DUSP1 Expression Induced by HDAC1 Inhibition Mediates Gefitinib Sensitivity in Non–Small Cell Lung Cancers

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Abstract

Purpose: Non–small cell lung cancer (NSCLC) is a leading cause of cancer-related death worldwide. Patients with NSCLC with EGFR-activating mutation benefit greatly by gefitinib, an EGFR tyrosine kinase inhibitor. However, acquired resistance limits its clinical use. Histone deacetylases (HDAC) are oncoproteins associated with cancer progression and drug resistance. Here, we disclosed that inhibition of HDAC1 induced protein phosphatase DUSP1 upregulation to overcome gefitinib-acquired resistance.

Experimental Design: The effect of HDAC1 inhibition restored gefitinib sensitivity was assessed by in vitro MTT and apoptotic assays, and in vivo xenograft and orthotopic lung cancer mouse models. Protein phosphatase array was used to detect DUSP1 expression. Immunohistochemical staining and quantitative PCR were used to analyze DUSP1 expression in clinical NSCLC specimens.

Introduction

Non–small cell lung cancer (NSCLC) is a main cause of cancer-related death worldwide (1). It is usually diagnosed at advanced stage and has poor prognosis (2). Recently, the discovery of EGFR-activating mutation not only brings a new insight into molecular classification, but also leads to a great success in clinical treatment (3, 4). EGFR tyrosine kinase inhibitor (TKI) gefitinib exerts excellent efficacy to improve survival of patients with NSCLC harboring EGFR-activating mutations (5, 6). However, acquired resistance develops after a period of treatment, which is largely caused by secondary EGFR T790M point mutation or by activation of other signaling pathway (7, 8). Although second-generation EGFR TKI, afatinib, could overcome resistance caused by T790M mutation to regain therapeutic effect, acquired resistance to afatinib emerges soon. Therefore, acquired resistance to EGFR TKI might involve signaling pathways beyond EGFR (9, 10).

Histone deacetylases (HDAC), which remove acetyl groups from histone allowing chromatin compaction and resulting in gene silence, are aberrantly overexpressed in various tumors leading to carcinogenesis, cancer progression, and clinical poor outcome (11). Thus, HDACs can be a therapeutic intervention for cancer treatment to reverse aberrant epigenetic states associated with cancer (11). HDAC inhibitor (HDACi) Vorinostat (SAHA) was approved by the FDA for the treatment of advanced and refractory cutaneous T-cell lymphoma (12). Although HDACi shows activity as a single agent to inhibit cell growth, proliferation, angiogenesis, and metastasis (13, 14), combination with other anticancer drugs is proved to be the most useful application (11). HDACi synergizes with cisplatin causing cell-cycle perturbation and apoptosis in lung cancer cells (15). It also shows synergistic effect with kinase inhibitors such as PI3K inhibitor LY294002 and imatinib (16, 17).

Dual specificity phosphatase (DUSP) family proteins, which can dephosphorylate both serine/threonine and tyrosine residues of their substrates, are made up of 11 members and three classes (18, 19). Class I DUSPs (DUSP1, 2, 4, and 5) are found in the

Results: Gefitinib-resistant NSCLC cells showed HDAC1 overexpression, and its knockdown sensitized resistant cells to gefitinib in vitro and in preclinical models through DUSP1 expression. Overexpression of DUSP1 in resistant cells restored gefitinib sensitivity by inhibiting EGFR signaling and inducing apoptosis, whereas its knockdown in sensitive cells conferred gefitinib resistance. A novel HDAC inhibitor, WJ-26210-2, in combination with gefitinib upregulated DUSP1 expression to exert in vitro and in vivo synergistic effect on inactivation of EGFR signaling, growth inhibition, and apoptosis. Clinically, high DUSP1 level was correlated with delayed emergence of gefitinib-acquired resistance.

