oct;41(7):1039-47. doi: 10.1097/MPA.0b013e31824b22a2.

Selective induction of apoptosis and autophagy through treatment with dandelion root extract in hu manpancreatic cancer cells.

Ovadje P[1], Chochkeh M, Akbari-Asl P, Hamm C, Pandey S.

Author information

Abstract

OBJECTIVES:

Pancreatic cancer has a 100% mortality rate; the aim of this study is to evaluate the efficacy of dandelion root extract (DRE) in inducing apoptosis and autophagy in aggressive and resistant pancreatic cancer cells.

METHODS:

The effect of DRE was evaluated using WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate) assay. Apoptotic cell death was confirmed by nuclear condensation by Hoechst staining and externalization of phosphatidylserine to the outer leaflet of the plasma membrane by Annexin-V binding assay. Loss of mitochondrial membrane potential was observed using the JC-1 (5,5',6, 6'-tetrachloro-1,1',3,3' tetraethylbenzimidazolylcarbocyanine iodide) dye.

The induction of autophagy was detected using a monodansylcadaverine assay and this was confirmed by immunofluorescence for light chain 3-II.

RESULTS:

BxPC-3 and PANC-1 pancreatic cells were sensitive to aqueous DRE.

This extract induces selective apoptosis in a dose- and time-dependent manner. Dandelion root extract caused the collapse of the mitochondrial membrane potential, leading to prodeath autophagy. Normalhuman fibroblasts were resistant at similar doses.

CONCLUSIONS:

We demonstrate that DRE has the potential to induce apoptosis and autophagy in human pancreatic cancer cells with no significant effect on noncancerous cells. This will provide a basis on which further esearch in cancer treatment through DRE can be executed.

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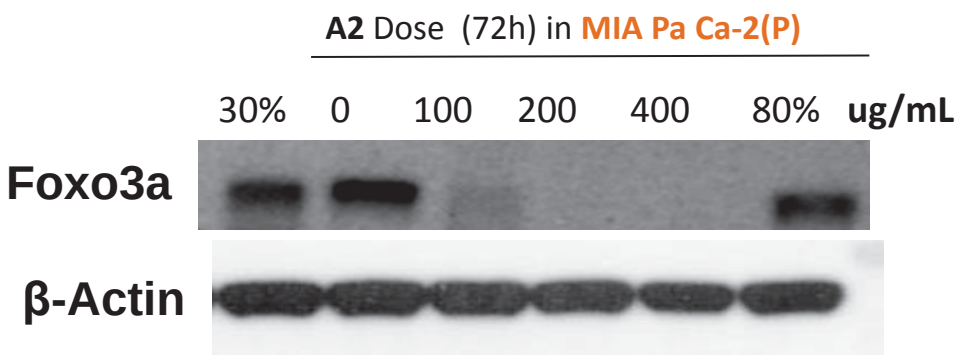
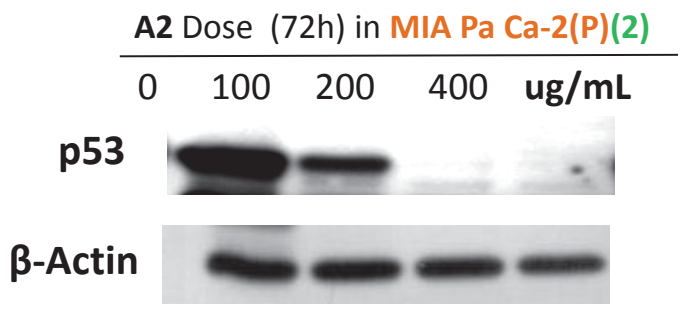
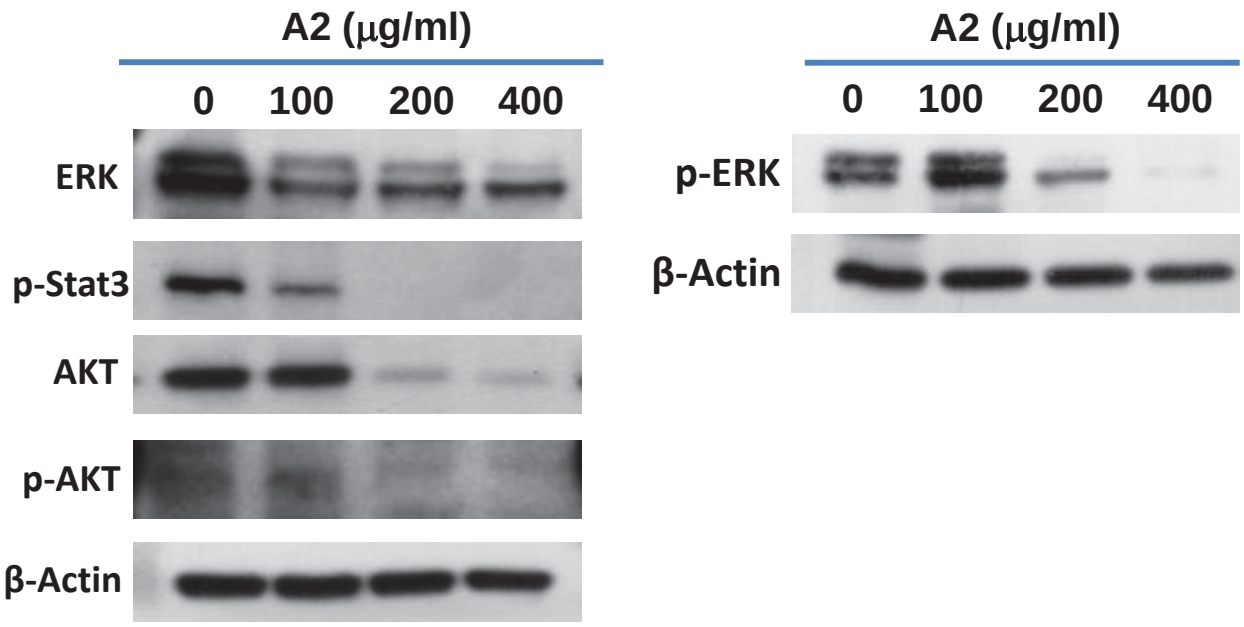




A2 decreases the activities of signaling proteins in MIA PaCa-2 pancreatic cancer cells (72 h of treatment)



MULTIPLE MOLECULAR TARGETING ANTICANCER EFFECTS ON MIA-PACA-2 HUMAN PANCREATIC CANCER CELLS BY KOREAN HERBAL RECIPE A2

Chih-Jung Yao[1, 2], Tae-Young Han[3], Wan-Ju Chao[4], Gi-Ming Lai[1, 2, 4]

[1]Division of Hematology and Medical Oncology, Wan Fang Hospital, Taipei Medical University, Taiwan
[2]Comprehensive Cancer Center of Taipei Medical University, Taiwan
[3]Banronginsu Oriental Medicine Clinic, Korea
[4]National Institute of Cancer Research, National Health Research Institutes, Taiwan

ABSTRACT

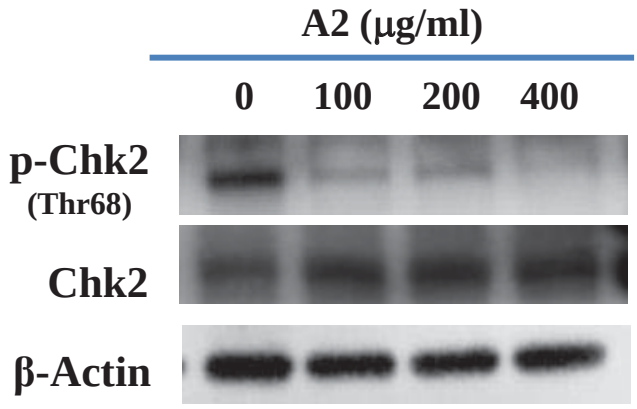
Purpose: A Korean herbal recipe A2 had been found to exert therapeutic effects in pancreatic cancer patients with "Shao Yin" constitution according to the oriental Sasang Constitutional Medicine. However, little is known about the underlying molecular mechanism. This study explores the effects of A2 on a poorly differentiated pancreatic cancer cell line MIA PaCa-2 to elucidate the molecular mechanism underlying its clinical effects.

Methods: The A2 was provided by Dr. Han, Banronginsu Oriental Medicine Clinic. Cell viability was determined by SRB assay. The cell-cycle distribution was analyzed by flow cytometry. Aldefluor® assays were used to analyze the cancer stem-like ALDH+ population. RT-PCR was used to evaluate the mRNA expression. Western blot and antibody against specific phosphorylated protein was employed to investigate the affected signaling pathways.

Results: After 72 h of treatment, A2 increased S phase percentage and induced apoptosis of MIA PaCa-2 cells accompanied by caspase-3 and -8 activation and Survivin inhibition. A2 decreased not only the signaling proteins like p-Stat3, p-AKT and p-ERK but also the potential targets for pancreatic cancer therapy such as c-Myc and Sirt1. Moreover, consistent with the decrease of ALDH+ population, repression of stemness genes (*Notch3, Jagged1* and *EpCAM*) and Vimentin and Snail that related to epithelial-mesenchymal transition (EMT) were observed in A2-treated cells.

Conclusions: During apoptosis induction in pancreatic cancer cells, A2 exerted effects on cell-cycle regulation, signaling inhibition as well as suppression of EMT and stemness markers. These multiple targeting effects might be responsible for the clinical benefits observed in A2-treated pancreatic patients.

A2 decreases the p-Chk2 (Thr68) in MIA PaCa-2 pancreatic cancer cells (72 h of treatment)



Chk2: Checkpoint kinase 2

- A2 increases S phase percentage and induces apoptosis of MIA PaCa-2 cells

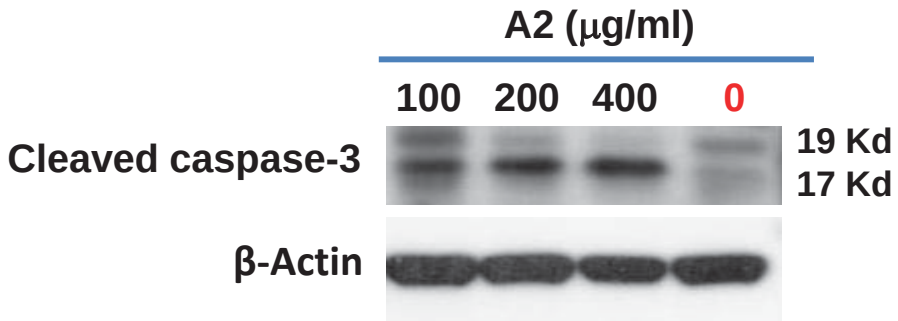
A2 decreases the oncogenic signaling in MIA PaCa-2 cells after 72 h of treatment

- A2 decreases the novel potential targets for pancreatic cancer therapy such as c-Myc, Sirt1 and MGMT.

- A2 inhibits EMT in MIA PaCa-2 cells

- **A2 decreases the ALDH-positive cancer-stem-like cells in MIA PaCa-2 pancreatic cancer cells**

A2 increases the cleaved caspase-3 of MIA PaCa-2 pancreatic cancer cells (72 h of treatment)

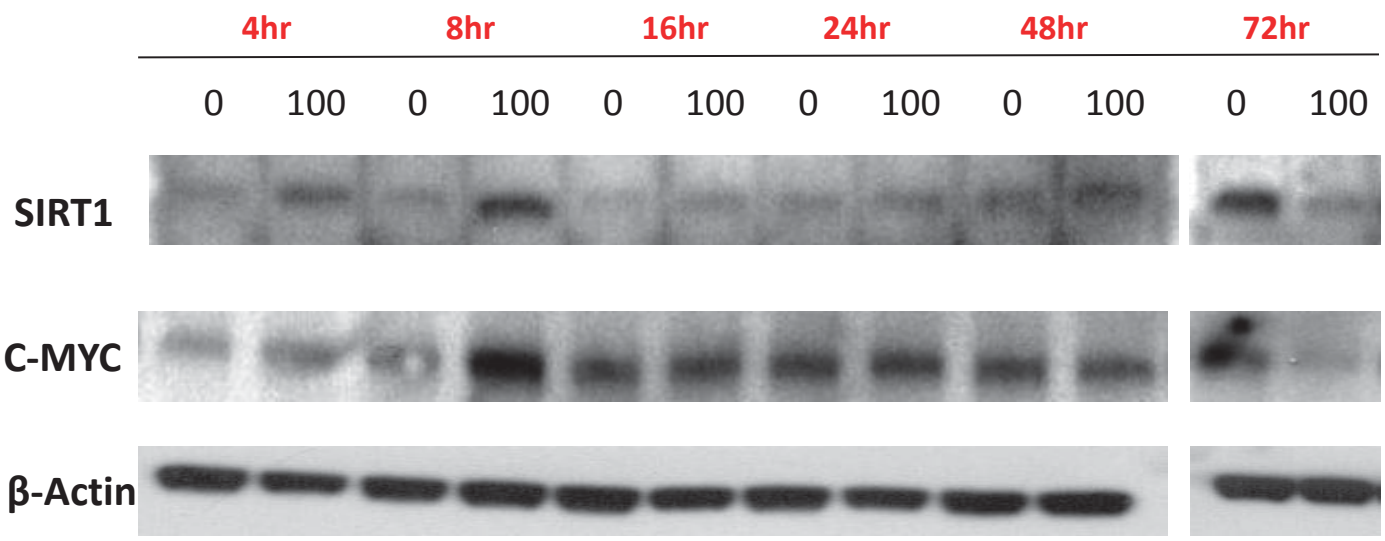


103.01.13w1

1. 不同濃度 A2 藥物對 MIA(P)胰臟癌細胞的影響-72hr(102.12.28.收細胞), Protein 量: 10 ug/10 uL(1ug/1uL)

	A2 in MIA PaCa-2(P) Cell										
Time	4hr		8hr		16hr		24hr		48hr		
M	0ug/mL	100ug/mL	0ug/mL	100ug/mL	0ug/mL	100ug/mL	0ug/mL	100ug/mL	0ug/mL	100ug/mL	M
6 uL	10 uL	10 uL	10 uL	10 uL	10 uL	10 uL	10 uL	10 uL	10 uL	10 uL	6 uL

A2 (ug/mL) in MIA Pa Ca-2(P) Pancrease cell



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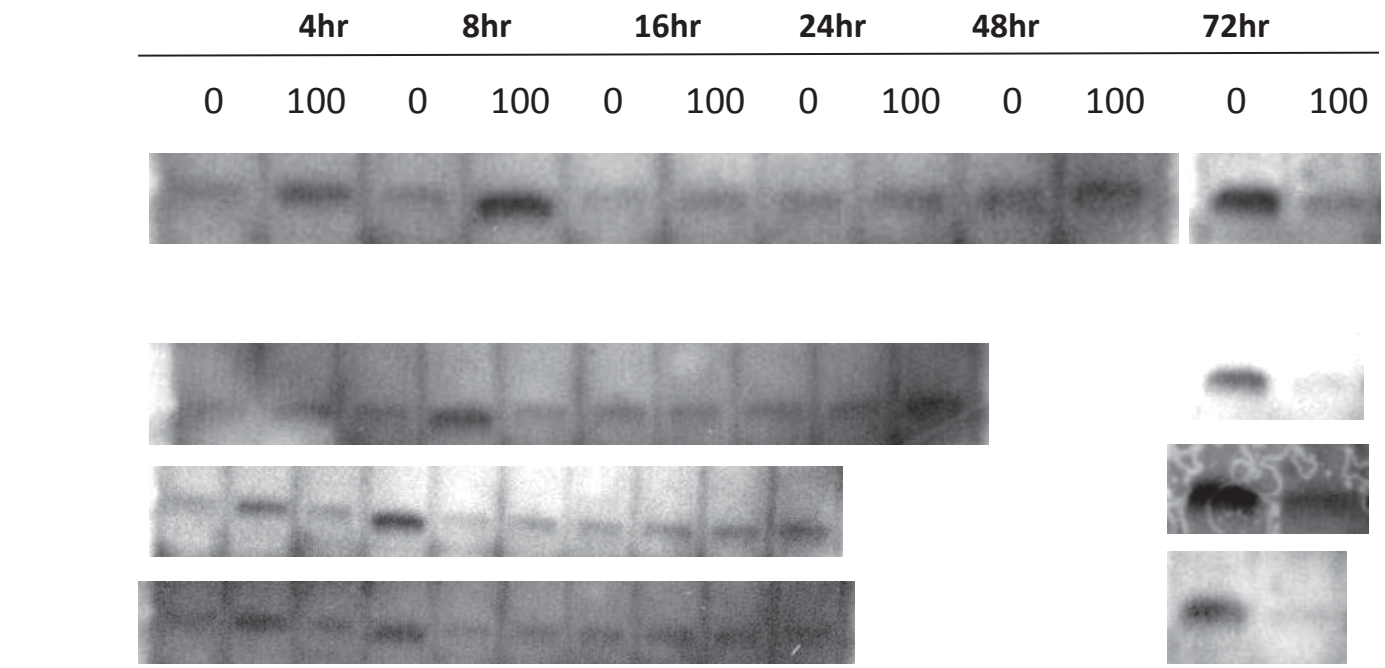
M	0ug/mL	100ug/mL	0ug/mL	100ug/mL	0ug/mL	100ug/mL	0ug/mL	100ug/mL	0ug/mL	100ug/mL	M
6 uL	10 uL	10 uL	10 uL	10 uL	10 uL	10 uL	10 uL	10 uL	10 uL	10 uL	6 uL

