國立嘉義大學生命科學院 104 年度
學生學術研究成果優良海報評選獲獎名單

時間：104 年 6 月 3 日

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Investigation the role of MSH2 expression in Hsp90 inhibitor enhancing pemetrexed-induced cytotoxicity in human lung cancer cells

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Abstract

Pemetrexed has demonstrated certain clinical activities against non-small cell lung cancer (NSCLC). However, no appropriate biomarkers have been recognized for predicting the clinical response to pemetrexed. DNA repair functionality plays a key role in chemosensitivity and cell death induction in squamous cell carcinoma, in which human MutS homolog 2 (MSH2), is a crucial element of the highly conserved DNA mismatch repair system. In response to stress stimuli that cause DNA damage, cells undergo cell cycle checkpoints to allow time for DNA repair, while the p38 MAPK pathway is involved in the induction of that. Until now, whether pemetrexed affects MSH2 expression in NSCLC is unknown. We hypothesized that the up-regulation of MSH2 is responsible for the cell's resistance to pemetrexed. In the other hand, elevated heat shock protein 90 (Hsp90) expression has been linked to poor prognosis in patients with NSCLC. Additionally, inhibition of Hsp90 functions affects multiple oncogenic substrates simultaneously and has shown obvious anti-cancer effects in vitro and in vivo. At first, we found that treatment with pemetrexed at 20–50 mM increased the MSH2 mRNA and protein levels in a MKK3/6–p38 MAPK signal activation-dependent manner. Furthermore, knockdown of MSH2 expression by transfection with small interfering RNA of MSH2 or the blockage of p38 MAPK activation by specific siRNA enhanced the cytotoxicity of pemetrexed. Combining the drug treatment with an Hsp90 inhibitor (17-AAG) resulted in an enhanced pemetrexed-induced cytotoxic effect, accompanied with the reduction of MSH2 protein and mRNA levels. The expression of constitutively active MKK6 (MKK6E) or HA-p38 MAPK vectors significantly rescued the decreased p38 MAPK activity, and restored the MSH2 protein and mRNA as well as cell survival in NSCLC cells co-treated with pemetrexed and Hsp90 inhibitor. On the basis of our data, suggested that combinatory treatment with pemetrexed and an Hsp90 inhibitor, or the inhibition of MSH2 expression, could provide new tools for overcoming the resistance to pemetrexed in patients with NSCLC, although the clinical relevance of these findings needs to be confirmed.

Aim

17-AAG → ?

Pemetrexed → MKK3/6 → p38 MAPK → MSH2 → Cell survival

Results

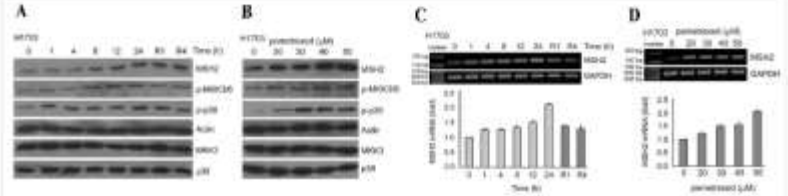


Fig. 1 Pemetrexed increased MSH2 and the p-MKK3/6 and p-p38 MAPK expression

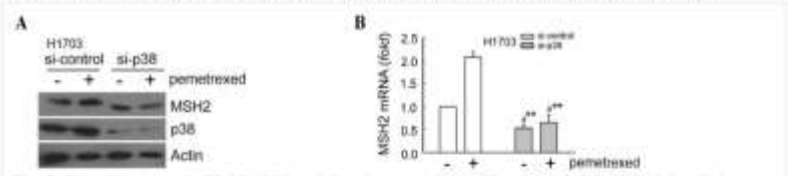


Fig. 2 Inactivation of p38 MAPK activity decreased MSH2 protein and mRNA levels in pemetrexed-exposed NSCLC cells

