Effect of insulin-like growth factor-1 on gamma H2AX induced by *cis*-diamminedichloroplatinum II in NSCLC

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Directed by Yoon Soo Chang

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<ABSTRACT>

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Because insulin-like growth factor-1 (IGF-1) counteracts the antineoplastic effect of cisplatin that induces DNA damage and cell death through the formation of several platinum-DNA adducts, we investigated the effects of IGF-1 on DNA double-strand breaks (DSBs) repair system induced by *cis*-diamminedichloroplatinum II (cisplatin). NCI-H1299 and H460 non-small cell lung cancer (NSCLC) cells treated with IGF-1 recovered from inhibited cell proliferation and apoptosis derived by cisplatin. Comet assay revealed that cotreatment with IGF-1 decreased tail length and movement indicating activation of the IGF system attenuates DNA damage. Bimodal expression of

p^{ser139}gamma H2AX (γH2AX) was suppressed by IGF-1, followed by diminished expression of $p^{ser1981}$Ataxia-telangiectasia mutated (ATM), and p^{ser427}ATM-Rad3-related (ATR). Phosphorylation of chk2 and chk1, activated by ATM and ATR respectively, also dwindled by cotreatment of IGF-1. Phosphorylation of p53 by DNA-dependent protein kinase catalytic sybunit (DNA-PKcs) after cisplatin and/or IGF-1 cotreatment was weak compared to chk1 and chk2 response. AG 1024, IGF-1R inhibitor, and *si*RNA of insulin receptor substrate-1 (IRS-1) augmented cisplatin-induced p^{ser139}γH2AX compared to cisplatin treatment alone. Cisplatin-induced translocation of IRS-1 into the nucleus with $p^{ser1981}$ATM was inhibited by IGF-1. In conclusion, cisplatin-induced γH2AX formation followed by DSBs repair system is inhibited by IGF-1 and reversed by suppression of the IGF system. So, we suggest that targeting agents against the IGF system may be a supplementary modality to conventional chemotherapy.

Key words : Non-small cell lung cancer; Cisplatin; Insulin-like growth factor-1; DNA repair system; Ataxia-telangiectasia mutated; ATM-Rad3-related; DNA-dependent protein kinase catalytic subunit; γH2AX

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Ⅰ. Introduction

More than 1 million new cases of lung cancer are diagnosed worldwide each year and it is the leading cause of cancer-related death in men and women globally [1, 2]. Only 15-25% of NSCLC (non-small cell lung cancer) patients can be treated by pulmonary resection with curative intent at the time of diagnosis and the others are treated with chemotherapy and/or radiotherapy [3]. Despite intensive efforts to control lung cancer mortality with surgery, radiation, and chemotherapy, the 5-year lung cancer patient survival rate of

7% in 1970 has only recently improved to 14% [4, 5].

Cis-diamminedichloroplatinum Ⅱ (cisplatin), 1 of the most commonly used chemotherapeutic agents for treatment of NSCLC, bonds with DNA to form at least 6 adducts, including intrastrand cross-links that exist as a large percentage of whole adducts, such as 1,2d (CpG) and 1,3d (GpXpG), and interstrand G-G cross-links, minor adducts that express approximately 5-10% [6-8]. Distorting DNA structure, platinum-DNA adducts induce DNA double-strand breaks (DSBs), which are 1 of the most dangerous forms of DNA damage and inhibit DNA replication and transcription, leading to irreversible DNA damage and cell death [8, 9]. DSBs activate molecules for DNA repair, such as Ataxia-telangiectasia mutated (ATM), ATM-Rad3-related (ATR), and DNA-dependent protein kinase catalytic subunit (DNA-PKcs) of the phosphatidylinositol 3-kinase (PIKK) family [8, 10]. ATM-mediated chk2 and ATR-activated chk1 lead to arrest cell cycle and DNA repair in response to DNA damage [11]. They are implicated in the p^{ser139}gamma H2AX (γH2AX) of