1.SIRT1

A2 (ug/mL) in MIA Pa Ca-2(P) Pancrease cell



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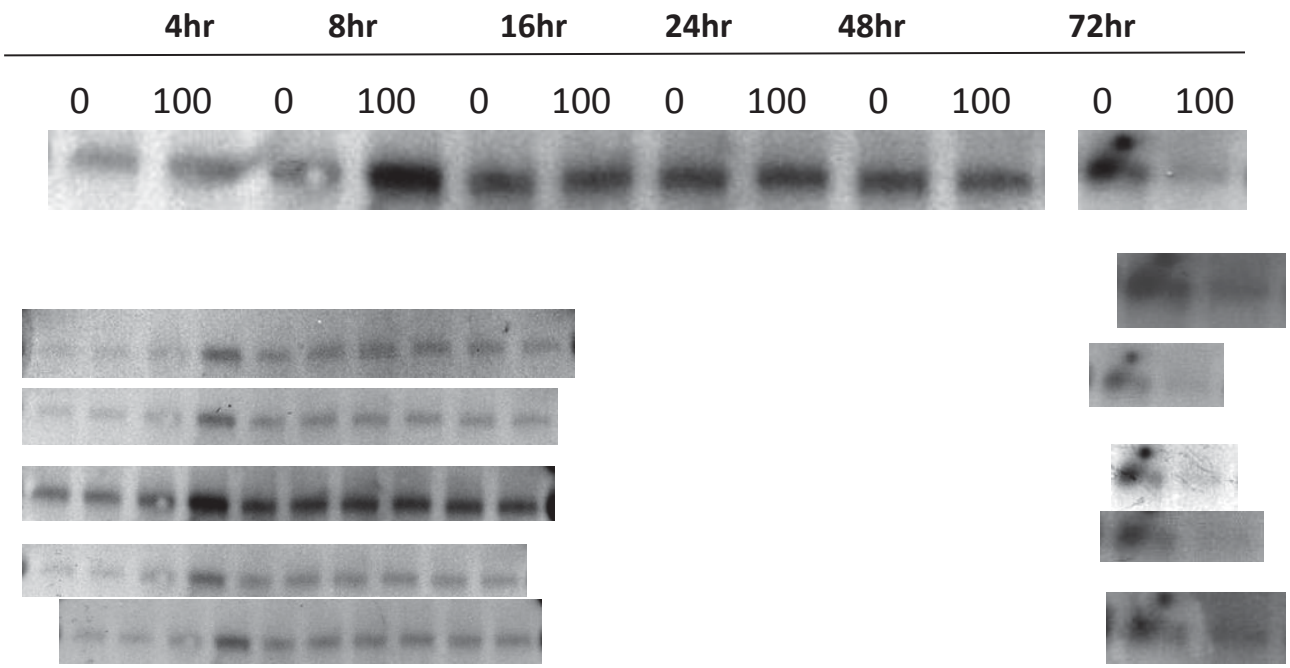
Time	4hr		8hr		16hr		24hr		48hr		
M	0ug/mL	100ug/mL	0ug/mL	100ug/mL	0ug/mL	100ug/mL	0ug/mL	100ug/mL	0ug/mL	100ug/mL	M
6 uL	10 uL	10 uL	10 uL	10 uL	10 uL	10 uL	10 uL	10 uL	10 uL	10 uL	6 uL

2.c-myc

A2 (ug/mL) in MIA Pa Ca-2(P) Pancrease cell



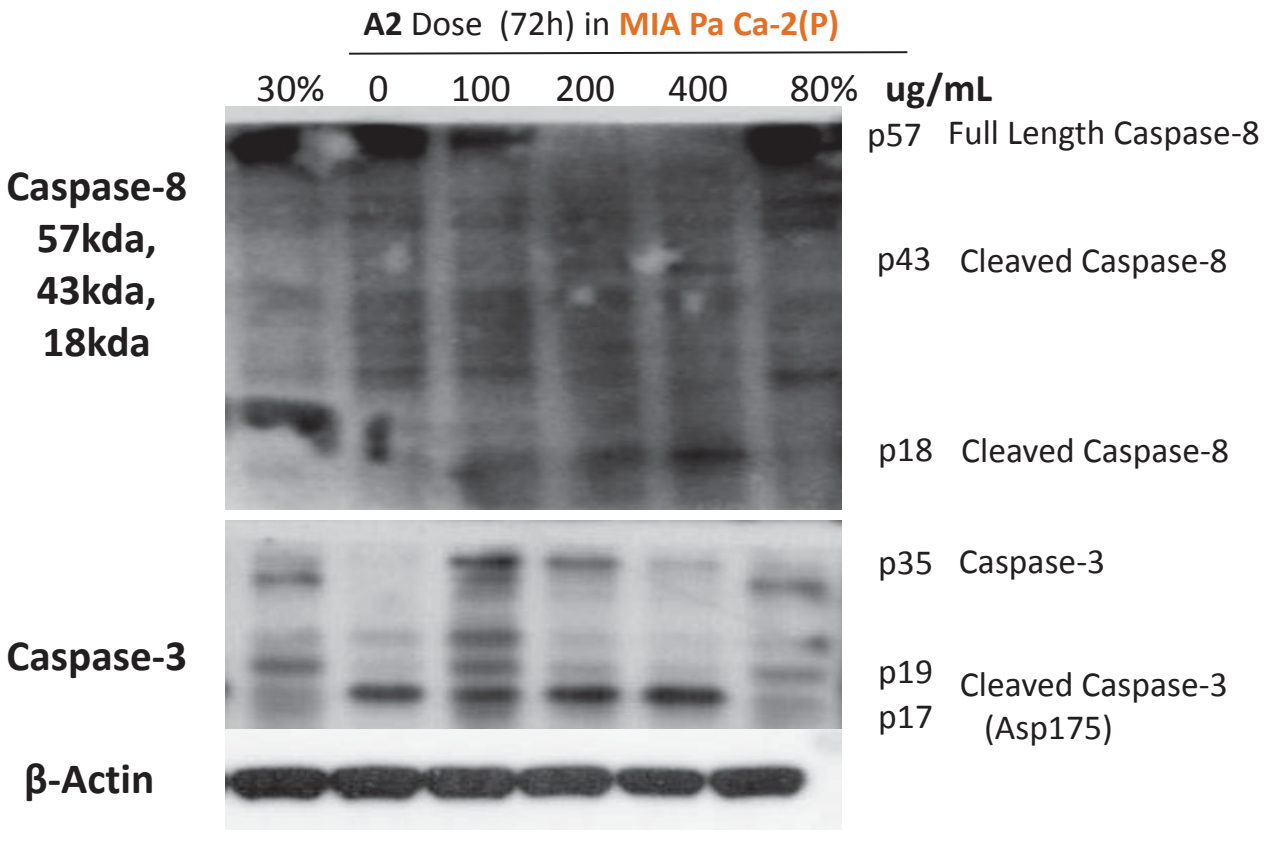
不同濃度 A2 藥物對MIA Pa Ca-2(P) 胰臟癌細胞的影響

Caspase pathway

第2次data

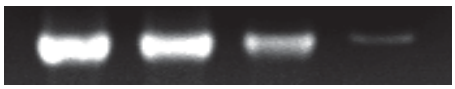
103.03.27

A2 (ug/mL) in MIA Pa Ca-2(P) Pancrease cell



A2 Dose (72h) in **MIA Ca Pa-2**

0 100 200 300 **ug/mL**



102.09.11

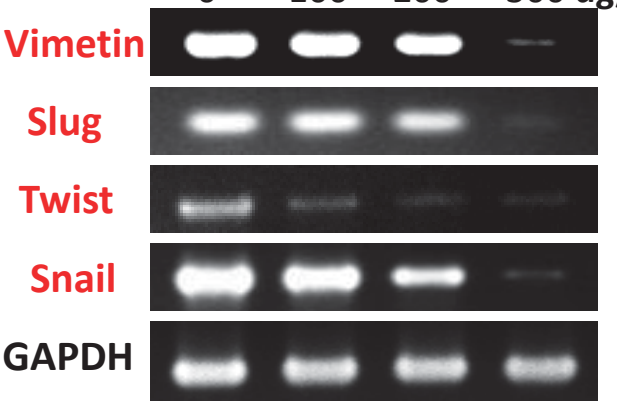
Vimetin

GAPDH 同102. 08.28 ?

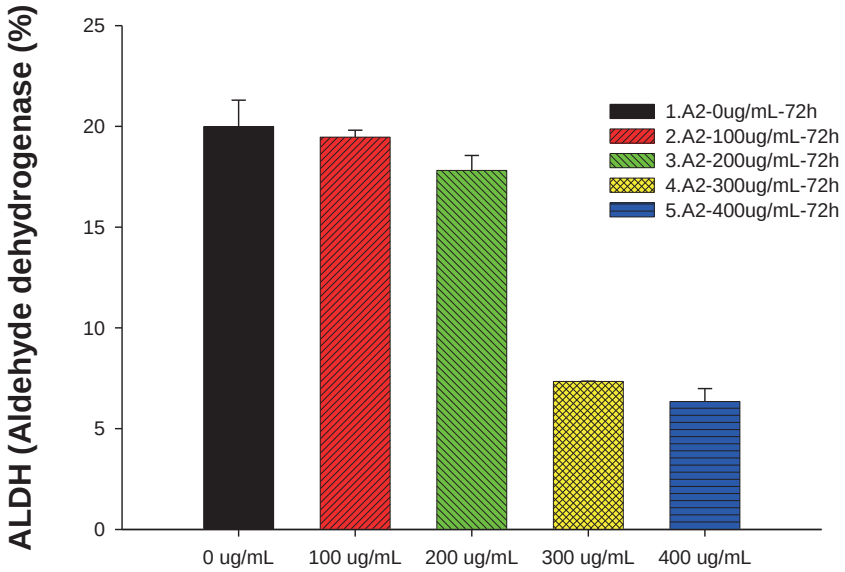
102.08.28

A2 Dose (72h) in **MIA Ca Pa-2(P)**

0 100 200 300 **ug/mL**



ALDH activity of A2 drug in MIA-Ca Pa-2 pancrease cancer for 72hr



A2 Concentration(ug/mL)

mean ± SD

A2 Conc	0 ug/mL	100ug/mL	200ug/mL	300ug/mL	400ug/mL
ALDH (%)	19.99 ± 1.31	19.47± 0.35	17.34± 0.74	7.34± 0.01	6.35 ± 0.64

Oncol Rep. 2014 Feb;31(2):755-62. doi: 10.3892/or.2013.2929. Epub 2013 Dec 16.

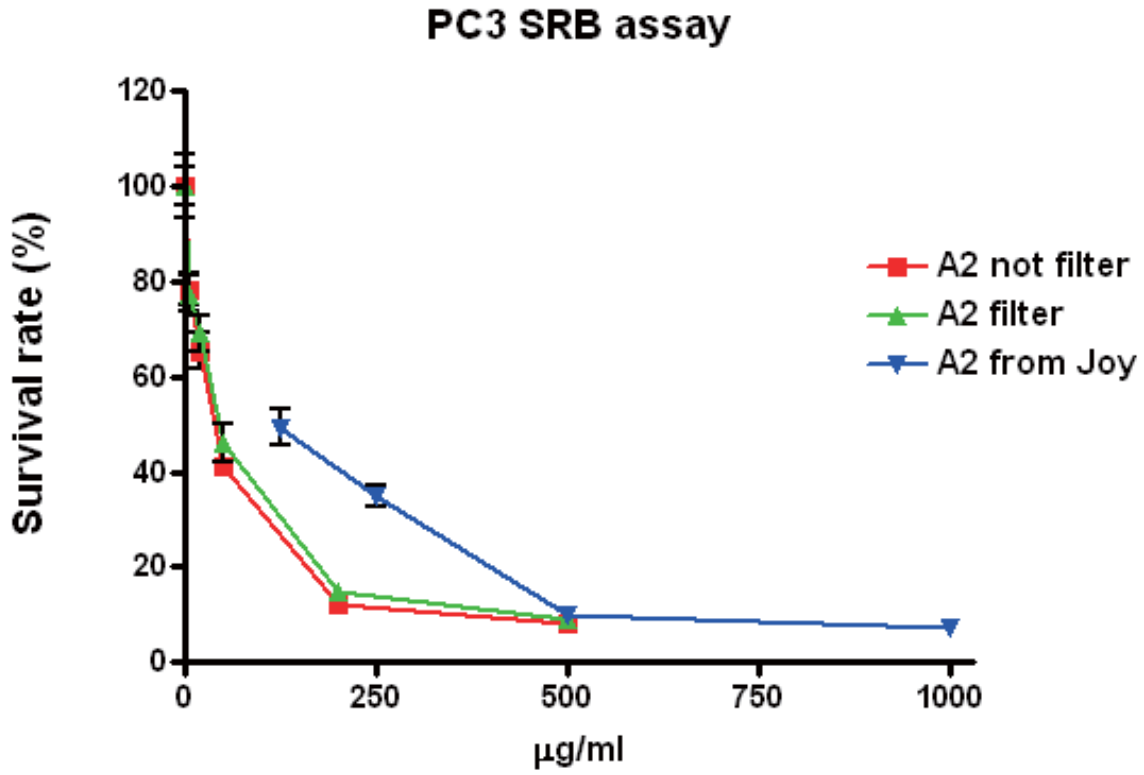
Licochalcone A induces apoptosis in KB human oral cancer cells via a caspase-dependent FasL signalingpathway.

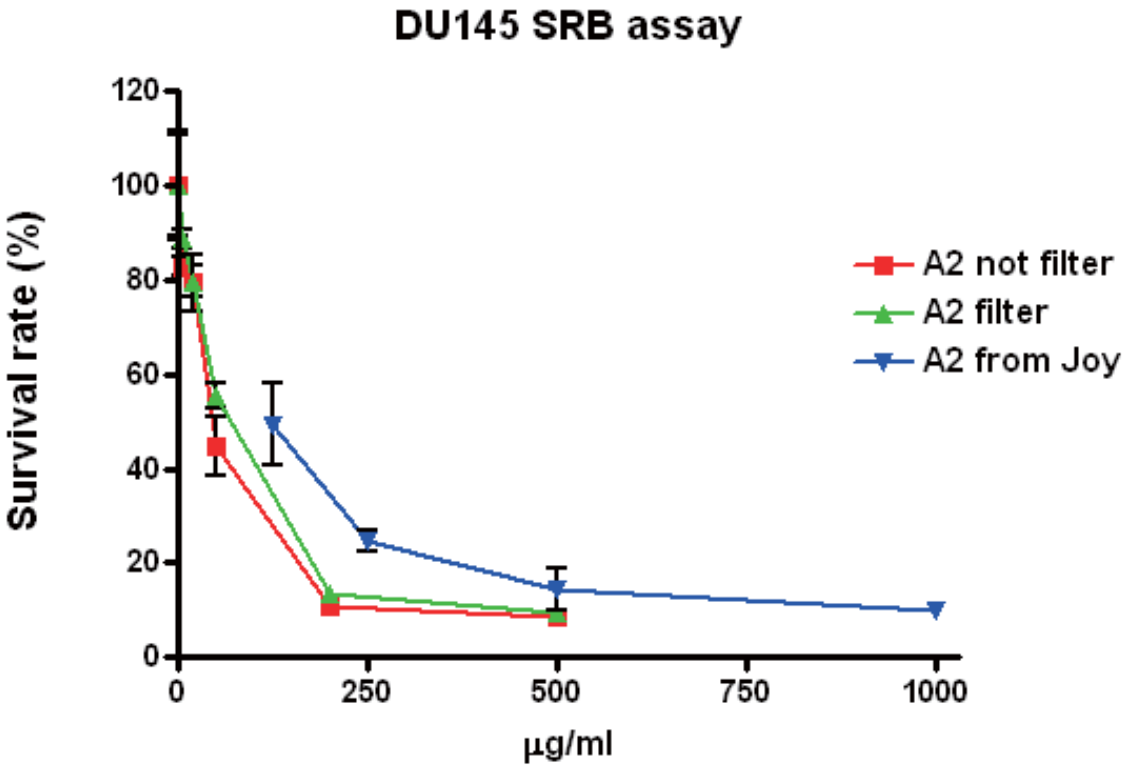
Kim JS[1], Park MR[1], Lee SY[1], Kim do K[1], Moon SM[2], Kim CS[2], Cho SS[3], Yoon G[3], Im HJ[4], You JS[5], Oh JS[1], Kim SG[1].

