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Proteomic investigating the cooperative lethal effect of EGFR and MDM2 inhibitors on ovarian carcinoma

Shing-Jyh Chang[a,1], En-Chi Liao[b,1], Hsin-Yueh Yeo[b], Wen-Hong Kuo[c], Hsin-Yi Chen[b], Yi-Ting Tsai[b], Yu-Shan Wei[b], Ying-Jen Chen[b], Yi-Shiuan Wang[b], Ji-Min Li[b], Chuan-Chi Shih[a], Chia-Hao Chan[a], Hsiu-Chuan Chou[d], Yung-Jen Chuang[b,**], Hong-Lin Chan[b,*]

[a] Department of Obstetrics and Gynecology, Hsinchu MacKay Memorial Hospital, Hsinchu, Taiwan
[b] Institute of Bioinformatics and Structural Biology and Department of Medical Science, National Tsing Hua University, Hsinchu, Taiwan
[c] Department of Surgery, National Taiwan University Hospital, Taipei, Taiwan
[d] Department of Biomedical Engineering and Environmental Sciences, National Tsing Hua University, Hsinchu, Taiwan

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ABSTRACT

With the concept of precision medicine, combining multiple molecular-targeting therapies has brought new approaches to current cancer treatments. Malfunction of the tumor suppressor protein, p53 is a universal hallmark in human cancers. Under normal conditions, p53 is degraded through an ubiquitin-proteosome pathway regulated by its negative regulator, MDM2. In contrast, cellular stress such as DNA damage will activate p53 to carry out DNA repair, cell cycle arrest, and apoptosis. In this study, we focused on ovarian carcinoma with high EGFR and MDM2 overexpression rate. We assessed the effects of combined inhibition by MDM2 (JNJ-26854165) and EGFR (gefitinib) inhibitors on various ovarian cell lines to determine the importance of these two molecular targets on cell proliferation. We then used a proteomic strategy to investigate the relationship between MDM2 and EGFR inhibition to explore the underlying mechanisms of how their combined signaling blockades work together to exert cooperative inhibition. Our results demonstrated that all four cell lines were sensitive to both individual and combined, MDM2 and EGFR inhibition. The proteomic analysis also showed that gefitinib/JNJ-treated CAOV3 cells exhibited downregulation of proteins involved in nucleotide biosynthesis such as nucleoside diphosphate kinase B (NME2). In conclusion, our study showed that the combined treatment with JNJ and gefitinib exerted synergistic inhibition on cell proliferation, thereby suggesting the potential application of combining MDM2 inhibitors with EGFR inhibitors for enhancing efficacy in ovarian cancer treatment.

1. Introduction

Ovarian cancer develops in the ovaries, the reproductive glands responsible for producing eggs and female hormones in women. Ovarian cancer accounts for high mortality rate among all gynecological malignancies and other gynecologic cancers, indicating the severe impact of the disease [1].

The murine double minute2 (*MDM2*) gene is a proto-oncogene encoding a nuclear protein that negatively regulates the transcriptional activation of p53 via the ubiquitination of p53 [2]. This regulation is important for maintaining low levels of p53 in order to sustain normal cell cycle progression and cell survival [3]. The level of MDM2 is elevated in ovarian cancer but extremely low in benign ovarian tumors and normal ovaries. Deregulation of the MDM2/p53 balance may lead to malignant transformation of normal cells and may also affect the chemosensitivity of tumor cells [4].

EGFR overexpression plays a significant role in cancer development and progression, especially in tumor invasiveness and aggressiveness. It has been detected in 30–98% of epithelial ovarian carcinomas [5], and is associated with poor prognosis and worse therapeutic response in ovarian cancer patients [6]. Specifically, the increased EGFR expression has been associated with high tumor grade, high cell proliferation index, and aberrant p53 expression [7].

Precision medicine is an emerging approach for cancer treatment. It

* Corresponding author. Institute of Bioinformatics and Structural Biology & Department of Medical Science, National Tsing Hua University, No.101, Kuang-Fu Rd. Sec.2, Hsinchu, 30013, Taiwan.
** Corresponding author. Institute of Bioinformatics and Structural Biology & Department of Medical Science, National Tsing Hua University, No.101, Kuang-Fu Rd. Sec.2, Hsinchu, 30013, Taiwan.
E-mail addresses: yjchuang@life.nthu.edu.tw (Y.-J. Chuang), hlchan@life.nthu.edu.tw (H.-L. Chan).
[1] Equal contribution of these two authors.