Conclusions: Decreased DUSP1 might be a mechanism responsible for gefitinib resistance, and DUSP1 might be a biomarker for gefitinib efficacy. HDACi inhibition–induced DUSP1 upregulation could be a promising strategy to overcome gefitinib-acquired resistance. Clin Cancer Res; 21(2); 428–38. ©2015 AACR.
Translational Relevance

Gefitinib is highly effective in non–small cell lung cancer (NSCLC) harboring EGFR-activating mutation. However, acquired resistance developing after 1 year of treatment is inevitable. Current understanding of resistance includes EGFR T790M mutation or MET amplification. Our study disclosed that DUSP1 downregulation might be a novel mechanism of gefitinib-acquired resistance, and HDAC1 inhibition could induce DUSP1 upregulation to sensitize resistant cells to gefitinib in vitro and in preclinical models. In addition, DUSP1 expression correlated with gefitinib sensitivity in a clinical study and could serve as a predictive biomarker. Therefore, our study provides not only mechanistic insights into gefitinib-acquired resistance but also a promising strategy to overcome it.

Materials and Methods

Cell lines

Human lung adenocarcinoma cells harboring EGFR-activating mutation, PC9 (exon 19 deletion), PC9/Iressa resistance (PC9/IR), and HCC827/Iressa resistance (HCC827/IR), showed HDAC1 overexpression, and its knockdown could sensitize resistant cells to gefitinib and induce DUSP1 expression in vitro and in preclinical models. Overexpression of DUSP1 in resistant cells restored gefitinib sensitivity, whereas its knockdown in sensitive cells conferred resistance. We also developed a novel HDACi, WJ-26210-2, which was more potent than SAHA to inhibit HDAC activity and cellular growth. WJ-26210-2 in combination with gefitinib overcame gefitinib resistance through upregulation of DUSP1, leading to the inhibition of cell viability and induction of apoptosis in vitro and in xenograft and orthotopic mouse models. Clinically, tumors with gefitinib-acquired resistance had decreased DUSP1 mRNA level compared with those from gefitinib-naïve patients, and patients harboring high DUSP1 expression tumors had favorable outcome while taking gefitinib as the first-line treatment. Our results not only provide a novel strategy to overcome gefitinib-acquired resistance, but also imply that DUSP1 might be a predictive biomarker for gefitinib treatment.
Because HDAC1 was overexpressed in resistant H1975, PC9/IR, and HCC827/IR cells and its knockdown could sensitize resistant cells to gefitinib, the effect of WJ-26210-2 combined with gefitinib on these resistant cells was examined. Cell viability of PC9/IR cells exposed to individual agent or combination at different ratio was analyzed by the median-effect method to determine the IC_{50} values and CI (Supplementary Fig. S4A, left). CI values at different ratio especially 1:1 were smaller than 1, indicating the synergism between WJ-26210-2 and gefitinib (Supplementary Fig. S4A, right and S4B). Therefore, 1:1 combination was used. The IC_{50} and CI values of WJ-26210-2 plus gefitinib were found to be smaller than those of SAHA plus gefitinib, indicating more potent of WJ-26210-2 than SAHA to combine with gefitinib (Supplementary Fig. S4C). Combination of 1 μmol/L WJ-26210-2 with 1 μmol/L gefitinib induced 80% reduction of cell viability in both H1975 and PC9/IR cells but not normal MEF cells (Supplementary Fig. S4C). Colony formation in resistant cells was also inhibited by combination but not either gefitinib or WJ-26210-2 alone (Supplementary Fig. S4D). These data demonstrated the synergism between WJ-26210-2 and gefitinib to suppress cell proliferation in gefitinib-resistant cells.

Corresponding to the inhibition on cell viability, WJ-26210-2 plus gefitinib facilitated dephosphorylation of p-EGFR and inhibition of AKT and ERK activation in H1975, PC9/IR, and HCC827/IR cells (Fig. 1D). Cleavages of PARP and pro–caspase-3, upregulation of BIM and downregulation of MCL-1 were also induced by combination but not gefitinib or WJ-26210-2 alone (Supplementary Fig. S4E). This event occurred in a time-dependent manner (Supplementary Fig. S4F). In addition, WJ-26210-2 was also more potent than SAHA in combination with gefitinib to inhibit EGFR signaling and induce cell apoptosis (Supplementary Fig. S4G). Therefore, WJ-26210-2 combining with gefitinib is more effective than SAHA to overcome gefitinib resistance.