Author information

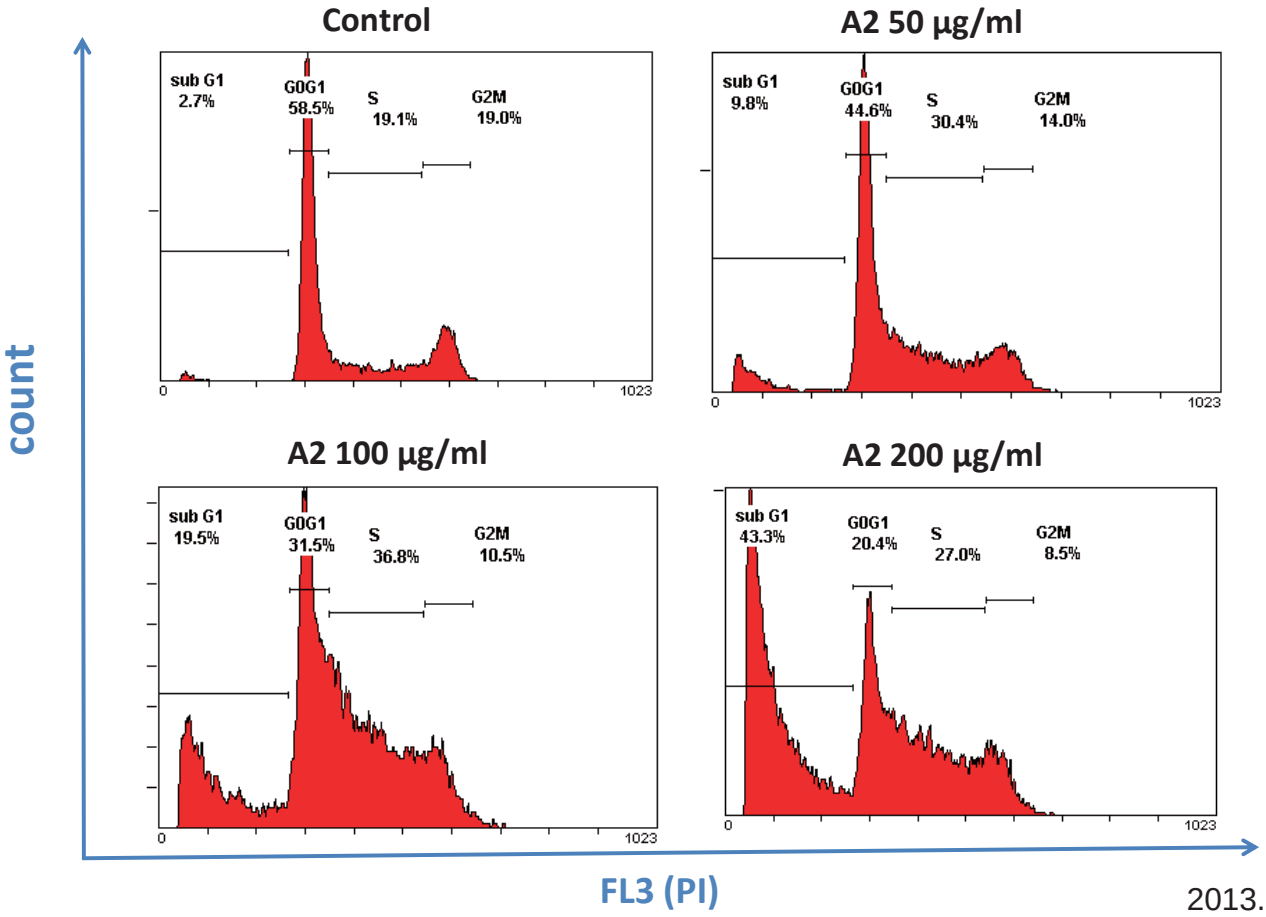
Abstract

Licochalcone A (Lico-A) is a natural phenol licorice compound with multiple bioactivities, including anti-inflammatory, anti-microbial, anti-fungal and osteogenesis-inducing properties. In the present study, we investigated the Lico-A-induced apoptotic effects and examined the associated apoptosispathway in KB human oral cancer cells. Lico-A decreased the number of viable KB oral cancer cells. However, Lico-A did not have an effect on primary normal human oral keratinocytes. In addition, the IC50 value of Lico-A was determined to be ~50 µM following dose-dependent stimulation.KB oral cancer cells stimulated with Lico-A for 24 h showed chromatin condensation by DAPI staining, genomic DNA fragmentation by agarose gel electrophoresis and a gradually increased apoptotic cell population by FACS analysis. These data suggest that Lico-A induces apoptosis in KB oralcancer cells. Additionally, Lico-A-induced apoptosis in KB oral cancer cells was mediated by the expression of factor associated suicide ligand (FasL) and activated caspase-8 and -3 and poly(ADP-ribose) polymerase (PARP). Furthermore, in the KB oral cancer cells co-stimulation with a caspase inhibitor (Z-VAD-fmk) and Lico-A significantly abolished the apoptotic phenomena. Our findings demonstrated that Lico-A-induced apoptosisin KB oral cancer cells involves the extrinsic apoptotic signaling pathway, which involves a caspase-dependent FasL-mediated death receptorpathway. Our data suggest that Lico-A be developed as a chemotherapeutic agent for the management of oral cancer.



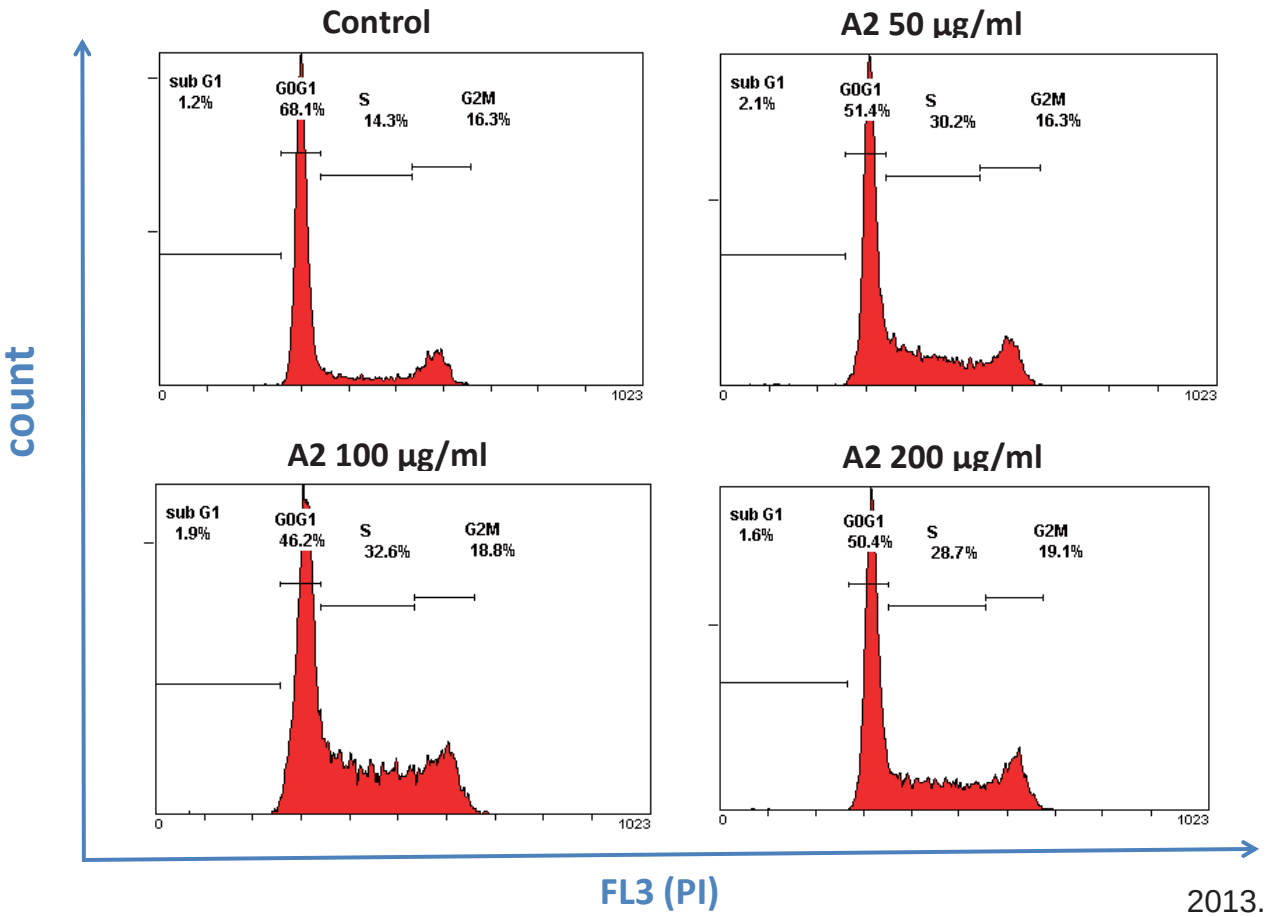


The cell cycle distribution of DU145 cells treated with food A2 for 72h

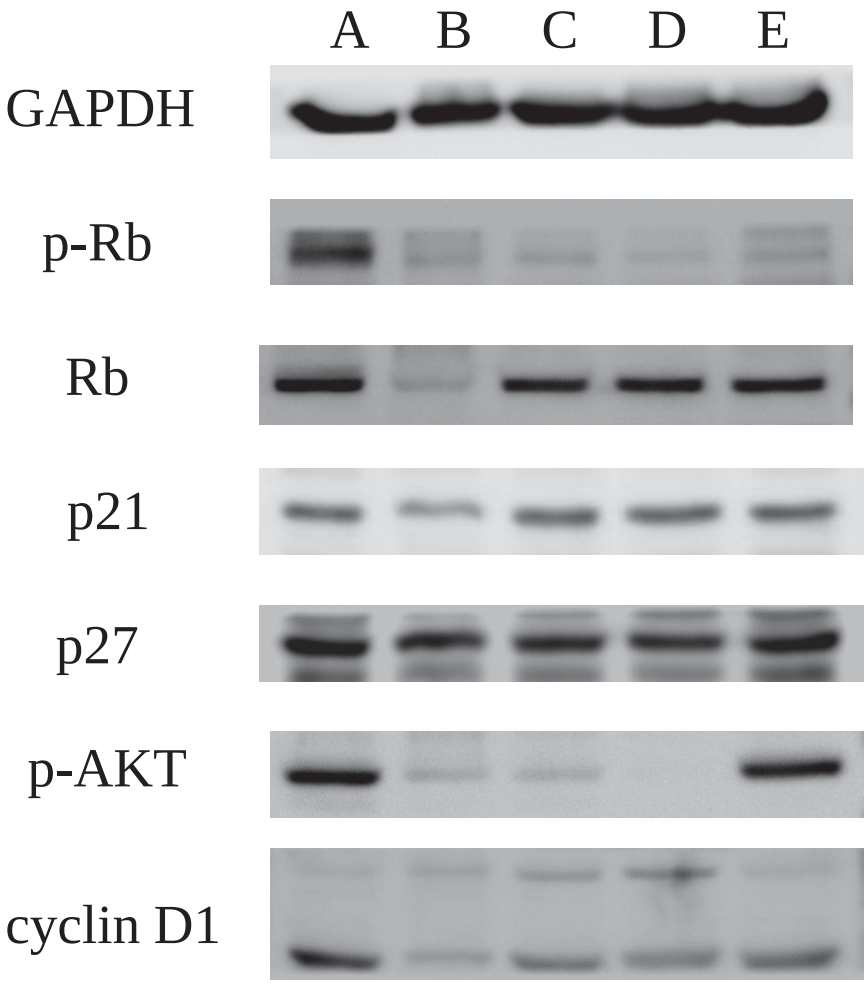


2013.6.5

The cell cycle distribution of PC3 cells treated with food A2 for 72h



2013.6.5



Cell line: PC3
Time: 72h
食用級A2

A: Control
B: food A2 50ug/ml
C: food A2 100ug/ml
D: foodA2 200ug/ml
E: Joy A2 250ug/ml

PS. 這次實驗的A2 50ug/ml 好像有問題

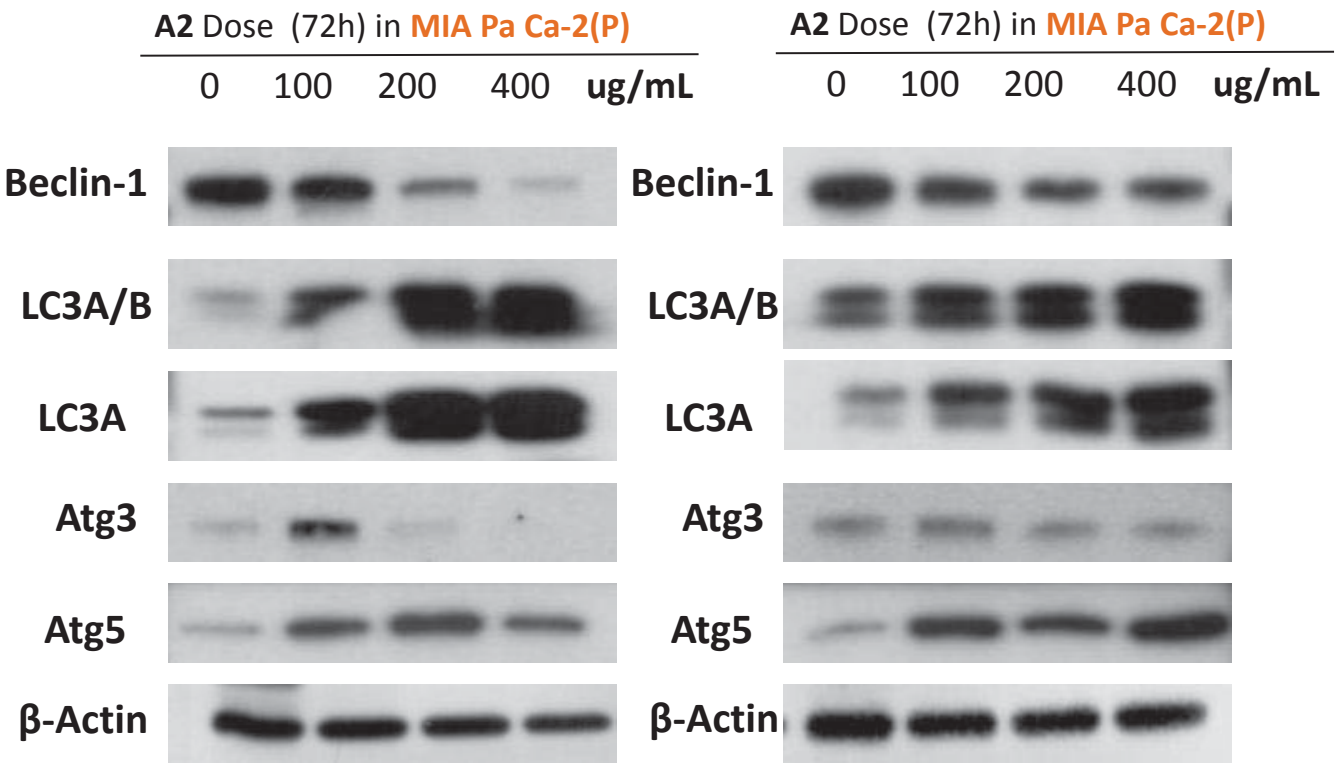
2013.5.31

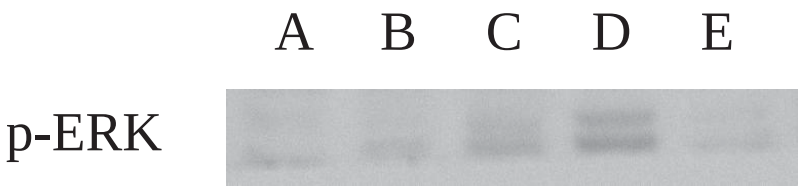
不同濃度A2藥物對MIA Pa Ca-2(P) 胰臟癌細胞的影響

103.06.13

7. Autophagy Signaling pathway

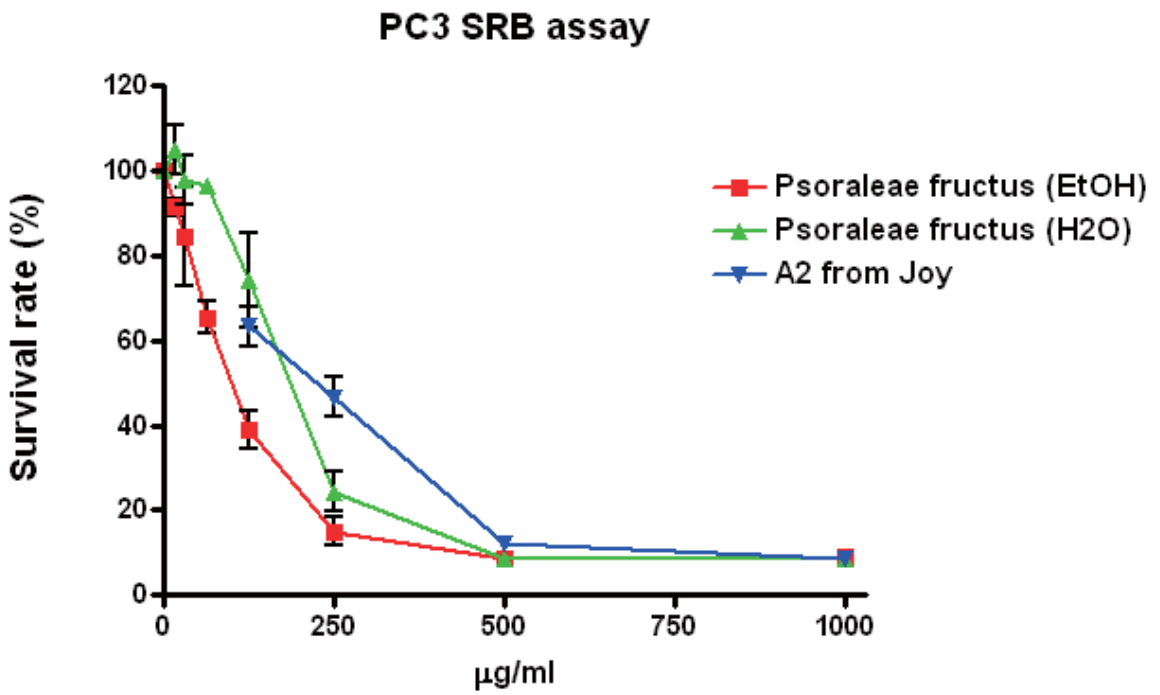
A2 (ug/mL) in MIA Pa Ca-2(P) Pancrease cell