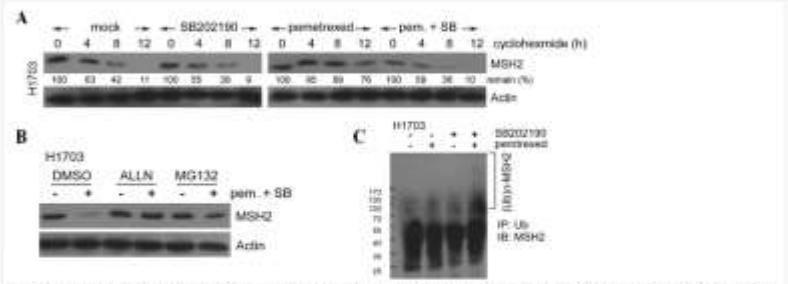


Fig. 3 MKK3/6–p38 MAPK signaling could protect MSH2 from ubiquitin-26S proteasome-mediated degradation

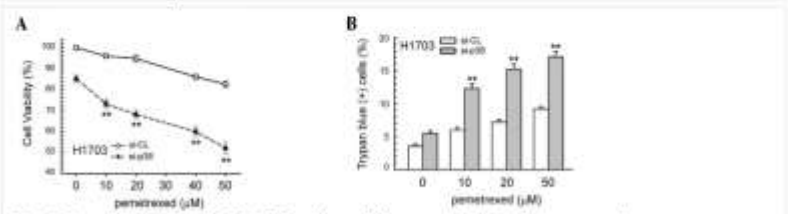


Fig. 4 Inactivation of p38 MAPK enhanced the cytotoxicity of pemetrexed

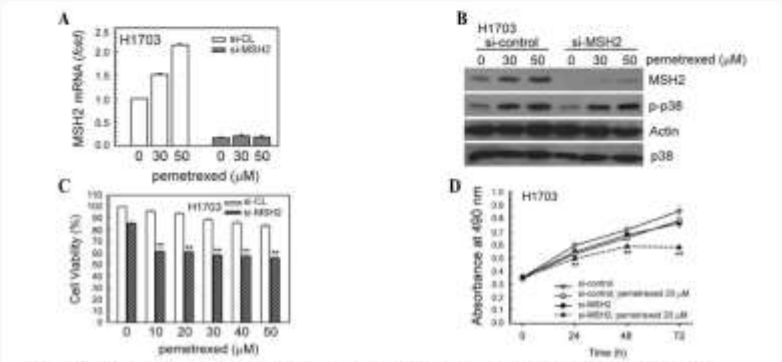


Fig. 5 Knockdown of MSH2 expression enhanced pemetrexed-induced cytotoxicity

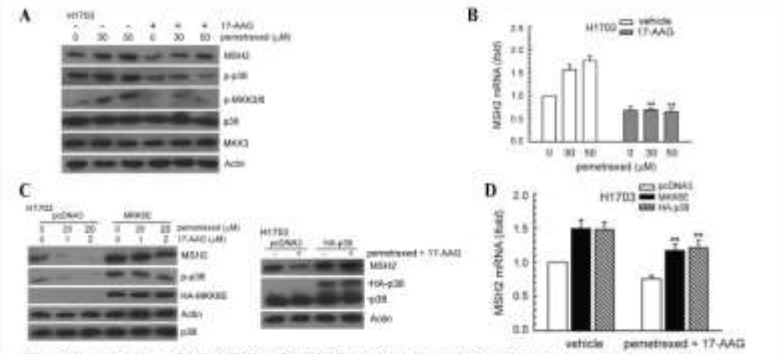


Fig. 6 Induction of MKK3/6–p38 MAPK phosphorylation by pemetrexed could be prevented by 17-AAG