**WJ compounds, novel HDAC inhibitors, inhibited cell growth and induced acetylation of histone-H3 and α-tubulin in various cancer cells**

We have synthesized a series of HDACis, which showed the ability to suppress in vitro activity of class I (HDAC1 and HDAC8) and class IIb (HDAC6) HDACs with greater capacity than SAHA (Supplementary Fig. S3A and Supplementary Table S1). To confirm their inhibition in cells, H1975, PC9, and PC9/IR cells treated with these compounds were analyzed by histone and α-tubulin acetylation. Among them, WJ-25591, WJ-26208-56, and WJ-26210-2 were the most potent to induce acetylation of histone-H3 and α-tubulin (Supplementary Fig. S3B). Parallel to their HDAC inhibition, WJ compounds effectively inhibited cell growth in PC3 cells (Supplementary Fig. S3C) and WJ-26210-2 was chosen as a lead compound (preparation of WJ-26210-2 was provided in Supplementary Materials and Methods). Its effect on the cell viability of NSCLC cells was assessed with IC_{50} values around 1 μmol/L (Supplementary Fig. S3D).

**WJ-26210-2 combined with gefitinib overcame gefitinib resistance by inhibiting EGFR signaling and inducing apoptosis**

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Induction of protein tyrosine phosphatase DUSP1 expression in resistant cells by combination of WJ-26210-2 and gefitinib

To explore the mechanism underlining combination-induced inhibition of EGFR signaling, H1975 cells were treated with a protein tyrosine phosphatase (PTP) inhibitor, sodium orthovanadate (Na3VO4). Inactivations of EGFR, ERK, and AKT were reversed by Na3VO4, indicating the involvement of phosphatase in this event (Fig. 2A). To get more insight into which protein phosphatase was involved, the Human Protein Phosphatases RT2 Profiler PCR array was performed to analyze the mRNA profiles. A high induction of DUSPs (DUSP26, DUSP1, and DUSP4), cytosolic phosphotyrosine-specific PTPs (PTPN7 and PTP4A1), transmembrane receptor-like PTPs (PTPRN2, PTPRFR, and PTPRE), protein phosphatase (catalytic subunit) PDP1, and PPEF1 was seen after cotreatment (Supplementary Table S3). These genes in H1975, PC9/IR, and HCC827/IR cells were validated by Q-PCR, and only DUSP1 was found to be increased in all these cells by WJ-26210-2 and enhanced by the cotreatment with gefitinib (Fig. 2B). RT-PCR also confirmed this effect (Supplementary Fig. S5A). Induction of DUSP1 expression was further demonstrated by immunofluorescence assay. Increased DUSP1 (red) expression and its colocalization with EGFR (green) were seen (Fig. 2C). SAHA combined with gefitinib and HDAC1 knockdown could also induce DUSP1 expression in gefitinib-resistant H1975 cells (Fig. 2D). These results suggested that DUSP1 was induced by HDACi WJ-26210-2 and SAHA through HDAC1 inhibition and enhanced by the cotreatment with gefitinib to overcome gefitinib resistance.

To further explore how DUSP1 was induced, the protein biosynthesis inhibitor, cycloheximide was used. It attenuated the upregulated DUSP1 and reversed the inactivated EGFR signaling, suggesting the neosynthesis of DUSP1 (Fig. 3A). We have found
that the binding of SP1 to the EGFR promoter was affected by SAHA (25), and there are several SP1-binding sites on the DUSP1 promoter. Therefore, SP1 inhibitor mithramycin A was used to examine whether SP1 was involved in WJ-26210-2 plus geﬁtinib-induced DUSP1 expression. Mithramycin A reversed the upregulation of DUSP1 mRNA and protein (Fig. 3B). Quantitative chromatin immunoprecipitation (ChIP–qPCR) assay was further performed to examine the binding of SP1 and HDAC1 to the DUSP1 promoter. The results showed increased SP1 and decreased HDAC1 binding to the promoter, indicating the involvement of SP1 and HDAC1 in the DUSP1 gene transcription (Fig. 3C).