the carboxyl terminal region, leading formation of p^{se139}γH2AX in DNA damage sites. The recruitment of DNA repair proteins and damage signal components within a few minutes and then, activate homologous recombination-directed DNA repair (HRR) and non-homologous end joining (NHEJ) [12-14]. Insulin-like growth factor-1 (IGF-1), a polypeptide growth factor hormone, promotes mitosis and metastasis, including NSCLC, leading to long-term regulation programs, such as cell proliferation and differentiation [15, 16]. Moreover, IGF-1 may influence the maintenance of genetic integrity and cell viability via DNA repair mechanism [17]. It is known that insulin-like growth factor-1 receptor (IGF-1R) activated by ligand protects cells from massive apoptosis and develops drug resistance in a variety of ways. However, the signaling pathway for cell survival has not yet been clearly described [18]. The mechanism against DNA damage divides into HRR and NHEJ. In HRR, it is generated by newly replicated DNA strands in proliferating cells but quiescent cells protect damaged DNA by binding to the end of DSBs.

Recently, it has been reported that cells activated by IGF-1 promote HRR by joining insulin receptor substrate-1 (IRS-1), 1 of the major substrates of IGF-1R, and Rad51 in DSBs sites [12]. In addition, IGF-1R initiates antiapoptic program through the IRS-1 pathway. Autophosphorylated IGF-1R in association with the binding of IGF-1 interacts with IRS-1. It leads to survival signaling pathway, namely, IRS-1 phosphorylation and serine phosphorylation of BAD, a Bcl-2 family protein by activated Akt/PKB through in with or without PI3 kinase, to inactivate the cell death program [19, 20]. Although cisplatin is frequently used for solid tumors, its response rate is limited and the molecular mechanism of chemoresistance is not fully explained. NSCLC and other cancers are related to IGF-1 concentration of the interior of the body [16]. Therefore, we investigated the molecular mechanisms on the relevancy of the IGF system of IGF-1 that indicates antagonism with DNA damage repair mechanisms induced by cisplatin in NSCLC.

II. MATERIALS AND METHODS

1. Cell culture and chemical agents

Non-small cell lung cancer NCI-H460, H1299, and A549 were grown in RPMI 1640 (American Type Culture Collection, Manassas, VA, USA) supplemented with 5% fetal bovine serum (FBS), and Cos7 was maintained in 10% Dulbecco's Modified Eagle's Medium (DMEM; American Type Culture Collection, Manassas, VA, USA) containing penicillin and streptomycin in a humidified atmosphere with 5% CO_2 at 37℃. Anti-ATR (N-19), -DNA-PKcs (C-19), goat anti-mouse IgG HRP, goat anti-rabbit IgG-HRP, mouse anti-goat IgG–HRP (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), and –ATM (clone AM9), - γH2AX (Ser^{139}) (Millipore, Charlottesville, VA, USA), -*p*ATR (Ser^{428}), -Chk1, -*p*Chk1 (Ser^{317}), -Chk2, -*p*Chk2 (Thr^{68}), -p53, -*p*p53 (Ser^{37}) (Cell Signaling Technology, Inc., Danvers, MA, USA), peroxide labeled anti-mouse, peroxide labeled anti-rabbit (GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA), and -*p*ATM (Ser^{1981}) (Rockland

Immunochemicals, Inc., Gilbertsville, PA, USA) were used.

2. Cell proliferation assay

Cells were placed into 96-well plates in culture medium. The next day, IGF-1 (EMD Chemicals Inc., San Diego, CA, USA) was stimulated for 48 hr and cisplatin (Sigma-Aldrich Co., St. Louis, MO, USA) was treated for 24 hr in 1% medium. Proliferation of cells stimulated by cisplatin 35 μM and IGF-1 50 ng/mL was reacted to 3-(4,5–dimethylthiazol–2–yl)-2,5-diphenbyltetrazolium bromide (MTT; AMRESCO, Inc., Solon, OH, USA) for 4 hr and measured by absorbance at 550 nm.

3. Apoptic assay

Apoptosis was discovered by using annexin V-FITC kit (Beckman Coulter, Inc., Fullerton, CA, USA). The 2 X 10^5 cells were stimulated by cisplatin 100 μM and/or IGF-1 50 ng/mL. After collecting and washing twice with cold

PBS, cells were resuspended in 1 X binding buffer, annexin V-FITC, and propidium iodide. Cell preparations were incubated on ice for 15 min in the dark. The samples were added to ice-cold binding buffer and analyzed by Beckman Coulter FC500 (Beckman Coulter, Inc., Fullerton, CA, USA).