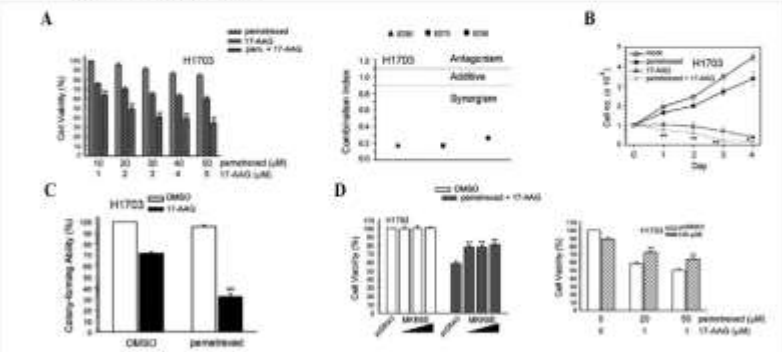
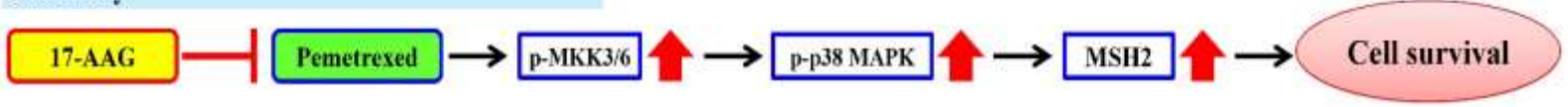


Fig. 7 17-AAG enhanced pemetrexed-induced cytotoxicity via the inactivation of p38 MAPK

Summary



Activation of aryl hydrocarbon receptor reduces carbendazim-induced cell death

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Abstract:

Carbendazim is being explored to be an anti-cancer drug, due to its ability to inhibit microtubule assemble in human cells. It depolymerize microtutules, therefore are able to inhibit mitosis and arrest cell cycle at G2/M phase, resulting in inhibiting cancer cell proliferation. Due to its promising preclinical antitumor activity, carbendazim is potential to be applied as anti-cancer drugs. Our data show that carbendazim induces mRNA and protein expression of cytochrome P450 1A1 (CYP1A1) and its promoter activity. In addition, carbendazim activates aryl hydrocarbon response element (AHRE), a binding site of the activated aryl hydrocarbon receptor (AhR). The carbendazim-induced CYP1A1 expression is blockd by AhR antagonsits, and is highly decreased in the AhR-signal deficient cells. These results demonstrated that carbendazim activates AhR, by which CYP1A1 expression is stimulated. In order to know whether the AhR-induced metabolic enzymes convert carbendazim into higher or less toxic metabolites, the flow cytometry to reveal subG0/G1 population and MTT assay to reveal proliferation, were applied to monitor the carbendazim-induced cell apoptosis. Results show that carbendazim induces less apoptosis in Hepa-1c1c7 cells than in both c4 and c12 cells. Pretreatment of β-NF, a synthetic potent AhR agonist and highly inducing CYP1A1 expression, decreases the carbendazim-induced cell death. In summary, our results also show that the toxicity of carbendazim decreased in cells with AhR signal. This information provides an important reference for the application of carbendazim in cancer chemotherapy.

Results

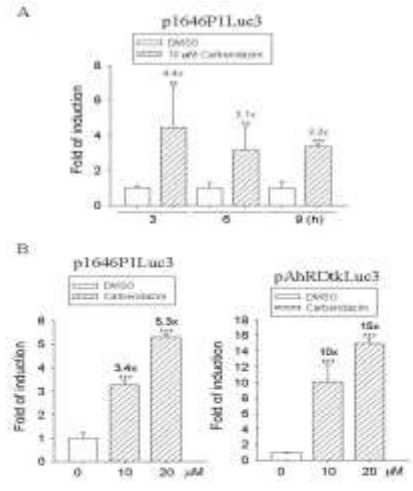

Fig. 1. Effect of carbendazim on transactivation activity of Cyp1a1 promoter and aryl hydrocarbon response element (AHRE). Luciferase reporters, p1646P1Luc3 and pAhRDtkLuc3, transfected in Hepa-1c1c7 cells are indicated on each chart. (A) Cells were treated with carbendazim (10 μM) for 3–9 h. (B) Cells were treated with carbendazim (10, and 20 μM) for 3 h. At the end of incubation with the test compounds, cells were harvested, and cell lysates were collected for an activity assay of luciferase and β-galactosidase. Results are expressed as the mean ± S.E.M., $n = 3$. ***$p < 0.001$, **$p < 0.01$.