DUSP1 was downregulated in geﬁtinib-resistant NSCLC cells and its overexpression sensitized resistant cells to geﬁtinib. Because DUSP1 seems to play an important role in mediating geﬁtinib sensitivity, its expression in five EGFR-mutant–expressing NSCLC cells was examined. Overexpression of DUSP1 was found in geﬁtinib-sensitive PC9 and HCC827 cells compared with resistant H1975, PC9/IR, and HCC827/IR cells (Fig. 4A, left). Three EGFR wild-type A549, CL1-0, and CL1-5 cells also resistant to EGFR TKI were examined with less DUSP1, compatible with the findings in EGFR-mutant cells. However, the expression pattern of HDAC1 in these three EGFR wild-type cells was not similar to that of in EGFR-activating mutation cells (Fig. 4A, right), suggesting...
that HDAC1/DUSP1 regulation contributes to acquired EGFR TKI resistance specifically in EGFR-mutant–expressing NSCLC cells. Transfection of DUSP1 into H1975 and PC9/IR cells induced dephosphorylation of EGFR and its downstream signaling in a dose-dependent manner (Fig. 4B, top). Phosphorylation of ErbB3 but not MET was also decreased (Supplementary Fig. S5B). The association between DIU1P and EGFR was demonstrated by coimmunoprecipitation (Fig. 4B, lower). To further investigate the role of DUSP1 in gefitinib sensitivity, H1975 and PC9/IR cells transfected with DUSP1 were found to be sensitized to gefitinib with IC50 values around 0.15 and 0.1 μmol/L, respectively, which was comparable with the effect of WJ-26210-2 plus gefitinib (Fig. 4C, top). In colony formation assay, DUSP1 transfection also sensitized H1975 and PC9/IR cells to gefitinib as the effect of WJ-26210-2 plus gefitinib in parental cells (Fig. 4C, lower). Similarly, PARP cleavage and MCL-1 downregulation were induced by gefitinib in these resistant cells transfected with DUSP1 or treated with WJ-26210-2, as the effect of gefitinib in PC9 cells (Fig. 4D, compared lane 8 and lanes 2, 3, 6; compared lane 16 and lanes 10, 11, 14, and 17). On the other hand, knockdown of DIU1P in gefitinib-sensitive PC9 and HCC827 cells conferred EGFR signaling and conferred resistance to gefitinib (Supplementary Fig. S5C). Sensitizing effect of WJ-26210-2 plus gefitinib in H1975 cells was abolished by DIU1P knockdown (Supplementary Fig. S5D). All these results indicated that DIU1P expression induced by HDAC inhibition did mediate gefitinib sensitivity by inducing apoptosis through inactivation of EGFR signaling.

HDAC1 knockdown sensitized H1975 cells to gefitinib in the mouse xenograft model

To validate the in vivo effect of HDAC1 on gefitinib resistance, H1975 cells infected with a lentivirus-expressing shRNA against HDAC1 were inoculated into the nude mice. Mice inoculated with H1975, H1975/vector, or H1975/shHDAC1 cells were treated with vehicle or gefitinib (6 groups, n = 7–8/group). Compared with H1975 and H1975/vector xenograft, tumor growth of HDAC1 knocked down H1975 xenograft was slightly retarded (Fig. 5A, left). Gefitinib treatment further suppressed the growth of HDAC1 knocked down H1975 xenograft (Fig. 5A, left and Supplementary Fig. S6A), indicating that HDAC1 knockdown could sensitize H1975 tumor to gefitinib inhibition. DIU1P expression was induced in H1975/shHDAC1 tumors and enhanced by the cotreatment with gefitinib (Fig. 5A, right and Supplementary Fig. S6B), indicating that knockdown of HDAC1 upregulated DIU1P expression to overcome gefitinib resistance in vivo.

Combination of WJ-26210-2 and gefitinib induced in vivo DIU1P expression in gefitinib-resistant NSCLC and inhibited lung cancer growth

To investigate whether combination of WJ-26210-2 and gefitinib suppressed gefitinib-resistant tumor growth, the orthotopic lung cancer model was used. By injection of luciferase-expressing H1975 cells into the right lower lobe of lungs, tumor growth in NOD/SCID mice (n = 6/group) was monitored by bioluminescence imaging. IVIS and ex vivo bioluminescence from excised lungs showed that combination inhibited lung tumor growth and nodule formation (Fig. 5B and Supplementary Fig. S6C). Hema-toxylin and eosin (H&E) staining further confirmed this finding, and IHC staining demonstrated DIU1P upregulation (Fig. 5C). In addition, the H1975 cells derived xenograft mouse model was also used to demonstrate that combination synergistically inhibited tumor growth compared with individual treatment (Fig. 5D, left and Supplementary Fig. S6D). All mice tolerated the treatment well without significant toxicity and showed stable body weights. Increased expression of DIU1P and reduced activation of EGFR, AKT, and ERK were also seen in tumor lysates from combination treatment (Fig. 5D, right and Supplementary Fig. S6E). These results indicated that WJ-26210-2 combined with gefitinib could overcome gefitinib resistance in vivo via the induction of DIU1P expression to reduce the EGFR signaling.