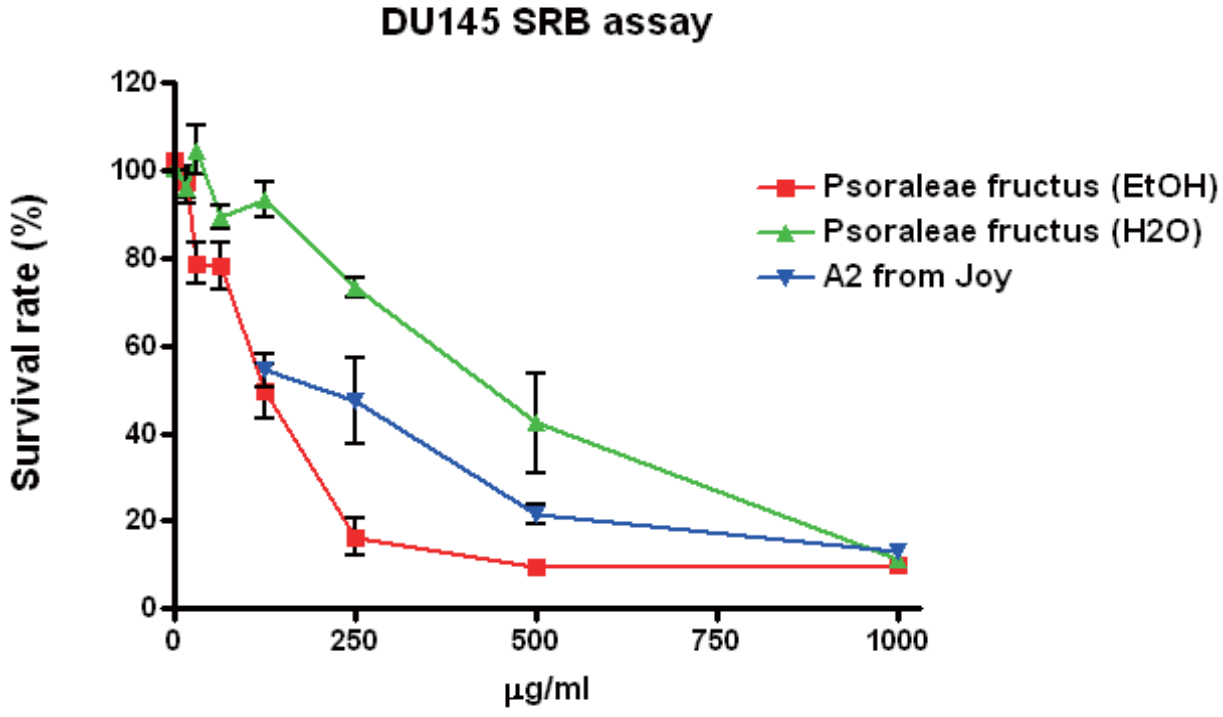




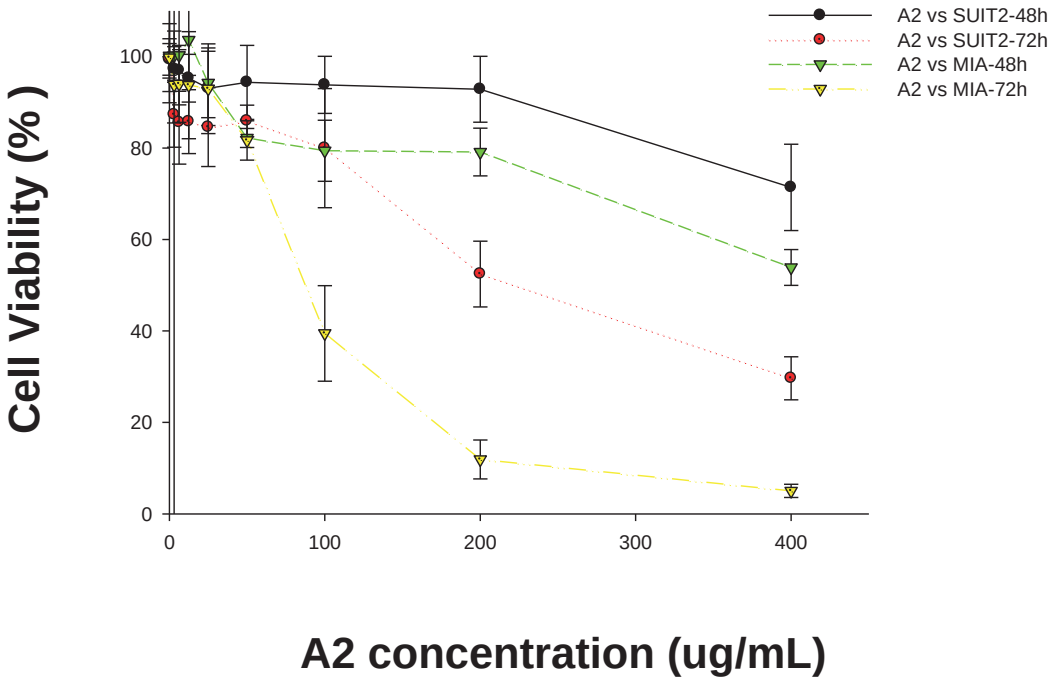
Cell line: PC3
Time: 72h
食用級A2

A: Control
B: food A2 50ug/ml
C: food A2 100ug/ml
D: foodA2 200ug/ml
E: Joy A2 250ug/ml


PC3 SRB assay
Survival rate (%)
120
100
80
60
40
20
0
0
250
500
750
1000
µg/ml
Psoraleae fructus (EtOH)
Psoraleae fructus (H2O)
A2 from Joy


DU145 SRB assay
Survival rate (%)
120
100
80
60
40
20
0
0
250
500
750
1000
µg/ml
Psoraleae fructus (EtOH)
Psoraleae fructus (H2O)
A2 from Joy

Cytotoxic activity of A2 drug in SUIT-2 and MIA-Ca Pa-2 ¡ pancreatic cancer for 48 and 72hr



102.06.26

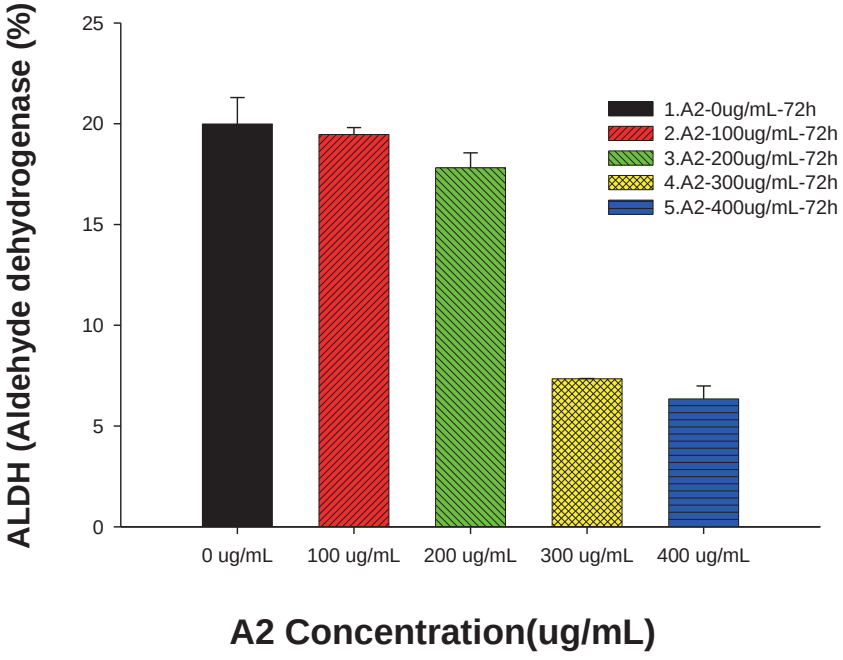
3-2. A2對 SUIT-2 胰臟癌細胞株之Cell cycle-72小時影響:

Treatment	Cell-cycle distribution							
72hr	Sub-G1 Phase		G0/G1 Phase(%)		S Phase(%)		G2/M Phase(%)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
A2- 0 ug/mL	0.67	0.14	56.71	1.29	15.92	0.17	26.29	1.16
A2- 50 ug/mL	1.15	0.05	50.02	0.76	24.31	0.04	25.09	0.69
A2-100 ug/mL	1.29	0.15	47.49	0.16	26.02	0.16	25.91	0.58
A2-200 ug/mL	3.00	0.08	41.13	0.06	24.54	0.52	26.74	0.08
A2- 300 ug/mL	4.02	0.47	44.03	1.93	29.49	0.30	21.88	2.02
A2- 400 ug/mL	19.75	0.00	46.08	0.00	24.40	0.00	10.62	0.00

3-3. A2對 MIA Ca Pa-2 胰臟癌細胞株之Cell cycle-72小時影響:

Treatment	A2對MIA-Ca Pa-2之Cell-cycle distribution							
72hr	Sub-G1 Phase		G0/G1 Phase(%)		S Phase(%)		G2/M Phase(%)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
A2- 0 ug/mL	2.34	0.32	58.87	0.72	19.22	0.79	19.27	0.33
A2- 50 ug/mL	2.59	0.24	52.81	0.47	19.88	3.38	23.52	0.71
A2-75 ug/mL	4.25	0.49	54.78	3.14	20.79	0.09	20.66	3.07
A2-100 ug/mL	1.24	0.16	35.63	0.69	38.05	0.57	25.76	0.58
A2- 200 ug/mL	24.93	5.71	37.56	1.08	26.01	3.23	12.45	3.46

ALDH activity of A2 drug in MIA-Ca Pa-2 pancrease cancer for 72hr



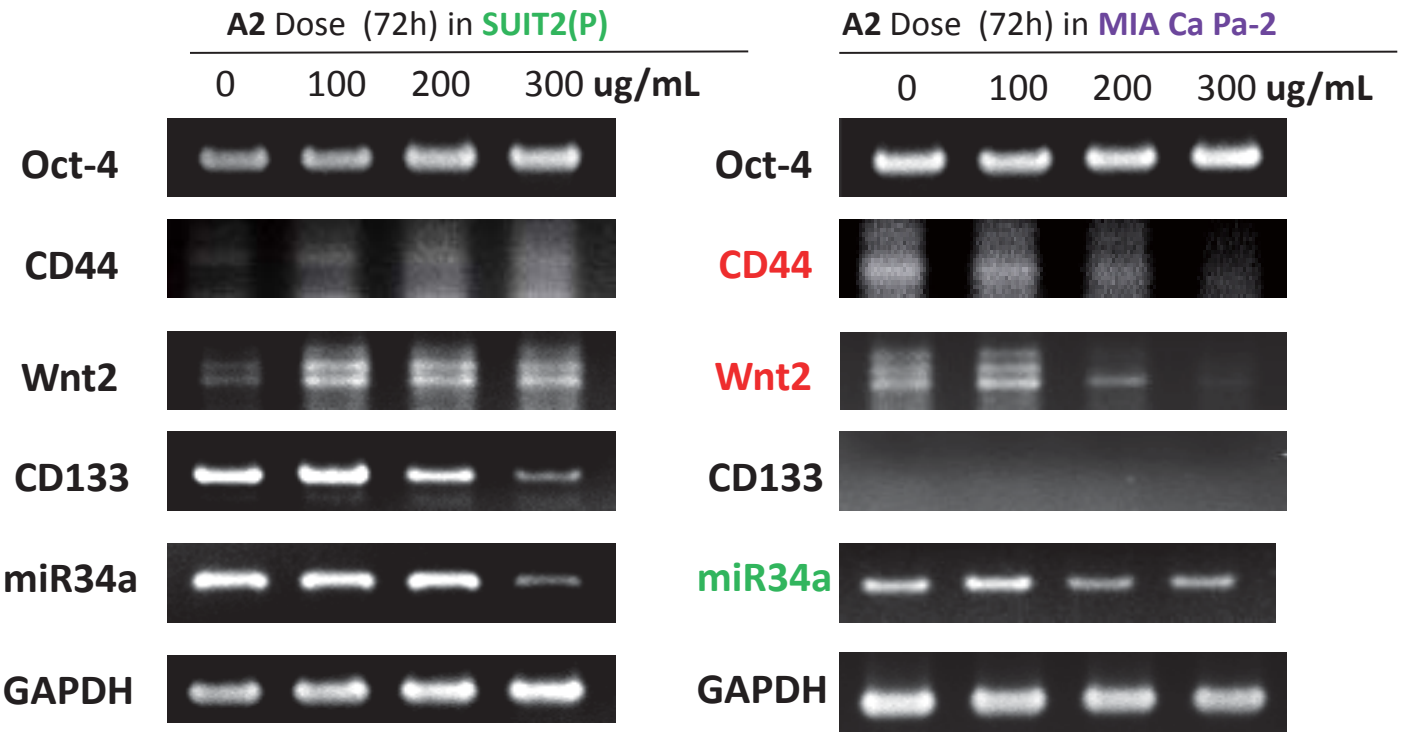
A2 Conc	0 ug/mL	100ug/mL	200ug/mL	300ug/mL	400ug/mL
ALDH (%)	19.99 ± 1.31	19.47± 0.35	17.34± 0.74	7.34± 0.01	6.35 ± 0.64

1-2. Stem cell marker

102.08.08

比較A2藥物對SUIT2(P) 與MIA-Ca Pa2(P)胰臟癌72h後之mRNA 結果:

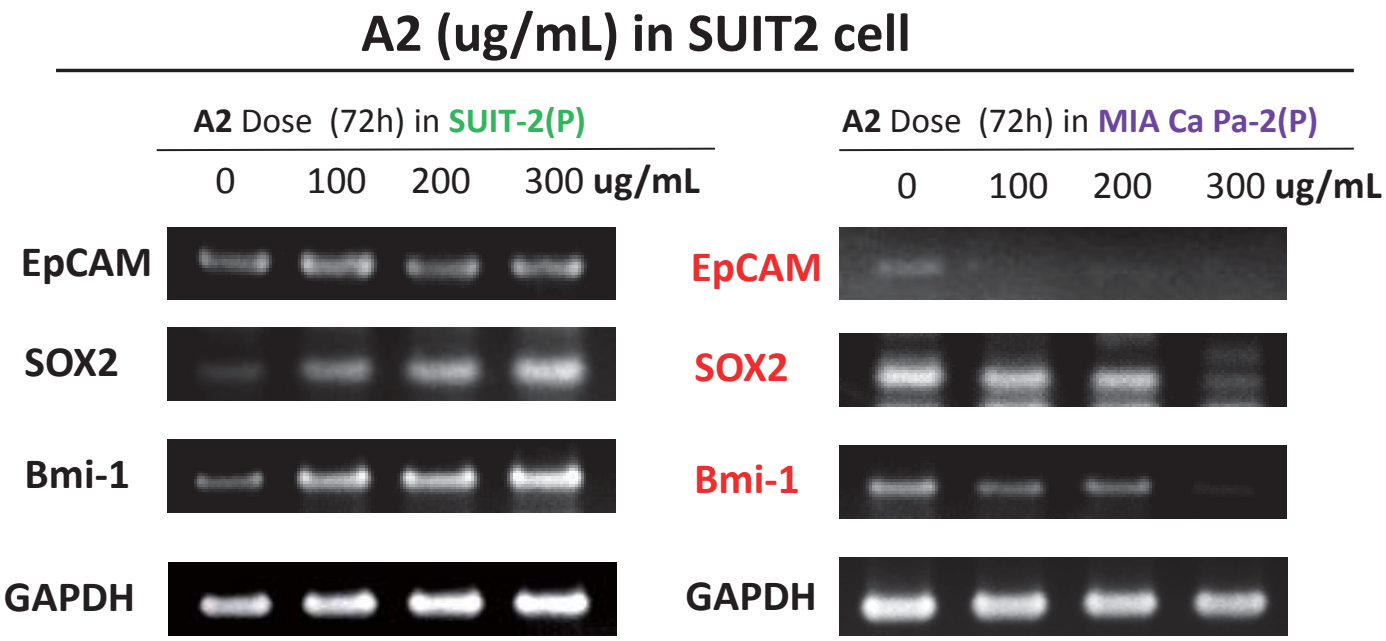
A2 (ug/mL) in Pancrese cell



1-4. Stem cell marker

102.08.26

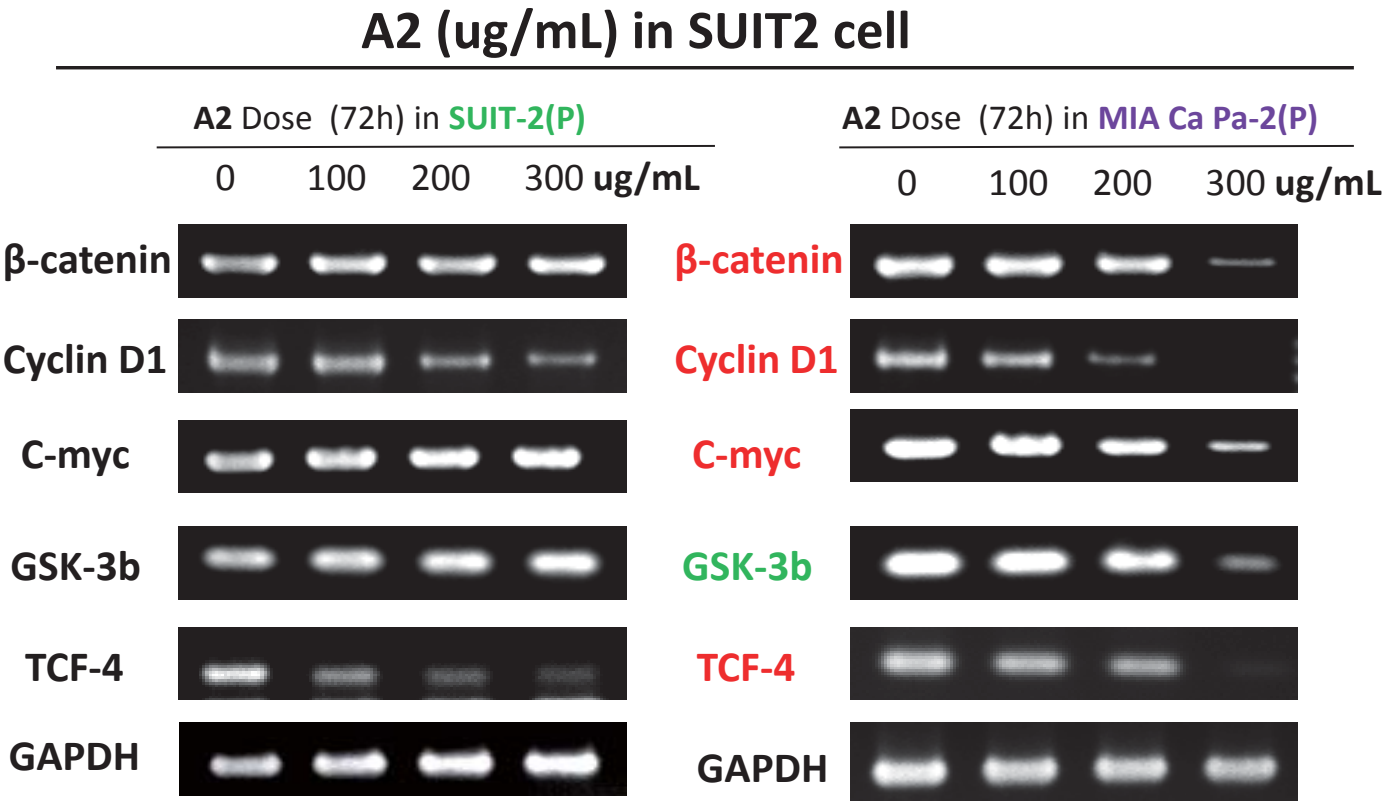
比較A2藥物對SUIT-2(P) 與MIA Ca Pa-2(P)胰臟癌72h之mRNA 結果:



3-2. β -catenin pathway

102.08.26

比較A2藥物對SUIT-2(P) 與MIA Ca Pa-2(P)胰臟癌72h之mRNA 結果:



2-2. Notch pathway

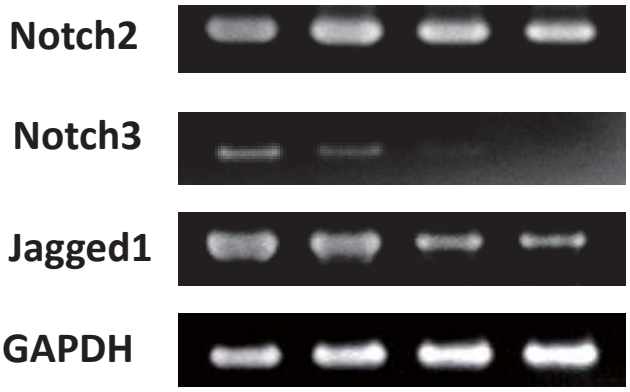
102.08.15

比較A2藥物對SUIT-2(P) 與MIA Ca Pa-2(P)胰臟癌72h之mRNA 結果:

A2 (ug/mL) in SUIT2 cell

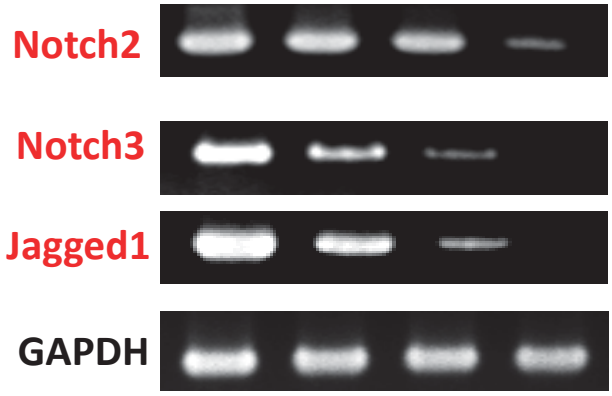
A2 Dose (72h) in SUIT-2(P)

0 100 200 300 **ug/mL**



A2 Dose (72h) in MIA Ca Pa-2(P)

0 100 200 300 **ug/mL**



5-2. EMT pathway

102.08.28

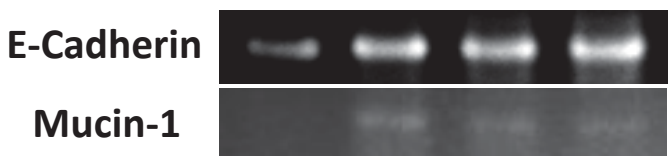
比較A2藥物對SUIT-2(P) 與MIA Ca Pa-2(P)胰臟癌72h之mRNA 結果:

A2 (ug/mL) in SUIT2 cell

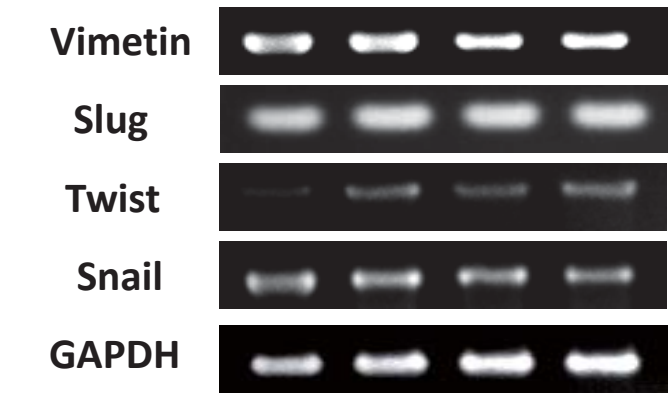
A2 Dose (72h) in SUIT-2(P)

0 100 200 300 **ug/mL**

Epithclial marker



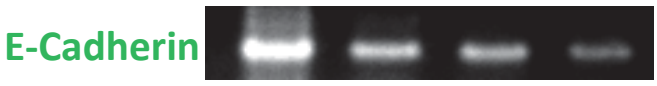
Mesenchymol marker



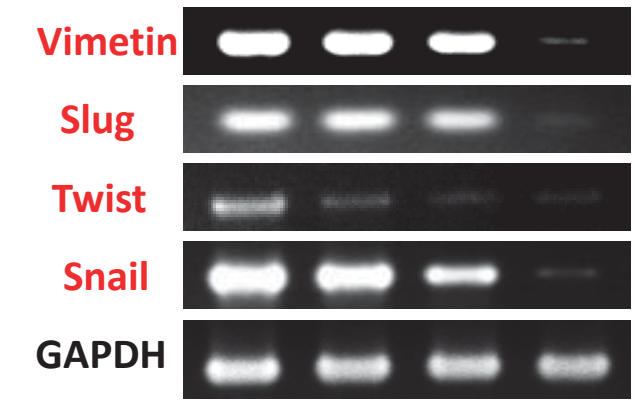
A2 Dose (72h) in MIA Ca Pa-2(P)

0 100 200 300 **ug/mL**

Epithclial marker



Mesenchymol marker



1-1. EMT marker

(第2次)

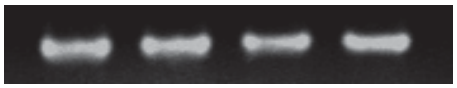
102.09.11

2.Vimetin

A2 (ug/mL) in SUIT2 cell

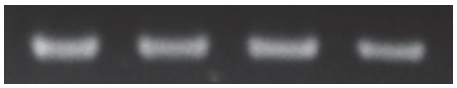
A2 Dose (72h) in **SUIT2(SP)**

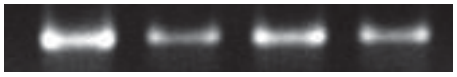
0 100 200 300 **ug/mL**



A2 Dose (72h) in **SUIT2(ALDH+)**

0 100 200 300 **ug/mL**



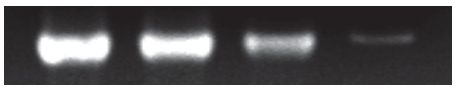


A2 Dose (72h) in **SUIT2(P)**

0 100 200 300 **ug/mL**

A2 Dose (72h) in **MIA Ca Pa-2**

0 100 200 300 **ug/mL**



Vimetin

4-1. CCL22

102.08.26

1.比較A2藥物對SUIT-2(P) 與SUIT-2(ALDH+) 胰臟癌72h之mRNA 結果:

A2 (ug/mL) in SUIT2 cell

A2 Dose (72h) in **SUIT-2(P)**

0 100 200 300 **ug/mL**

CCL22

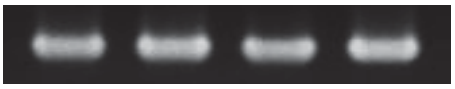
GAPDH

A2 Dose (72h) in **SUIT-2(ALDH+)**

0 100 200 300 **ug/mL**

CCL22

GAPDH



2.比較A2藥物對SUIT-2(P) 與MIA Ca Pa-2(P)胰臟癌72h之mRNA 結果:

A2 (ug/mL) in SUIT2 cell

A2 Dose (72h) in **SUIT-2(P)**

0 100 200 300 **ug/mL**

CCL22

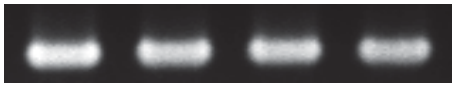
GAPDH

A2 Dose (72h) in **MIA Ca Pa-2(P)**

0 100 200 300 **ug/mL**

CCL22

GAPDH



1.不同濃度A2藥物對MIA Pa Ca-2(P) 胰臟癌細胞的影響

1. EMT Signaling pathway

102.11.18

A2 (ug/mL) in MIA Pa Ca-2(P) Pancrease cell

A2 Dose (72h) in MIA Pa Ca-2(P)

0 100 200 400 **ug/mL**

Mesenchymol marker

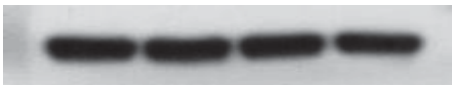
Vimetin

Snail

Epithclial marker

E-Cadherin

β-Actin



不同濃度A2藥物對MIA Pa Ca-2(P) 胰臟癌細胞的影響

2. Transcription protein Signaling pathway

102.12.03

A2 (ug/mL) in MIA Pa Ca-2(P) Pancrease cell

A2 Dose (72h) in MIA Pa Ca-2(P)

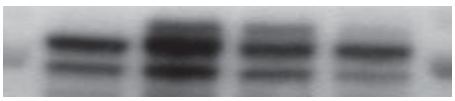
0 100 200 400 **ug/mL**

Src

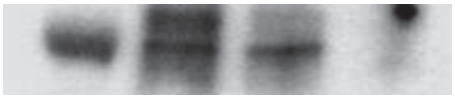
ERK

pStat3

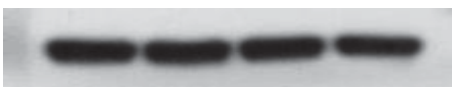
GSK3- β



P-P70 S6 kinase



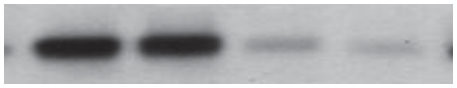
β-Actin



A2 Dose (72h) in MIA Pa Ca-2(P)

0 100 200 400 **ug/mL**

AKT

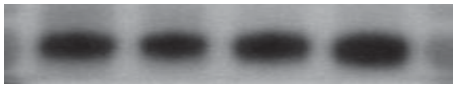


P-AKT

C-myc

NFkB

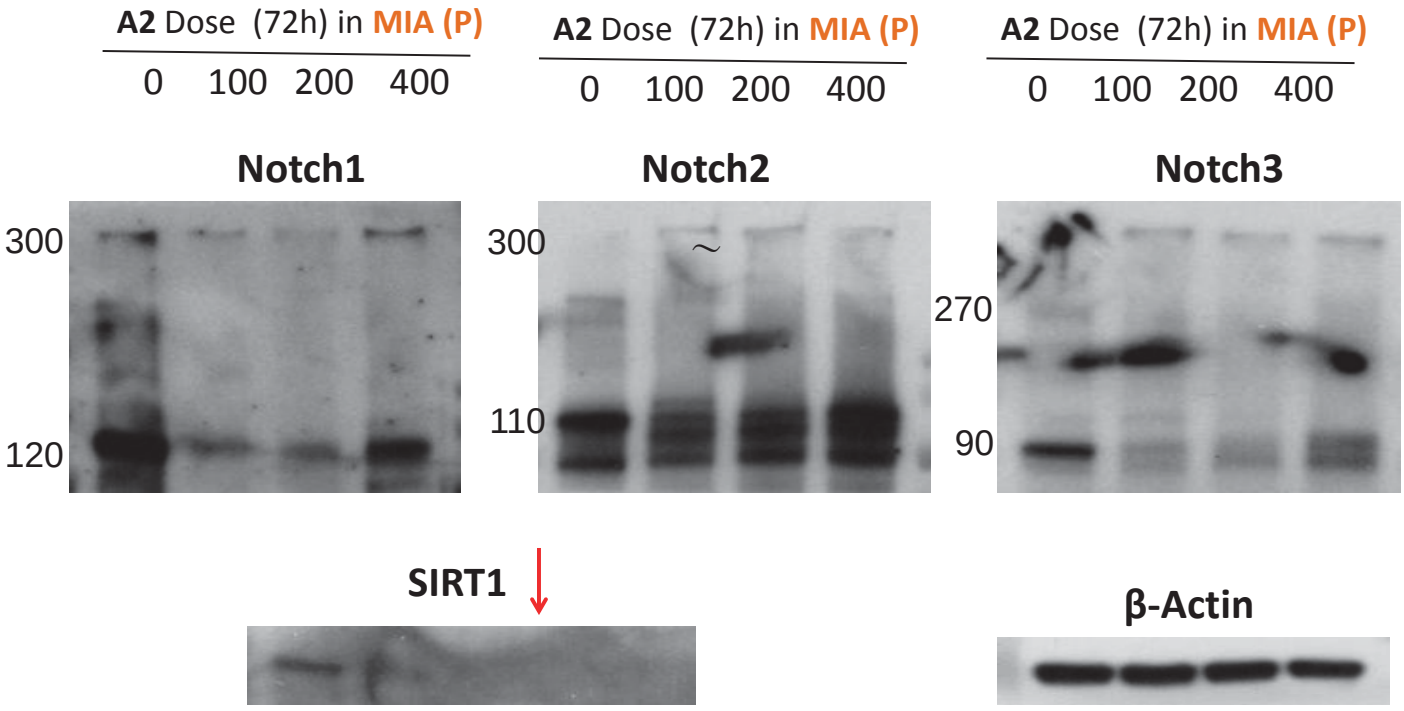
CD44



1.不同濃度 A2 藥物對MIA Pa Ca-2(P)(P) 胰臟癌細胞的影響
～3. Notch pathway + Self renewal

102.11.28

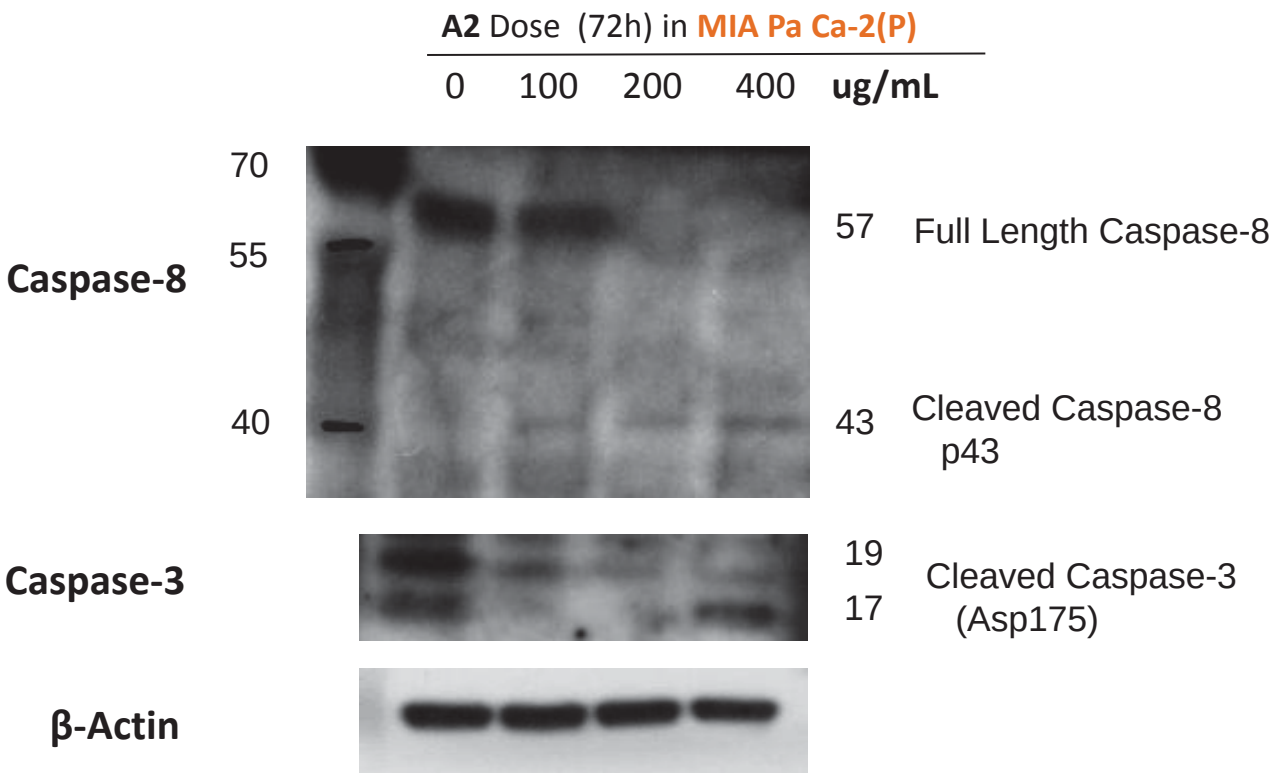
A2 (ug/mL) in MIA Pa Ca-2(P) Pancrease cell