4. Comet assay

Comet assay was performed using Trevigen's Comet Assay kit (Trevigen, Inc., Gaithersburg, MD, USA). The cells grown in 6-well plate were induced by cisplatin 35 μM and/or IGF-1 50 ng/mL.. After trypsinized cell pellet was washed by 1 X PBS (Ca^{2+} and Mg^{2+} free), 75 ul of resuspended cells with molten LMAgarose were fixed onto pre-coated glass slide. Cells were lysed by ice-cold lysis solution and immersed alkaline solution. The slides were subjected to electrophoresis in 1 X TBE buffer. The slides were immersed in 70% ethanol prior to staing, and DNA of dried samples were stained with SYBR®Green I staining solution. Fluorescently stained nucleotide images

were captured using Nikon ECLIPSE 80i (Nikon Photo Products Inc., Azumabashi, Japan) at 494 nm.

5. Transfection of small interfering RNA (*si*RNA)

For silencing of IRS-1 expression, oligonucleotide pairs were designed sense 5'–CGGUCACUACAUUUUGUCUtt–3', and antisense 5'-AGACAAAAUGUAGUGACCGtt-3' of pre-designed *si*RNA (Ambion, Inc., Austin, TX, USA) and sense 5'-GGCUACAUGAUGAUGUCCtt-3' and antisense 5'-GGACAUCAUCAUGUAGCCAtt-3' of custom *si*RNA (Ambion, Inc., Austin, TX, USA). Cells were cultured in medium containing 5% FBS without penicillin and streptomycin. Cells in 100-mm dishes at 30-50% cofluence were replaced with opti-MEM® Ⅰ (Gibco BRL, Carlsbad, CA, USA) and transfected. Before transfection, lipofectamine™ 2000 (Invitrogen Corporation, Carlsbad, CA, USA), pre-designed, and custom *si*RNA in a dose-dependent manner were diluted with Opti-MEM®. After 5min,

lipofectamine™ 2000 and *si*RNA were mixed for 20min in room temperature and cells transfected by combined mixtures were incubated at 37℃ in a CO_2 incubator for 24 hr.

6. Western blotting

The 50-70% confluent NSCLC cells were treated by AG 1024 0.4 μM (Sigma-Aldrich Co., St. Louis, MO, USA), cisplatin 35 μM, IGF-1 50 ng/mL, and *si*RNA 40 nM. Whole cell lysates were prepared modified RIPA buffer. For Western blotting, equal amounts of protein were separated on sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE gel), and transferred to nitrocellulose membrane. The blocked membrane on 5% skim milk for 1hr was probed with primary antibody at 4℃ overnight and then detected with horseradish peroxidase-conjugated secondary antibody IgG for 1hr at room temperature. The membranes were visualized by the enhanced ECL kit (GE Healthcare Bio-Sciences Corp, Piscataway, NJ, USA).

7. Immunoprecipitation

For immunoprecipitation, stimulated and unstimulated cells were harvested by modified RIPA buffer. All protein samples were diluted with PBS to 1 mg/mL, added 7-10 μl of IRS-1 antibody, and then incubated at 4℃ overnight. The mixtures were captured by adding agarose-conjugated protein A (EMD Chemicals, Inc., San Diego, CA, USA) at 4℃. After 1h all immune complexes were washed with modified RIPA buffer and resuspended 2 X sample buffer. The beads were dissociated by boiling for 5 min at 95-100℃. The supernatant fractions transferred to fresh tubes were analyzed by Western blotting as indicated above.