Figure 3.

Mechanism of DUSP1 induction. A, H1975 cells were treated with 1 μmol/L gefitinib and 1 μmol/L WJ-26210-2 in the presence of 10 μmol/L cycloheximide (CHX) for 24 hours. Total cell lysates were analyzed by Western blot analysis detected by antibodies against EGFR signaling molecules with β-actin as loading control. B, H1975 cells were treated with 1 μmol/L gefitinib and 1 μmol/L WJ-26210-2 in the presence of various concentrations of mitthramycin A (MTM) for 24 hours. The mRNA level and protein expression of DIU1P were detected by Q-PCR and Western blot analysis, respectively (n = 3, Student t test; * P < 0.05). C, the binding of SP1 and HDAC1 to the DIU1P promoter was detected by ChIP-qPCR using anti-SP1 and anti-HDAC1 antibodies. Data were analyzed by Q-PCR and plotted as the percentage (% of input DNA (Student t test; * P < 0.05).
Patients with NSCLC with decreased DUSP1 expression associated with gefitinib-acquired resistance and poor progression-free survival

To determine the clinical significance of DUSP1 expression in gefitinib-acquired resistance of patients with NSCLC, 48 specimens of malignant pleura effusion, including 22 pre-gefitinib and 26 post-gefitinib specimens (acquired resistance), and their DUSP1 mRNA levels were analyzed by Q-PCR (Fig. 6A, left; Supplementary Table S4). The \( \Delta C_t \) value from 0.33 to 6.03 with a median of 3.5 was used to classify patients into the DUSP1 high-expression or low-expression group. Fourteen of 22 specimens in the pre-gefitinib group had higher DUSP1 mRNA, whereas 17 of 26 in the post-gefitinib group had lower DUSP1 mRNA (Fig. 6A, right). The association of low DUSP1 level and the post-gefitinib group was significant (\( \chi^2 \) test, \( P = 0.043 \)), indicating the correlation between decreased DUSP1 mRNA level and gefitinib-acquired resistance. In addition, 47 specimens from patients undergone surgical resection taking gefitinib as their first-line treatment after tumor recurrence were collected. DUSP1 expression was analyzed by IHC staining with positive control of expression in normal kidney tubules and negative control of staining by normal rabbit IgG (Fig. 6B, a and b). Among 47 patients, 26 with higher DUSP1 expression had longer progression-free survival (PFS; Fig. 6C, Kaplan–Meier method, \( P = 0.024 \)). Therefore, DUSP1 may serve as a predictive marker for gefitinib efficacy and its decrease may be associated with gefitinib resistance.

Discussion

EGFR-targeted therapy provide not only a new treatment paradigm but also a new biologic insight into NSCLC, in which gefitinib exerts a durable PFS and significantly prolongs overall survival in patients with EGFR-activating mutation (5, 26).
However, the emergence of acquired resistance is inevitable and remains the major obstacle. The emergence of acquired resistance to afatinib suggests the involvement of other signaling pathways beyond EGFR per se, such as c-Met amplification, PIK3CA mutations, BRAF mutations, or small-cell cancer transformation (9, 10, 27). In our study, downregulated DUSP1 was seen in gefitinib–acquired–resistant H1975, PC9/IR, and HCC827/IR cells, and DUSP1 induction by HDAC1 inhibition could sensitize resistant cells to gefitinib in vitro and in vivo. Furthermore, in clinical NSCLC specimens, DUSP1 mRNA level was decreased in tumor with
gefitinib-acquired resistance and high expression of DUSP1 was correlated with longer gefitinib efficacy duration. Our results imply that HDAC1 inhibition to induce DUSP1 upregulation could be a strategy to overcome gefitinib-acquired resistance. DUSP1 might serve as a predictive biomarker for gefitinib treatment and its decrease might be one of the mechanisms responsible for gefitinib-acquired resistance.