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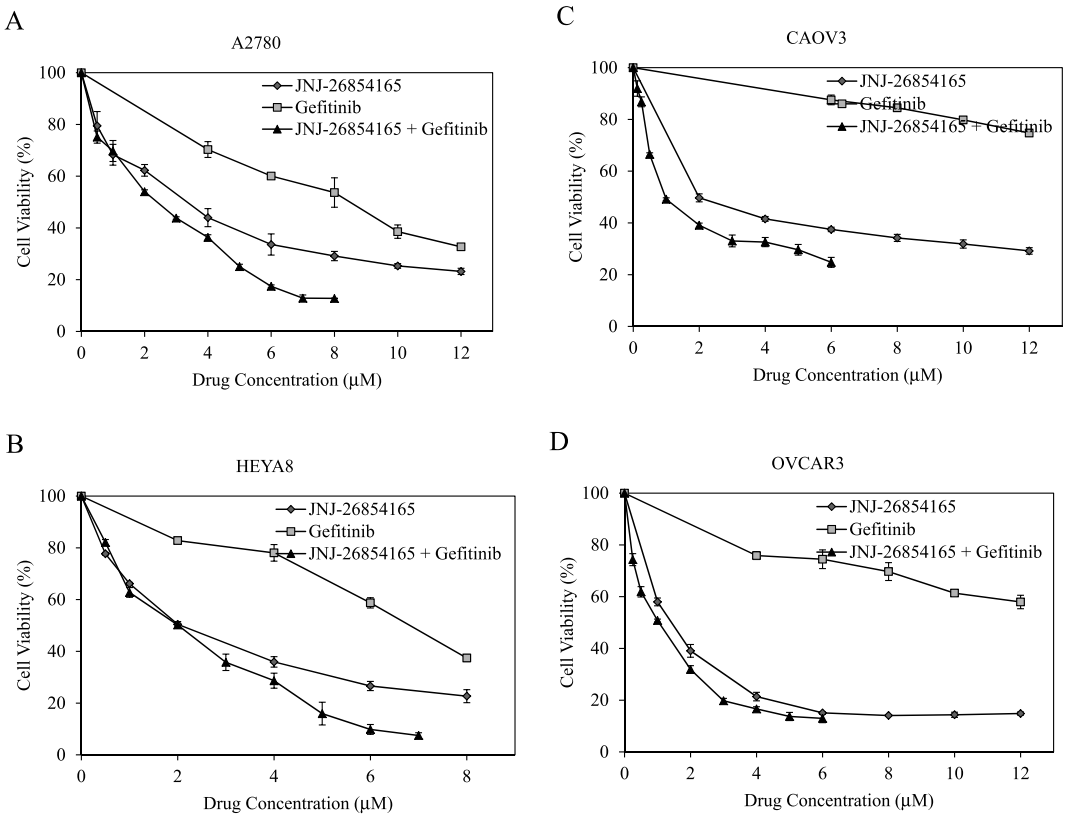

Fig. 1. Dose-response curves of ovarian cancer cell lines towards MDM2 and EGFR inhibition. Cell growths were evaluated using WST Cell Proliferation Assay after 72 h drug treatment. **(A)** A2780, **(B)** HEYA8, **(C)** CAOV3, **(D)** OVCAR3, were treated with different dose range of gefitinib or JNJ, alone and in combination. Combined treatments were composed of both drugs at a fixed ratio (1:1).

advocates that antitumor medication should be tailored to variations in each person's genome, thus taking individual variability in cancer progression and drug response into account. The cocktail therapy that uses different combinations of cancer drugs is a common clinical approach to facilitate improvement in response rates without the long waiting time as in the case of novel drug development. Thus, it is important to understand the biological mechanisms of carcinogenesis and to comprehend how cancer cells respond to select drug combinations.

In the current research, we selected EGFR and MDM2 as our molecular targets as both proteins are overexpressed in ovarian cancer cases. In 2004, *Bianco* et al. demonstrated that the inhibition of EGFR and MDM2 could exert a cooperative antitumor effect on hormone-independent prostate cancer [8]. Unfortunately, the underlying mechanism of how these two proteins interact to present the synergistic effect remains unclear. Due to the lack of information on how EGFR and MDM2 cooperate, we aimed to explore the relationship between EGFR and MDM2 and to investigate whether the combined action of EGFR and MDM2 will cause significant inhibitory effects on ovarian cancer cell lines. In addition, the current study uses a proteomic approach combining lysine 2D-DIGE and MS to investigate the inhibitory effects of EGFR and MDM2 on ovarian cancer cells.

JNJ-26854165 (JNJ), also known as Serdemetan, is an oral MDM2 inhibitor developed for interfering with the MDM2-p53 interaction. It binds to the RING domain of MDM2, which is responsible for E3 ubiquitin ligase activity. Such inhibitors enable the activation of p53 and induce apoptosis in the cancer cells [9]. The use of EGFR inhibitors for treating ovarian cancer has been clinically investigated in targeted therapy. Gefitinib (Iressa, ZD1839) is an FDA-approved orally active EGFR tyrosine kinase inhibitor (EGFR-TKI). However, gefitinib generates variable clinical outcomes suggesting that new prognostic biomarkers for patient selection and usage of drug combinations are needed [10].

In this study, four ovarian cancer cell lines were used to monitor the sensitivity of both MDM2 and EGFR inhibition. In addition, a proteomic analysis was also performed to investigate the cellular protein expression of CAOV3 in ovarian cells with gefitinib/JNJ treatment.

2. Materials and methods

2.1. Chemicals and reagents

Generic chemicals were purchased from Sigma-Aldrich (St. Louis, USA), while reagents for 2D-DIGE were purchased from GE Healthcare (Uppsala, Sweden). All primary antibodies were purchased from Genetex (Hsinchu, Taiwan) and anti-mouse, and anti-rabbit secondary antibodies were purchased from GE Healthcare (Uppsala, Sweden). All the chemicals and biochemicals used in this study were of analytical grade. Gefitinib (LC Laboratories) and JNJ-26854165 (AdooQ