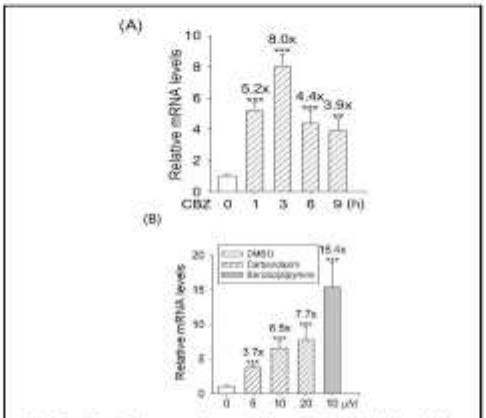

Fig. 2. Cyp1a1 expression profiles of mRNA induced by carbendazim (CBZ) and benzo[a]pyrene (BaP). (A) Hepa-1c1c7 cells were treated with carbendazim (10 μM) until they were collected at 0-9 h for RNA analysis. (B) Hepa-1c1c7 cells were treated with carbendazim (1–20 μM) for 3 h. Total RNA were harvested for the analysis. The expression of Cyp1a1 mRNA was analyzed by quantitative PCR as described in Experimental Procedures. Results are expressed as the mean (SEM, n) 3. * compared to the bar indicated by the arrow. ***$p < 0.001$, **$p < 0.01$.

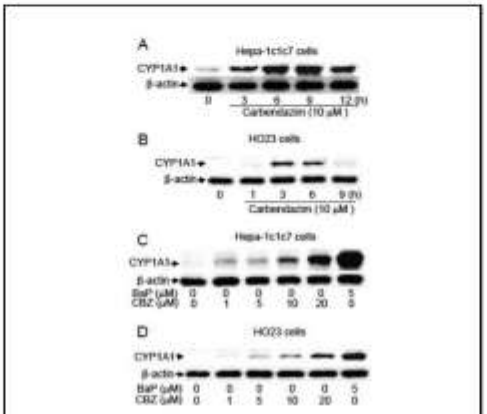

Fig. 3. CYP1A1 expression profiles of protein induced by carbendazim. (A and B) HO23 and Hepa-1c1c7 cells were treated with carbendazim (10 μM) for 1–12 h. (C and D) HO23 and Hepa-1c1c7 cells were treated with carbendazim (1–20 μM) for 3 and 6 h, respectively. The expression of CYP1A1 protein was analyzed by Western blots.

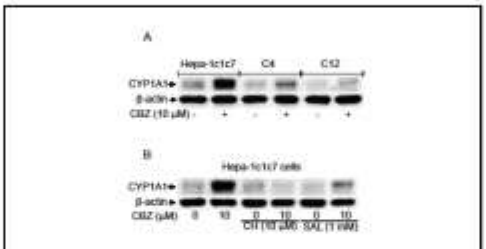

Fig. 4. Effect of aryl hydrocarbon receptor (AhR) signal on the expression of cytochrome P450 1A1 (CYP1A1) induced by carbendazim (CBZ). (A) Hepa-1c1c7, c4, and c12 cells were treated with CBZ (10 μM) for 6 h. (B) Hepa-1c1c7 cells were treated with either 10 μM CH-223191 (CH) or 1 mM salicylamide (SAL) for 1 h, followed by treatment with CBZ for 6 h. The CYP1A1 protein expression of their cell lysates was analyzed by Western blots. Both the c4 and c12 cell lines were derived from Hepa-1c1c7 and are deficient in Arnt and the AhR, respectively.