Overexpression of HDACs might mediate drug resistance like cisplatin, etoposide, and vincristine (28, 29). HDACi MS-275 has been reported to restore gefitinib sensitivity in lung cancer cells through upregulation of E-cadherin expression (30), and SAHA could restore BIM function and gefitinib sensitivity in resistant NSCLC cells harboring the BIM deletion polymorphism (31). In our study, HDAC1 was overexpressed in gefitinib-resistant H1975, PC9/IR, and HCC827/IR cells, and its knockdown could overcome gefitinib resistance and induce DUSP1 expression in vitro and in vivo. Though SAHA has been approved clinically for cutaneous T-cell lymphoma (12), its activity against solid tumor is limited. We developed a novel HDACi, WJ-26210-2, which was more potent than SAHA with less toxicity against normal cells in clinical relevant dose. WJ-26210-2 in combination with gefitinib exerted promising activity to overcome gefitinib resistance in vitro and in preclinical models through induction of protein phosphatase DUSP1. The association between DUSP1 and EGFR was demonstrated (Fig. 4B). Therefore, we are the first to demonstrate that DUSP1 induced by HDAC1 inhibition might restore gefitinib sensitivity in TKI-resistant cells through inactivation of EGFR signaling to induce apoptosis. HDACi entinostat in combination with erlotinib could improve the outcome of patients with NSCLC with high E-cadherin expression in the clinical trial (32). We also found that EGFR was downregulated by HDACi through transcription inhibition in colon cancer cells and through c-Cbl induction to decrease protein stability in NSCLC cells (25; unpublished data). Because HDACi can inactivate EGFR signaling through multiple mechanisms, its combination with EGFR TKI deserves further clinical development.

Protein kinase and phosphatase could maintain homeostasis of cellular signaling such as the MAPK signaling pathway. Therefore, dysregulation of phosphatase activity or loss of its expression may contribute to cancer progression or drug resistance (33). Overexpression of DUSP1 has been reported to inhibit proliferation and metastasis of NSCLC cells in vitro and in vivo, and correlate with favorable outcome (34, 35). Its expression was inversely correlated with disease severity in colon, bladder, and prostate cancer (36). In our study, DUSP1 expression was induced by HDAC1 inhibition and enhanced by the combination with gefitinib to inhibit gefitinib-resistant tumor growth in preclinical animal models. Overexpression of DUSP1 in NSCLC-resistant cells could overcome gefitinib resistance, whereas its knockdown in sensitive cells conferred gefitinib resistance. In patients with NSCLC, decreased DUSP1 mRNA expression was associated with gefitinib-acquired resistance, and high DUSP1 expression correlated with longer PFS of first-line gefitinib treatment. Therefore, DUSP1 expression might be not only associated with reducing gefitinib resistance, but also served as a predictive biomarker for gefitinib efficacy. HDACi WJ-26210-2 could induce DUSP1 upregulation to sensitize gefitinib-resistant cells to gefitinib. The mechanism of upregulation of DUSP1 involving SP1 was also demonstrated. The relationship between DUSP family and drug resistance has been reported. Deficiency of DUSP4 caused Ras–ERK pathway activation leading to chemotherapy resistance and poor outcome in breast cancer (37). In contrast, DUSP1 expression confers resistance to chemotherapy through dephosphorylation of JNK leading to antiprototic effect (38). Therefore, the role of DUSP family in drug resistance needs to be further investigated.
and cellular context as well as treatment agents might also be considered.

In conclusion, knockdown of HDAC1 could sensitize gefitinib-resistant cells to gefitinib and induce DUSP1 expression in vitro and in vivo. Combination of HDACi with gefitinib was found from NSCLC with gefitinib-acquired resistance, and DUSP1 overexpression predicted prolonged PFS of gefitinib. These data suggested that DUSP1 plays a crucial role in gefitinib sensitivity, and combination of HDAC inhibition with gefitinib is a promising strategy to overcome gefitinib-acquired-resistant NSCLC. Our study not only explored an additional mechanism for acquired resistance in NSCLC harboring EGFR-activating mutation, but also provided an effective combinational approach to overcome gefitinib-acquired resistance.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors' Contributions
Conception and design: Yun-Chieh Lin, Yu-Chin Lin, C.-C. Chen Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Yun-Chieh Lin, J.-Y. Shih, S.-W. Chao

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