1.不同濃度 A2 藥物對MIA Pa Ca-2(P) 胰臟癌細胞的影響
～4-1. Caspase pathway

102.11.28

A2 (ug/mL) in MIA Pa Ca-2(P) Pancrease cell

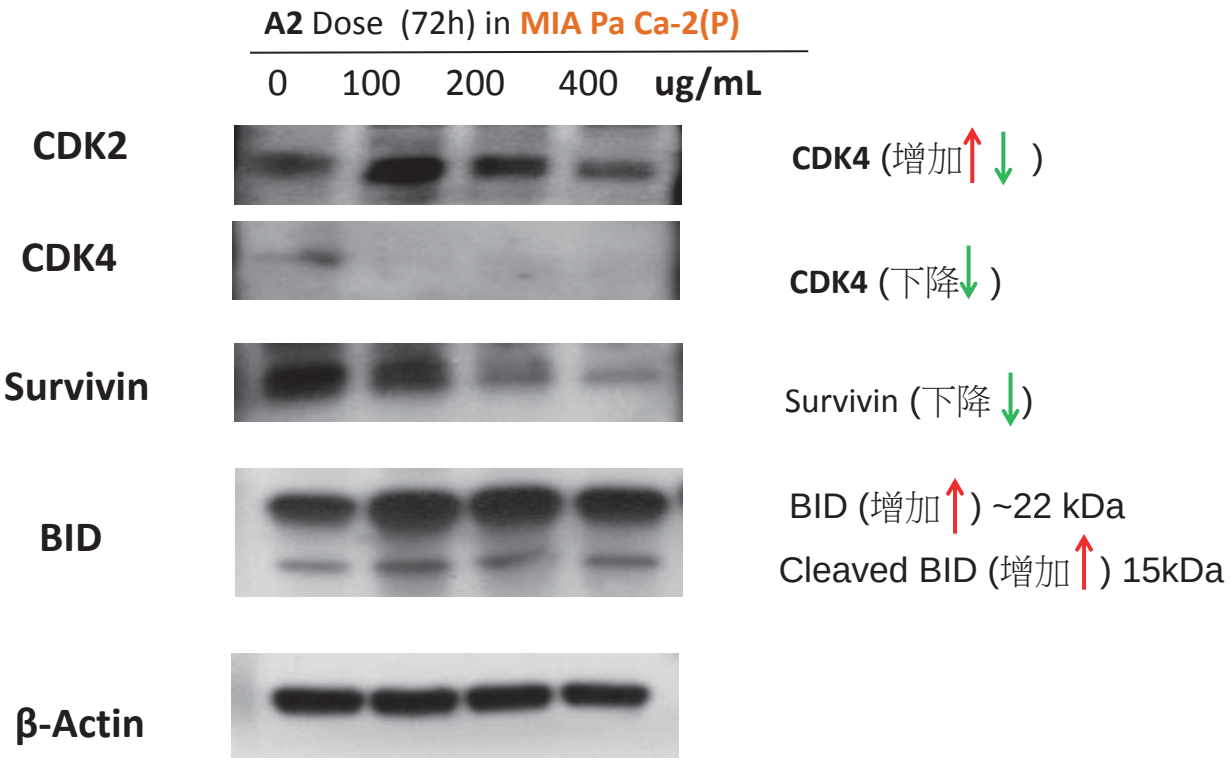


1.不同濃度 A2 藥物對MIA Pa Ca-2(P) 胰臟癌細胞的影響

～ 4-2. cell cycle ,Proliferation,Antiapoptic pathway

102.12.03

A2 (ug/mL) in MIA Pa Ca-2(P) Pancrease cell



1. A2對胰臟癌細胞(MIA Pa Ca-2)的影響

不同時間處理A2

Protein expreassion

1. SIRT1
2. C-MYC

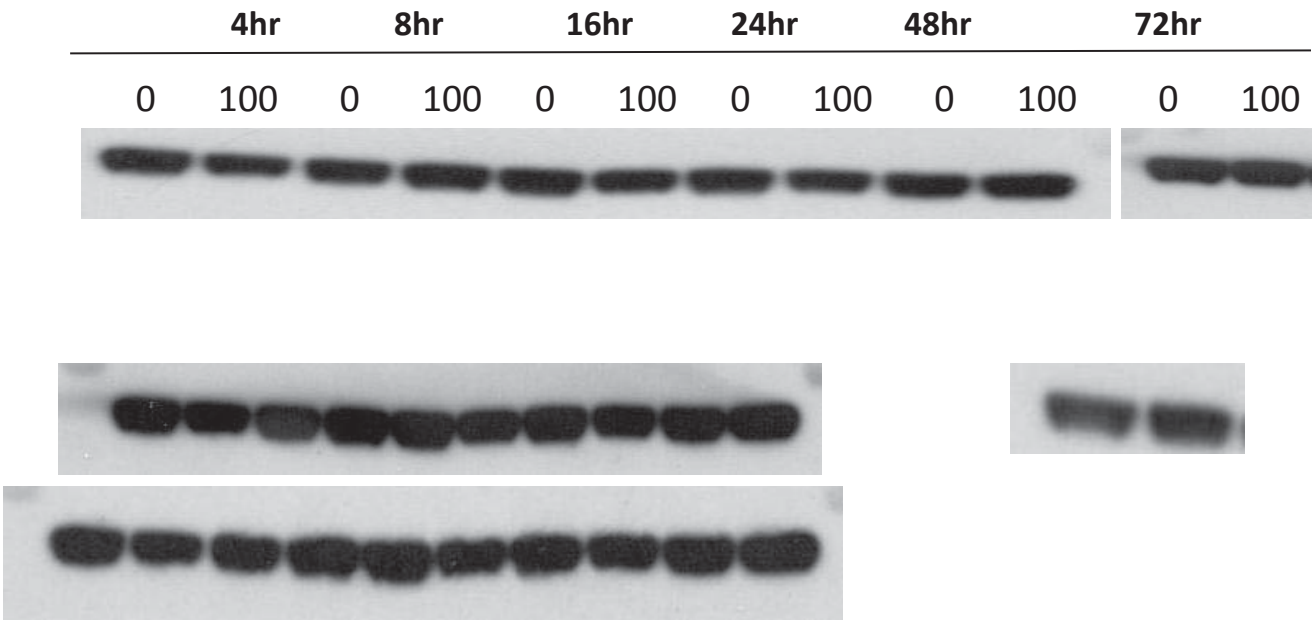
Cell: 1. MIA Pa Ca-2 (P)
Drug : A2
Dose:0、100ug/mL
Time: 4 、 8 、 16 、 24 、 48 、 72h

1. 不同濃度 A2 藥物對 MIA(P)胰臟癌細胞的影響-72hr(102.12.28.收細胞), Protein 量: 10 ug/10 uL(1ug/1uL)

	A2 in MIA PaCa-2(P) Cell										
Time	4hr		8hr		16hr		24hr		48hr		
M	0ug/mL	100ug/mL	0ug/mL	100ug/mL	0ug/mL	100ug/mL	0ug/mL	100ug/mL	0ug/mL	100ug/mL	M
6 uL	10 uL	10 uL	10 uL	10 uL	10 uL	10 uL	10 uL	10 uL	10 uL	10 uL	6 uL

3. β-Actin

A2 (ug/mL) in MIA Pa Ca-2(P) Pancrease cell



1. A2對胰臟癌細胞(MIA Pa Ca-2)的影響

不同時間處理A2

Protein expreassion

1. SIRT1
2. C-MYC

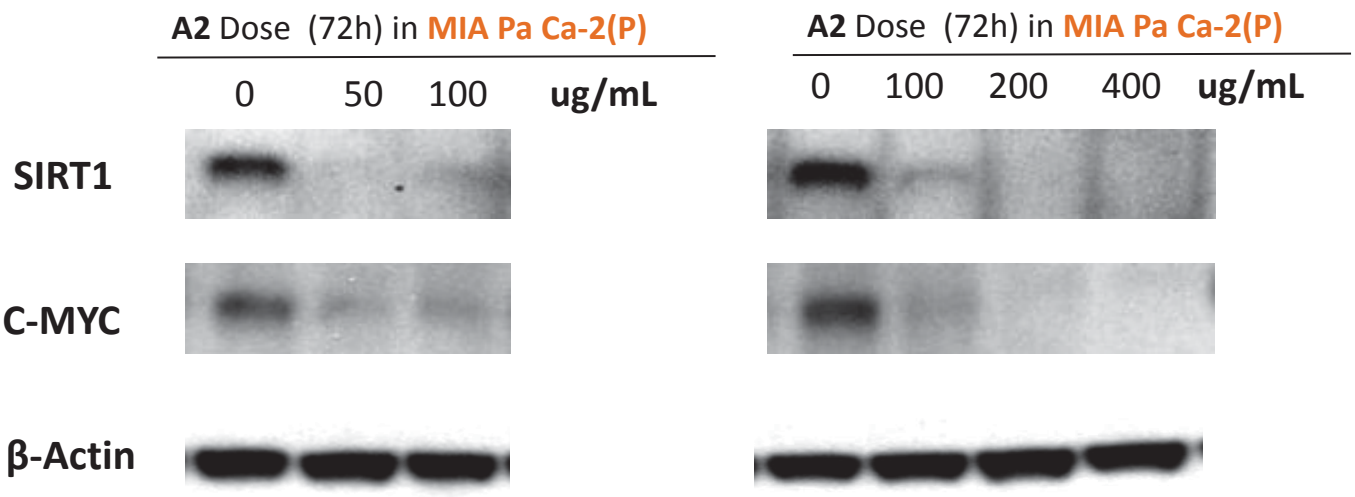
Cell: 1. MIA Pa Ca-2 (P)
Drug : A2
Dose:0、50 、 100ug/mL
Time: 72h

Cell: 1. MIA Pa Ca-2 (P)
Drug : A2
Dose:0、100、200u 、 400 ug/mL
Time: 72h

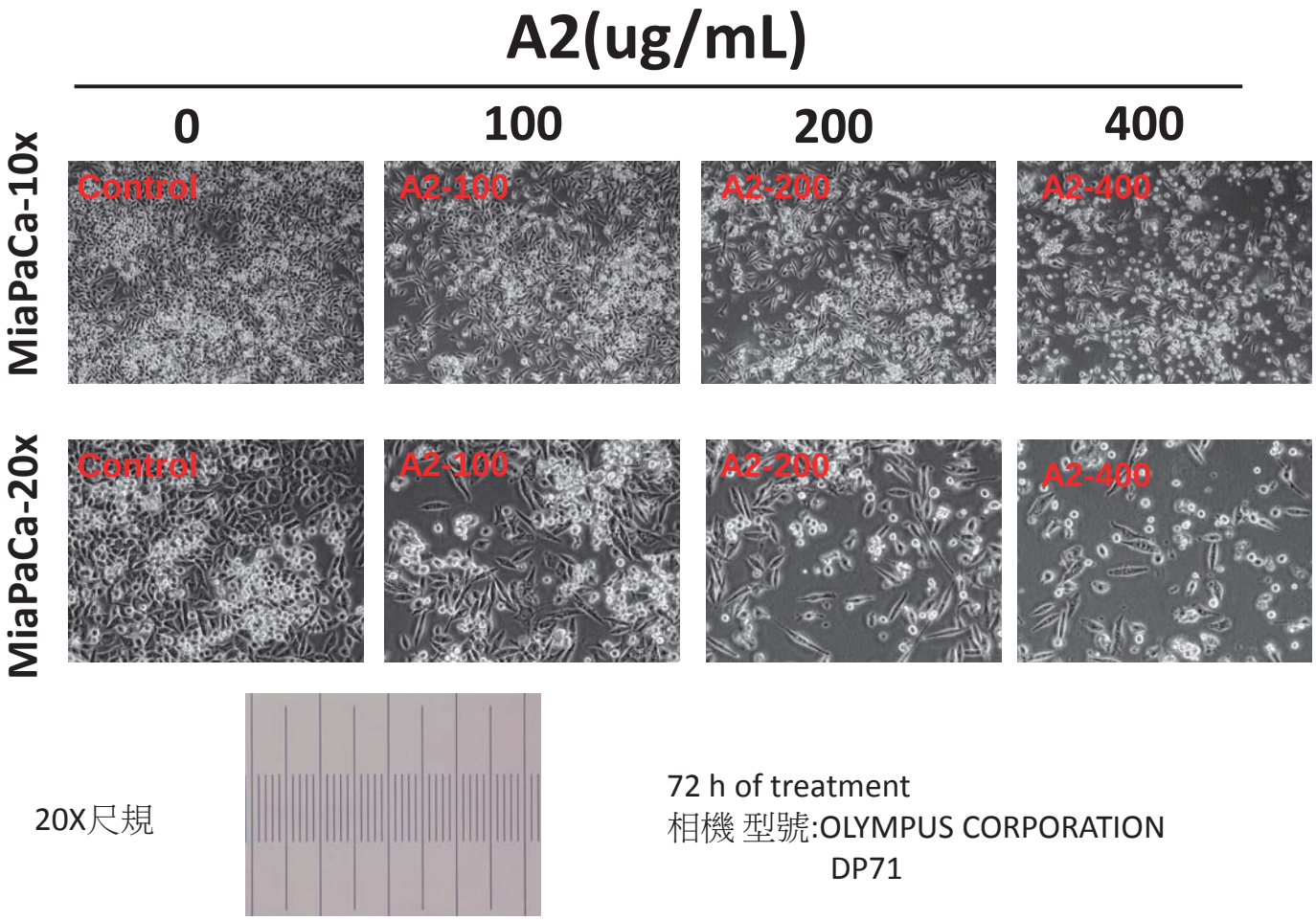
103.01.13w1

A2 in MIAPaCa-2(P) Cell						
72hr			72hr			
0ug/mL	50ug/mL	100ug/mL	0ug/mL	100ug/mL	200ug/mL	400ug/mL
10 uL	10 uL	10 uL	10 uL	10 uL	10 uL	10 uL

A2 (ug/mL) in MIA Pa Ca-2(P) Pancrease cell



103.03.28



20X尺規

72 h of treatment
相機 型號:OLYMPUS CORPORATION
DP71

1.不同濃度 A2 藥物對MIA Pa Ca-2(P) 胰臟癌細胞的影響

～4-1. Caspase pathway

第一次data

102.11.28

A2 (ug/mL) in MIA Pa Ca-2(P) Pancrease cell

A2 Dose (72h) in MIA Pa Ca-2(P)

0 100 200 400 **ug/mL**

Caspase-8

70

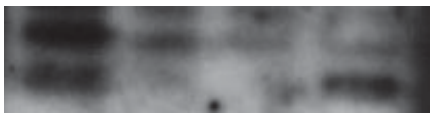
55

40

57 Full Length Caspase-8

43 Cleaved Caspase-8 p43

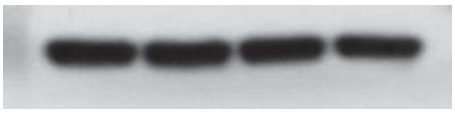
Caspase-3



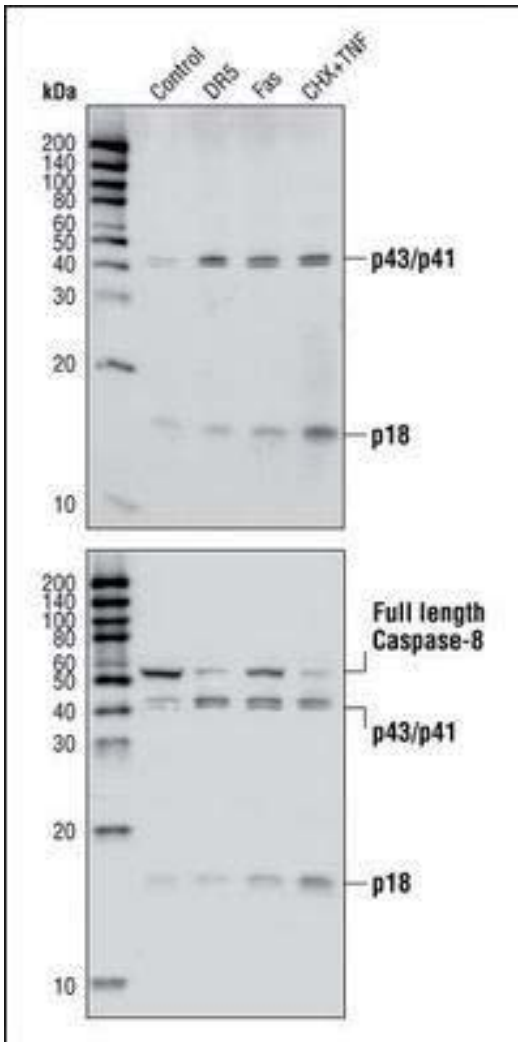
19

17 Cleaved Caspase-3 (Asp175)

β-Actin



Caspase-8
57kda,
43kda,
18kda



Molecular Cancer Therapeutics mct.aacrjournals.org
doi: 10.1158/1535-7163.TARG-13-A251
Mol Cancer Ther November 2013 12; A251
Abstract A251: MGMT inhibition decreases survivin expression in pancreatic cancer.

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Author Affiliations

Abstract
Survivin is an antiapoptotic gene negatively regulated by p53 tumor suppressor. Survivin is overexpressed in human cancers and one of its functions is to reduce the efficacy of chemotherapy. We report that O6-benzylguanine (BG), a potent inhibitor of MGMT (O6-methyl guanine DNA methyl transferase), a DNA repair protein, curtails the expression of survivin in pancreatic cancer cells. We also show that BG, either alone or in combination with gemcitabine, inhibits survivin and PCNA expression both at the mRNA and protein levels in Panc1 and L3.6pl cells. Further, BG, either alone or in combination with gemcitabine, significantly decreases survivin promoter activity in these cells. We also show that these combinations decrease survivin protein expression in pancreatic tumors derived from orthotopic xenografts developed in nude mice. Immunohistochemistry confirms the reduced expression of survivin and PCNA in tumor sections after BG or BG plus gemcitabine treatments. We also show the enhanced recruitment of p53 protein to the survivin promoter in BG-treated pancreatic cancer cells. Further, BG promotes p53 function leading to growth inhibition. Taken together our data suggests that BG induced partial restoration of p53 activity suppresses survivin function and enhances gemcitabine effect in pancreatic cancer.

Most pancreatic patients were treated with A2 combined with chemotherapy agents such as gemcitabine and cisplatin

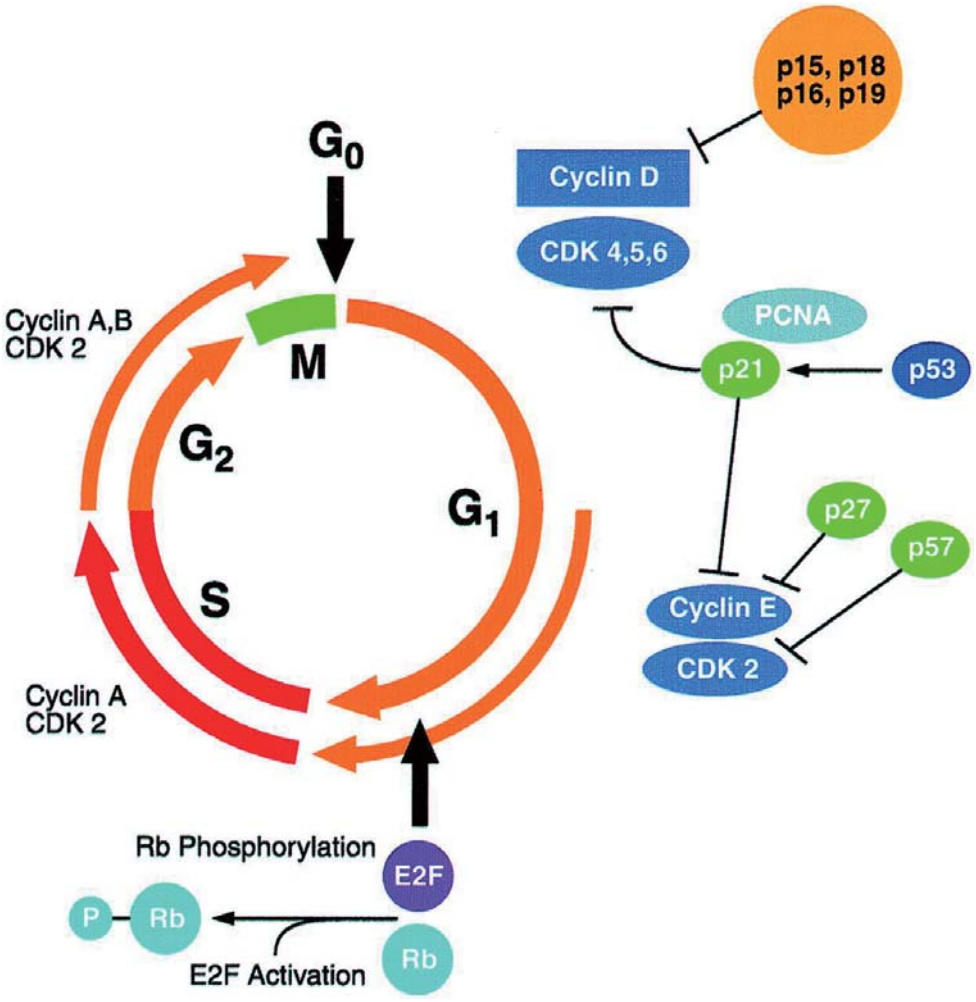
The EFFECTS of A2 on MGMT, Myc and SIRT1 lead to chemosensitization?
Provide the underlying mechanism for the clinical effects?