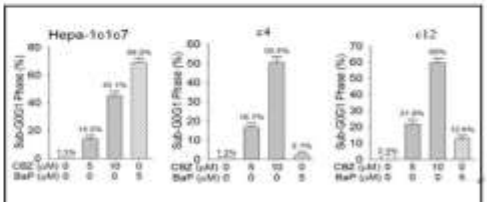

Fig. 5. The aryl hydrocarbon receptor (AHR)-signal regulated carbendazim (CBZ)-induced cell death. Hepa-1c1c7, c4, and c12 cells were treated with CBZ (5 and 10 μM) and benzo[a]pyrene (BaP) (5 μM) for 24 h. Afterwards, cells were subjected to staining with propidium iodide and analyzed by flow cytometry. Cell death was determined as the percentage of signals to the left of the G1 peak (sub G0/G1 population). Cells that had died were determined as shown on the charts. Data are the mean ± SD, n = 3. * Indicates a comparison with DMSO-treated cells. *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$.

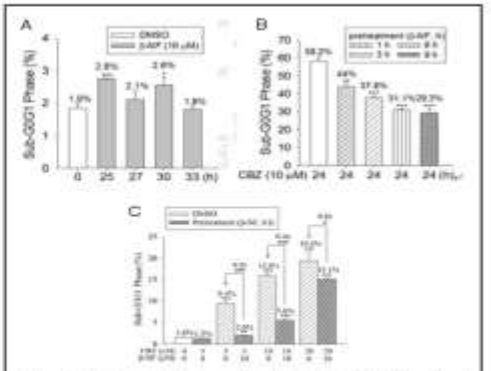

Fig. 6. β-NF neutralizes carbendazim (CBZ)-induced cell death. (A) Hepa-1c1c7 cells were treated with β-NF (10 μM) for 25-33 h. (B) Hepa-1c1c7 cells were pretreated with β-NF (10 μM) for 1-9 h, followed by treatment with CBZ (10 μM) for 24 h. (C) HepG2 cells were pretreated with β-NF (10 μM) for 8 h, followed by treatment with CBZ (5, 10 and 20 μM) for 24 h. Afterwards, cells were subjected to staining with propidium iodide and analyzed by flow cytometry. Cell death was determined as the percentage of signals to the left of the G1 peak (sub G0/G1 population). Cells that had died were determined as shown on the charts. Data are the mean ± SD, n = 3. * Indicates a comparison with DMSO-treated cells. *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$.

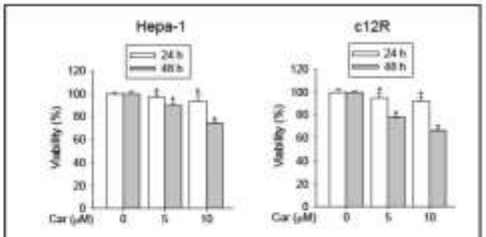

Fig. 7. Concentration-dependent inhibition of carbendazim on cell viability. Hepa-1 and c12 cells were cultured with the test compounds, for 24 and 48 h. Cell viability was analyzed by MTT assay. The data represent the mean±S.E.M. of at least three separate experiments. An asterisk (*) indicates a significant difference from the control ($p < 0.05$).

Conclusion

Carbendazim activated AhR and induced AhR-targeting gene, CYP1A1, a phase I metabolic enzyme. It is predictable that carbendazim potentially induces other phase I, and phase II and III metabolic enzymes. This report provides a model for the studies of AhR-induced metabolism. In addition, it reveals the toxicity model of carbendazim in tissues.

Investigation of the molecular mechanism of tumorigenesis in 5-Fu- and Oxaliplatin-resistant human colorectal cancer stem cells

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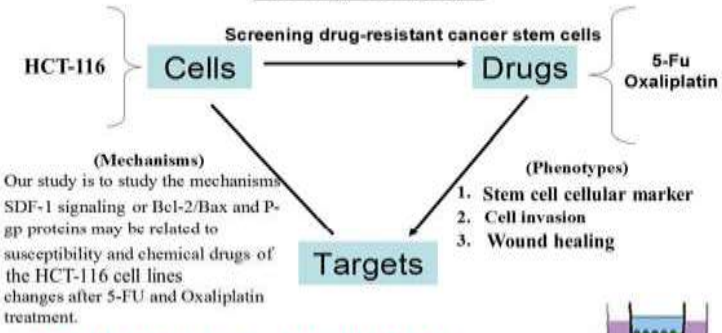
INTRODUCTION

Colorectal cancer in Taiwan is already the highest cancer incidence rate, and third cancer mortality rate. Chemotherapy treatments have made great contributions to the inhibition of cancer recurrence, and metastasis. But, Chemotherapy is often resistant in about half of the patients, resulting in cancer recurrence and progression. A lot of evidence are finding to suggest that cancer stem cells will lead chemoresistance and cause tumor sustained growth. However, colorectal cancer stem cells(CSCs) for drug resistance mechanisms remain unclear.

MOTIVE

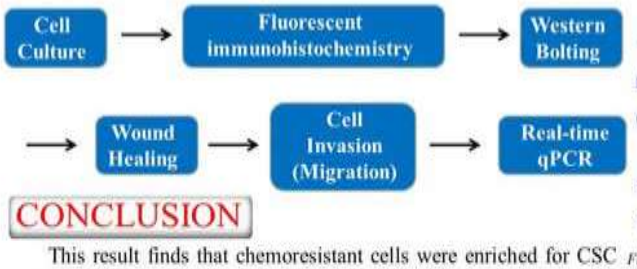
In this research, we use the mainstay chemotherapeutic drug, 5-Fluorouracil (5-FU) and Oaxliplatin (OxR) were selected with the colon cancer cell line HCT-116. The cancer stem cell lines were screened for colorectal cancer by the use of chemical resistant cloning method to analysis of whether a cancer stem cell characteristics. Study of the effect of SDF-1 on cancer stem cells signaling and uPA expression and the regulation of the Bcl-2/Bax and P-gp proteins may be related to resistant chemotherapy drug resistance mechanism.

Study Design



MATERIALS & METHODS

Colorectal cancer (CRC)	Cancer stem cell (CSC)
HCT-116	HCT-116/OxR HCT-116/5FU



CONCLUSION

This result finds that chemoresistant cells were enriched for CSC markers and showed higher tumorigenic capacity in vitro. Next, we will be determined that effects of 5-FU-resistant and OxR-resistant colorectal cancer stem cells were associated with overexpressed Bcl-2/Bax, P-gp and uPA proteins via activation of CXCL12 (SDF-1)/ CXC chemokine receptor 4 (CXCR4). These findings provide further insight into the molecular mechanism and suggest promising biomarkers for clinic therapy of colorectal cancer patients outcome of patients with lymph node metastasis.

REFERENCE

1. Anthony D. Yang, Fan Fan, E. Ramsay Camp, et al. (2006) Chronic Oxaliplatin Resistance Induces Epithelial-to-Mesenchymal Transition in Colorectal Cancer Cell Lines. *Clin Cancer Res.* 12:4147-4153.
2. You-Kyung Jeon, et al. (2010) Promoter hypermethylation and loss of CD133 gene expression in colorectal cancers. *World J Gastroenterol.* 16(25): 3153-3160.
3. Sanchita Roy, Adhip P N Majumdar.(2012). Signaling in colon cancer stem cells. *Journal of Molecular Signaling.* 7:11.