24. Isacoff WH, Moss RA, Pecora AL, Fine RL. Temozolomide/capecitabine therapy for metastatic neuroendocrine tumors of the pancreas. A retrospective review. J Clin Oncol, ASCO Annual meeting proceedings part I. 2006;24:14023.
25. F D, Fine RL, Schreibman SM. Effective treatment of neuroendocrine tumors with temozolomide and capecitabine. J Clin Oncol, ASCO Annual meeting proceedings. 2005;23:4216.

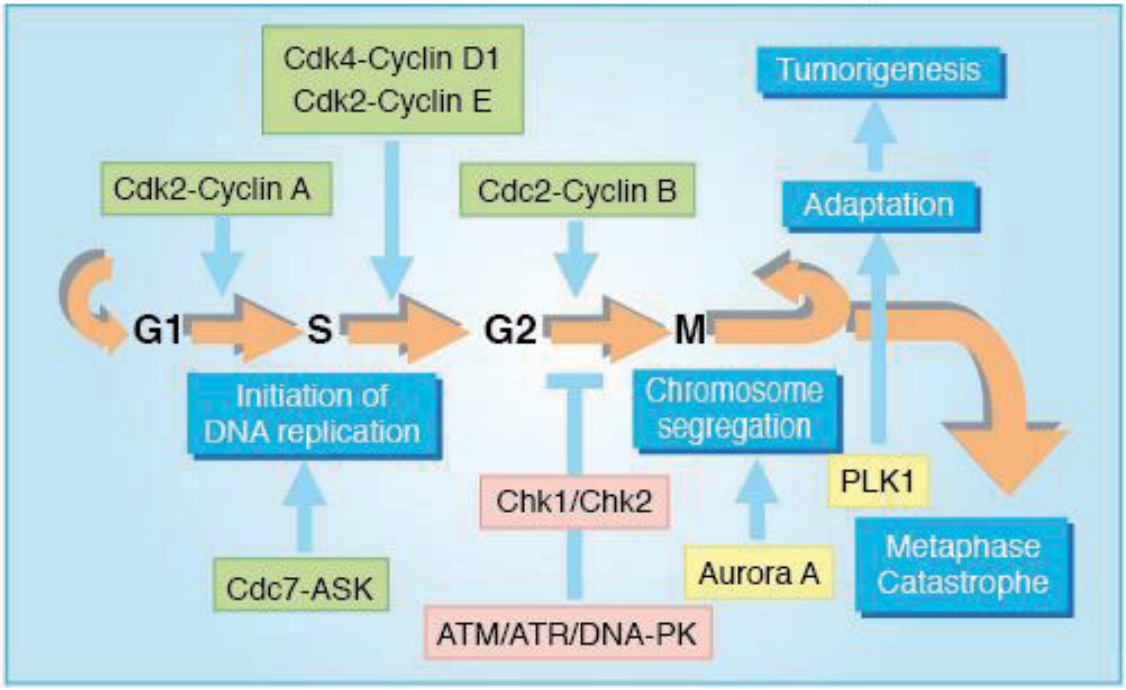
SIRT1 RNAi knockdown induces apoptosis and senescence, inhibits invasion and enhances chemosensitivity in pancreatic cancer cells

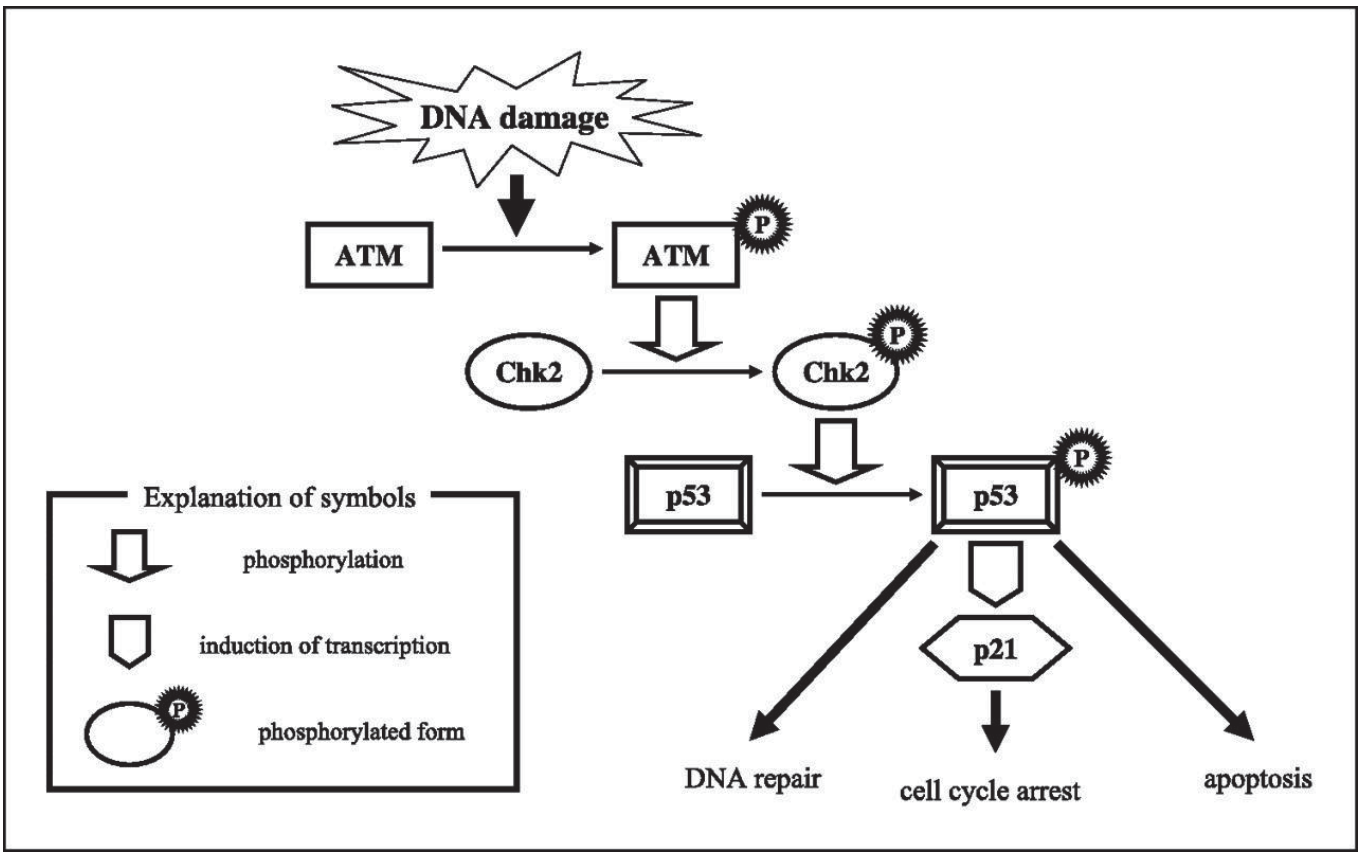
Gene Therapy **18**, 920-928 (September 2011) | doi:10.1038/gt.2011.81

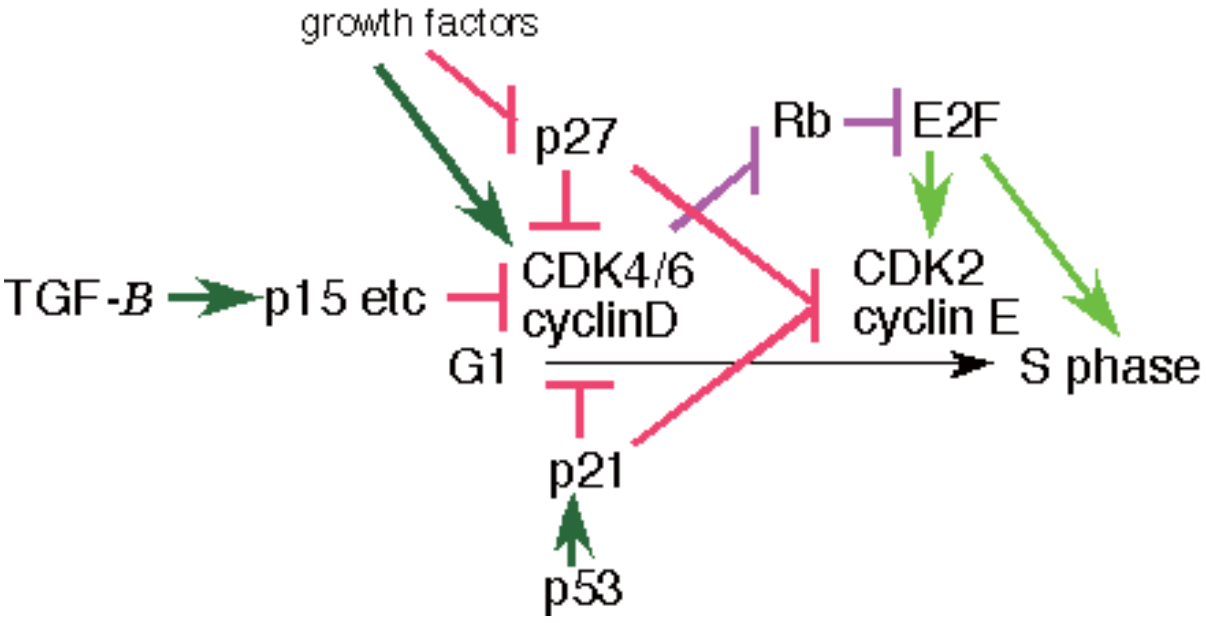
The NAD+-dependent deacetylase, sirtuin 1 (SIRT1), has been recently been suspected to have a role in tumorigenesis. We investigated the expression of SIRT1 in pancreatic cancer and the effect of SIRT1-targeted RNA interference (RNAi) on cell proliferation and tumor formation in a pancreatic cancer cell line, PANC1. The expression of SIRT1 was investigated in 49 specimens of pancreatic cancer and adjacent normal pancreatic tissues. SIRT1 was overexpressed in pancreatic cancer tissues at both the mRNA and protein levels, with increased SIRT1 positivity associated with tumors from patients over 60 years old, tumors larger than 4 cm, higher TNM (extent of tumor (T), the extent of spread to lymph nodes (N), and presence of distant metastasis (M)) stage or the presence of lymph node or hepatic metastases. The PANC-1 was stably transfected with a SIRT1 small hairpin RNA (shRNA) expression plasmid and compared with untransfected and PANC-1-negative RNAi cells. Proliferation of PANC-1–SIRT1–RNAi cells was significantly reduced, accompanied by increased rates of apoptosis, G1 arrest and senescence. Furthermore, FOXO3a expression was markedly upregulated in PANC-1–SIRT1–RNAi cells, but no significant difference in p53 expression was observed. The invasive ability of PANC-1–SIRT1–RNAi cells was markedly reduced in vitro, which was linked to increased E-cadherin and reduced-MMP expression. Additionally, PANC-1–SIRT1–RNAi cells had a significantly reduced capacity to form tumors in vivo compared with untransfected and PANC-1-negative RNAi cells. These results suggest that SIRT1 may promote cell proliferation and tumor formation in pancreatic cancer, and downregulation of SIRT1 using shRNA could provide a novel therapeutic treatment.

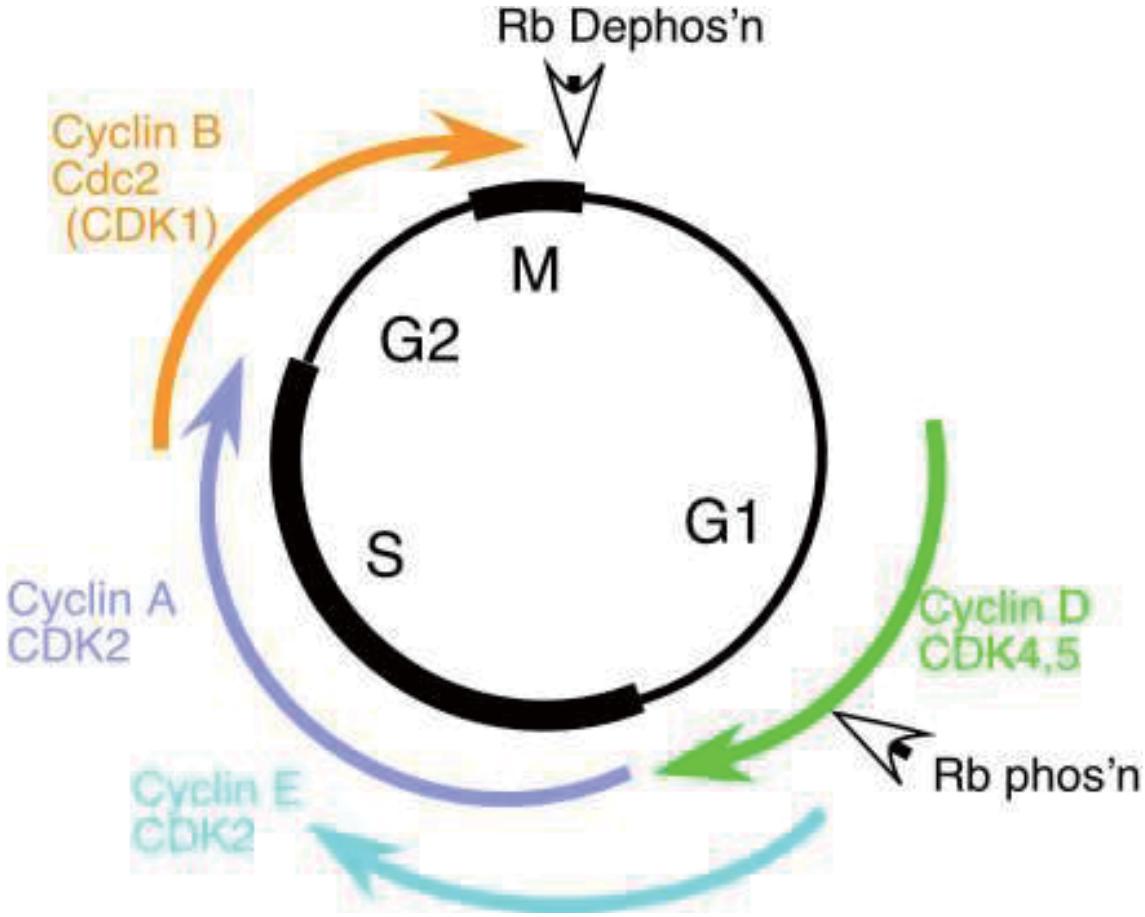


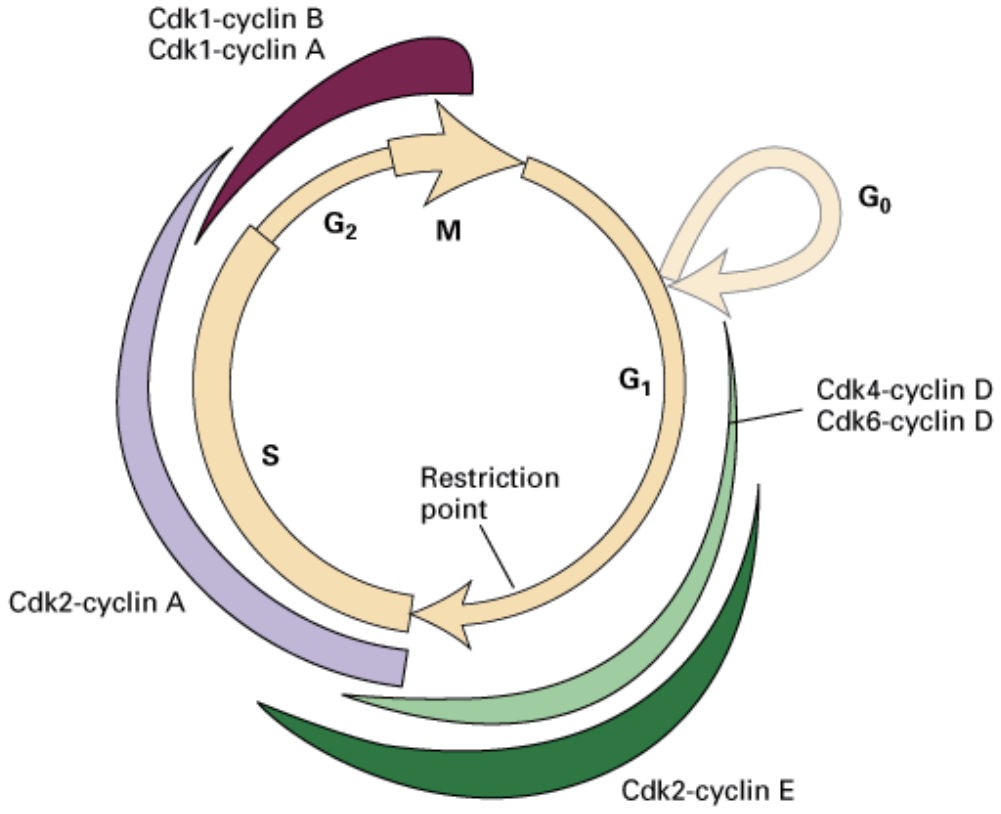
■ Serine/Threonine Kinases associated with cell cycle control