RESULTS

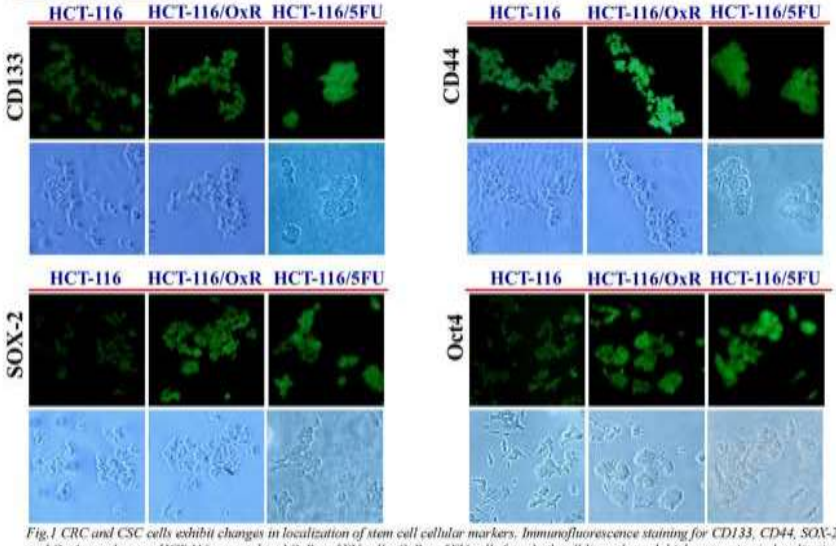
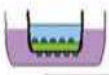


Fig.1 CRC and CSC cells exhibit changes in localization of stem cell cellular markers. Immunofluorescence staining for CD133, CD44, SOX-2 and Oct4 was done on HCT-116 parental and OxR or 5FU cells. OxR or 5FU cells from both cell lines showed high expression in localization of cellular markers.

Fig.2 CRC and CSC cells of stem cell cellular markers by Western blotting analysis.



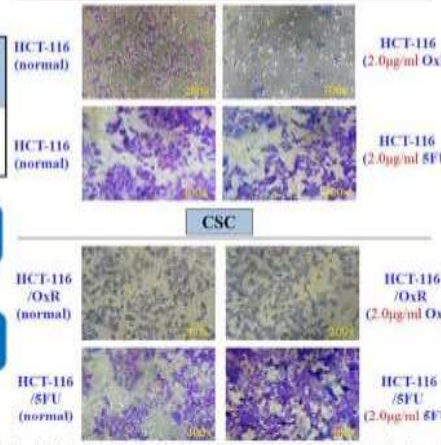


Fig.4 CSC cells have increased migratory and invasive capacity. Boyden chamber and modified Boyden chamber assays were done to compare the migratory and invasive capabilities of CRC and CSC cells.

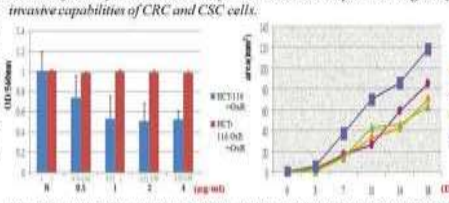


Fig.5 Compared drug resistance differentially expressed of the CRC and CSC cells in vitro/ in vivo.

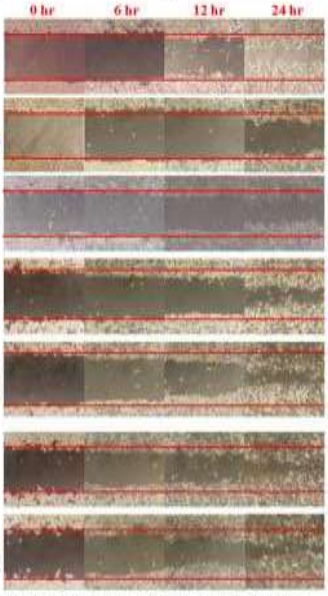


Fig.3 Wound-healing assay showed that the comparison of CRC and CSC cells.

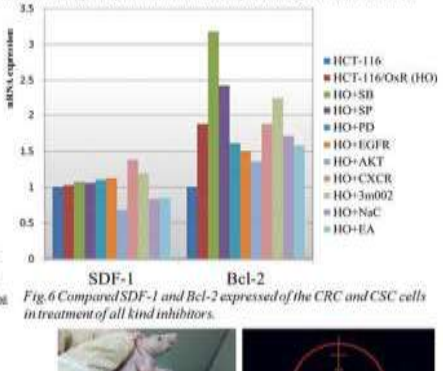


Fig.6 Compared SDF-1 and Bcl-2 expressed of the CRC and CSC cells in treatment of all kind inhibitors.