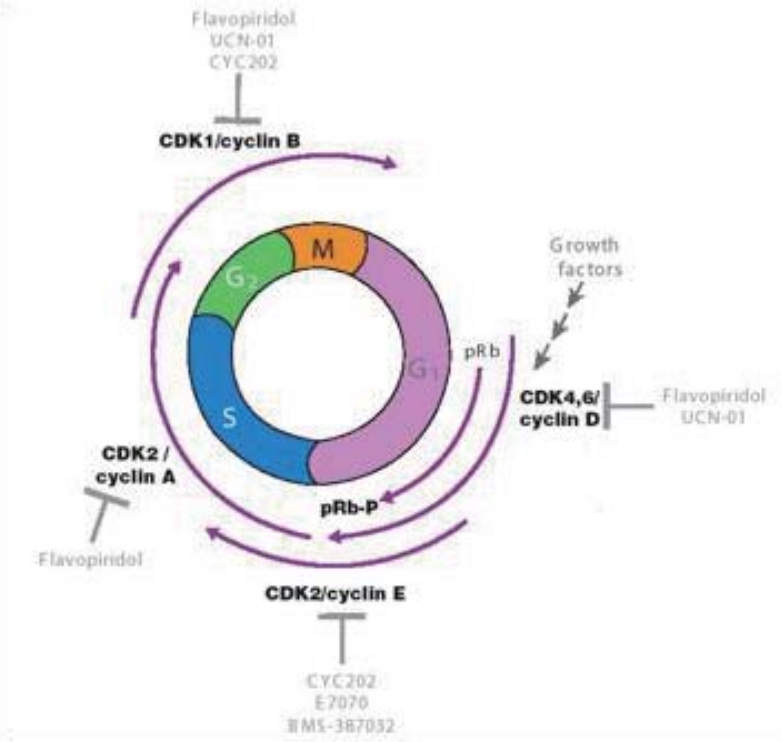



DNA damage
ATM
ATM
P
Chk2
Chk2
P
p53
p53
P
p21
DNA repair
cell cycle arrest
apoptosis
Explanation of symbols
phosphorylation
induction of transcription
phosphorylated form
P


growth factors
p27
Rb
E2F
TGF-B
p15 etc
CDK4/6
cyclinD
CDK2
cyclin E
G1
S phase
p21
p53


Rb Dephos'n
Cyclin B
Cdc2
(CDK1)
M
G2
G1
S
Cyclin A
CDK2
Cyclin D
CDK4,5
Rb phos'n
Cyclin E
CDK2


Cdk1-cyclin B
Cdk1-cyclin A
G2
M
G0
G1
Cdk4-cyclin D
Cdk6-cyclin D
S
Restriction
point
Cdk2-cyclin A
Cdk2-cyclin E

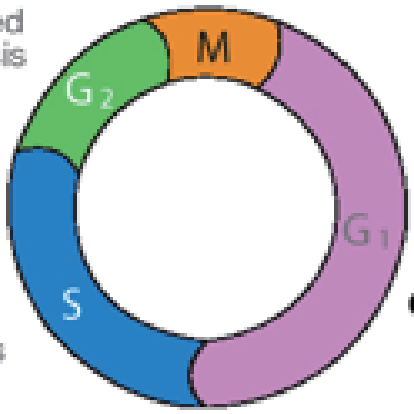


G_2 phase (gap 2)

- Cell growth continues
- Proteins are synthesised in preparation for mitosis
- Repair of errors that occurred during DNA replication

M phase (mitosis)

- Daughter chromosomes separate
- Usually followed by cytokinesis (cell division)

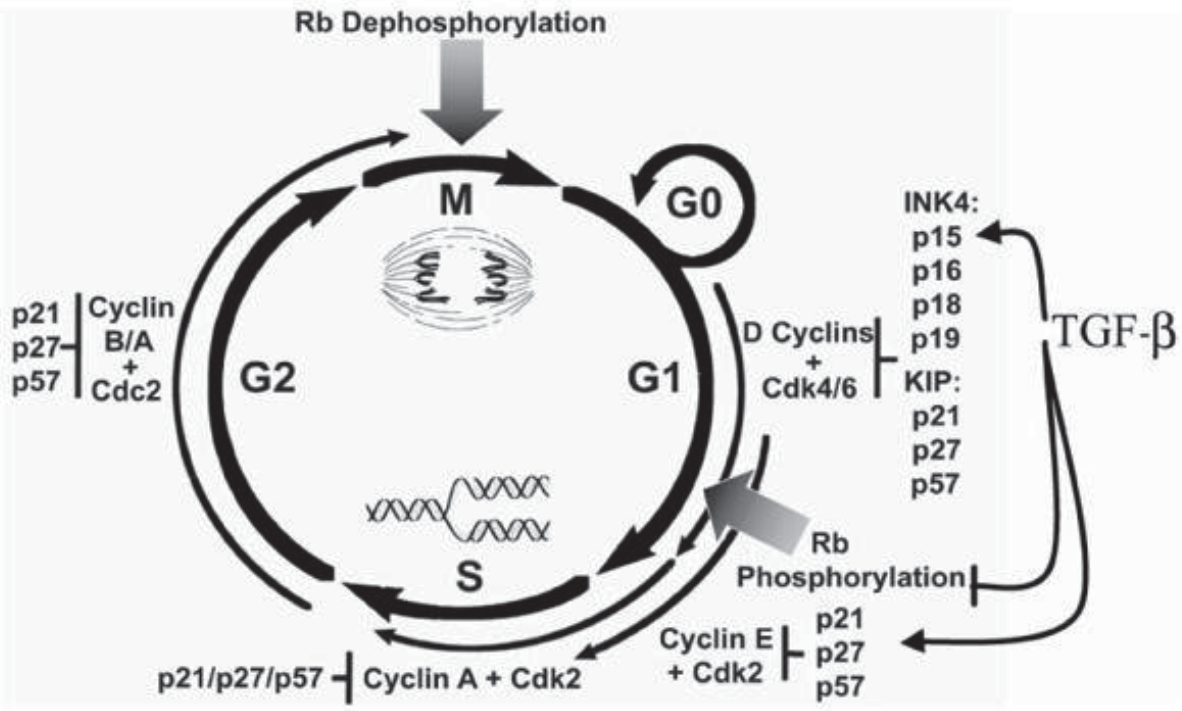


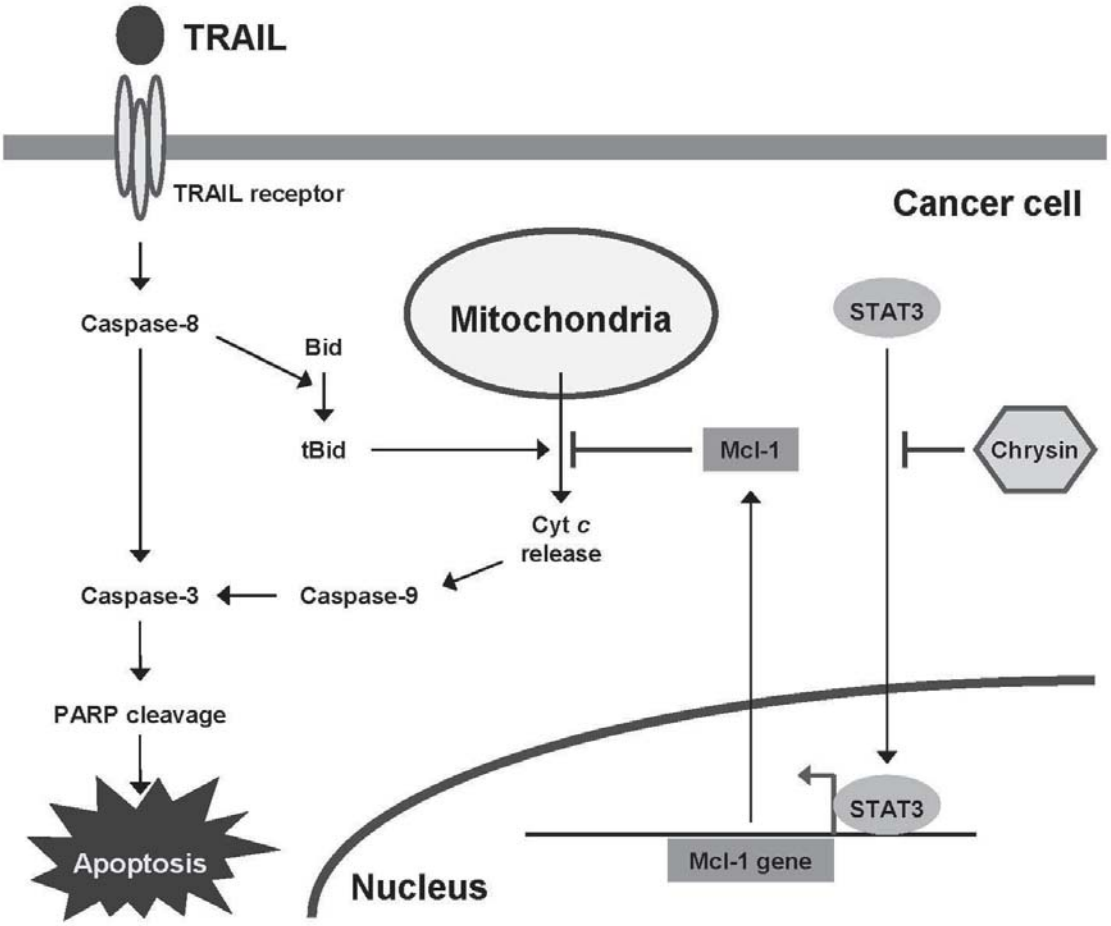
S phase (synthesis)

- DNA replication occurs to duplicate the cell's genome

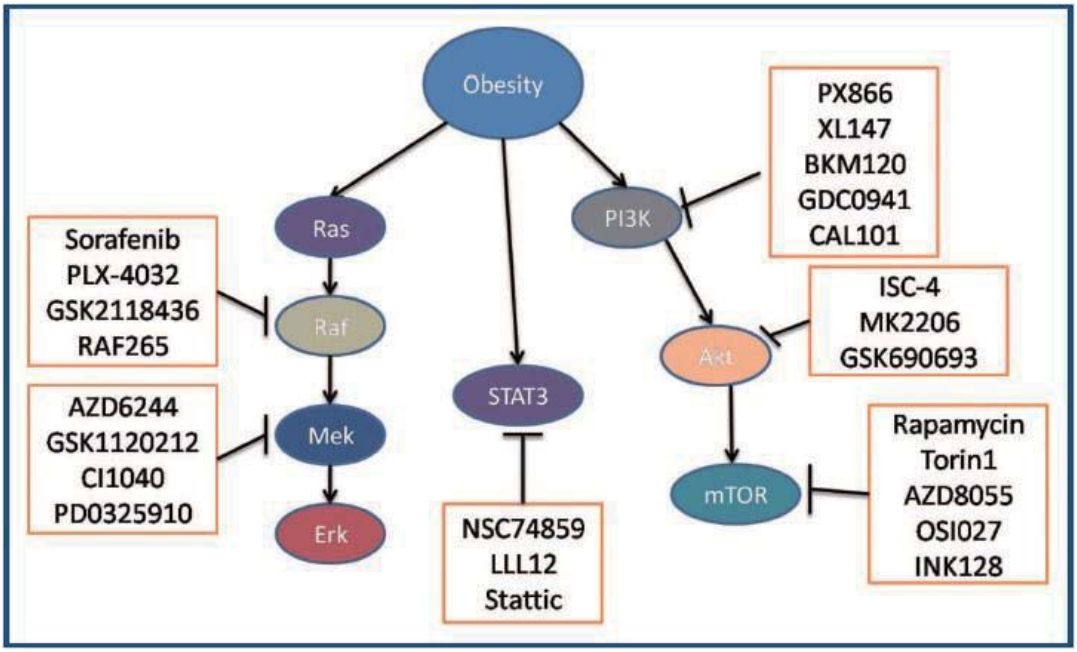
G_1 phase (gap 1)

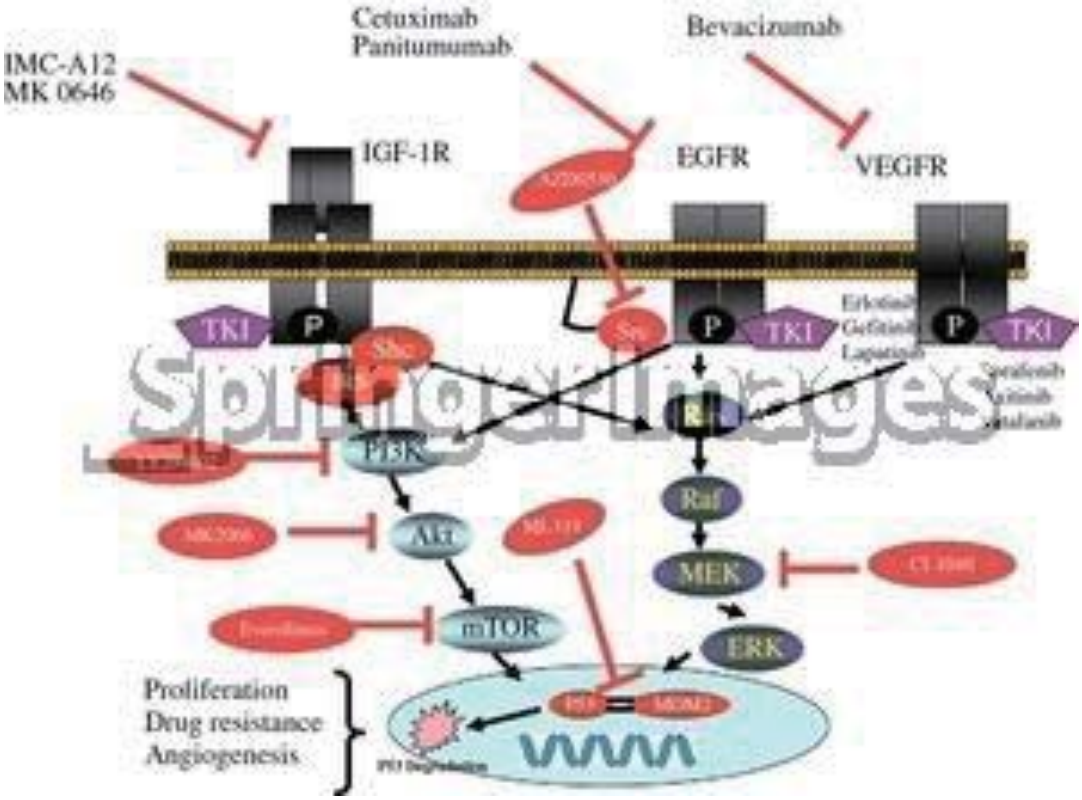
- The cell is metabolically active and growth occurs
- Cells commit to enter the cycle and prepare to duplicate their DNA

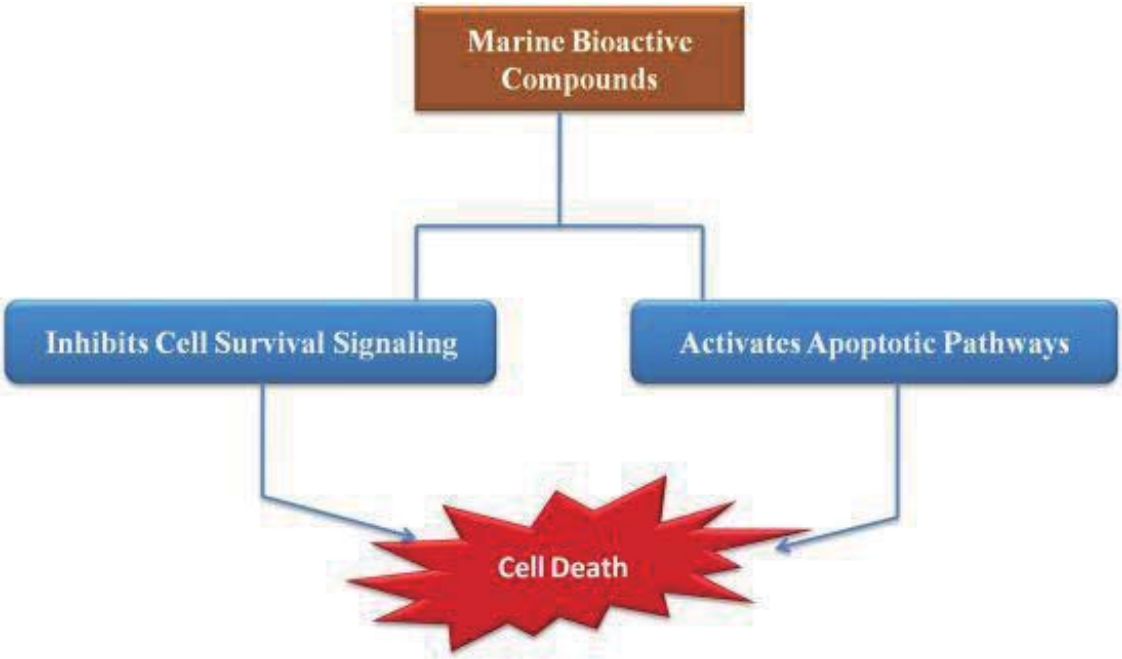




Int J Oncol. 2013 Jul;43(1):329-37.


Obesity
Ras
Raf
Mek
Erk
PI3K
Akt
mTOR
STAT3
Sorafenib
PLX-4032
GSK2118436
RAF265
AZD6244
GSK1120212
CI1040
PD0325910
NSC74859
LLL12
Stattic
PX866
XL147
BKM120
GDC0941
CAL101
ISC-4
MK2206
GSK690693
Rapamycin
Torin1
AZD8055
OSI027
INK128


IMC-A12
MK 0646
Cetuximab
Panitumumab
Bevacizumab
IGF-1R
EGFR
VEGFR
TKI
P
Shc
PI3K
Akt
Raf
MEK
ERK
mTOR
Proliferation
Drug resistance
Angiogenesis


Marine Bioactive Compounds
Inhibits Cell Survival Signaling
Activates Apoptotic Pathways
Cell Death