8. Immunocytochemistry

Coverslips (BD, Franklin Lakes, NJ, USA) were used to grow 1 X 10^6 cells. Before immunostaining, cells were maintained at 35 μM cisplatin and 50 ng/mL IGF-1 for 8 hr. PBS-washed cells were fixed in 4% formaldehyde for

20 min, permeabilizied in 0.1% triton X-100 for 10 min, and blocked by 1% bovine serum albumin (BSA; GE Healthcare Bio-Sciences Corp, Piscataway, NJ, USA) for 1 hr at room temperature. Cells incubated by primary antibody at 4℃ overnight were detected by Alexa fluor 488 goat anti-mouse (Molecular Probes Inc., Eugene, OR, USA) and goat anti-rabbit TRITC secondary antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) for 1 hr at room temperature. Cells were counterstained by 4', 6'-Diamidino-2-phenylindole dihydrochloride (DAPI; Sigma-Aldrich Co., St. Louis, MO, USA) at a dilution of 1:3000, mounted, and analyzed by ZEISS LSM 510 META (Carl Zeiss MicroImaging, Inc., Thornwood, NY, USA).

III. RESULTS

1. Antineoplastic effects of cisplatin are counteracted by IGF-1.

For 24 hr treatment cisplatin inhibited cell proliferation in a dose-dependent manner and IC50 in NCI–H1299, H460, A549 and Cos7 cells were estimated about 33.3, 33.7, 36.0 and 32.4 μM, respectively. Treatment of IGF-1 facilitated proliferation that reached a plateau at a concentration 100 ng/mL in NSCLC cells. (data not shown). The cells treated with cisplatin for 24 hr exhibited suppressed proliferation that was amplified by 48 hr treatment. H1299 cells displayed prompt recovery of proliferation by IGF-1 treatment, showing a higher recovery rate at 24 hr than 48 hr; however, H460 revealed a higher recovery rate at 48 hr than 24 hr, and A549 cell showed weak recovery of proliferation by IGF-1 treatment. These findings indicate that although there is a difference in the degree of response to IGF-1, it plays an important role in recovery of proliferation from damage

induced by cisplatin (Fig. 1).

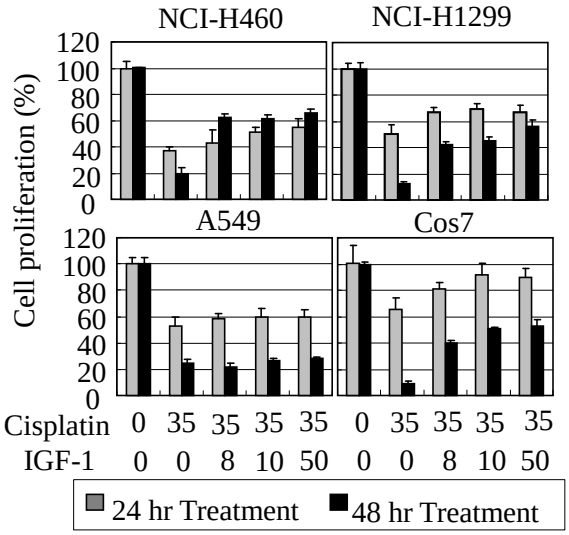

Fig. 1. Cisplatin-induced cell growth inhibition is recovered by IGF-1 in a dose-dependent manner. NSCLC cells were treated by cisplatin and various doses of IGF-1 and proliferation assay was performed. In H460, H1299, and Cos7, 35 μM of cisplatin-inhibited cell proliferation by about 50% and the addition of 50 ng/mL of IGF-1 restored cell growth by about 20% at 24 hr and 40% at 48 hr. In A549 cells, cell proliferation was recovered by about 10% at 24 hr and minimal change was observed at 48 hr with IGF-1 treatment.

2. Apoptosis and DNA damage derived from cisplatin are diminished by IGF-1 treatment.

The effect of IGF-1 on cisplatin-induced apoptosis were evaluated by FACS analysis using annexin V-FITC and propidium iodide. NSCLC cells were treated with 50 ng/mL of IGF-1 and 100 μM of cisplatin and incubated overnight. In H460 cells, cisplatin treatment induced about 25% of early apoptosis, but pretreatment with IGF-1 recovered about 10% of early apoptosis (Fig. 2A). To quantify DNA damage, comet assay was performed. In H460, H1299, and A549 NCLC cells, DNA contents of control and IGF-1-treated cells remained in range of the nucleus whereas those in the cells exposed by 35 μM cisplatin showed significant tail length and mobility, which were partially suppressed by cotreatment of 50 ng/mL IGF-1 (Fig. 2B).

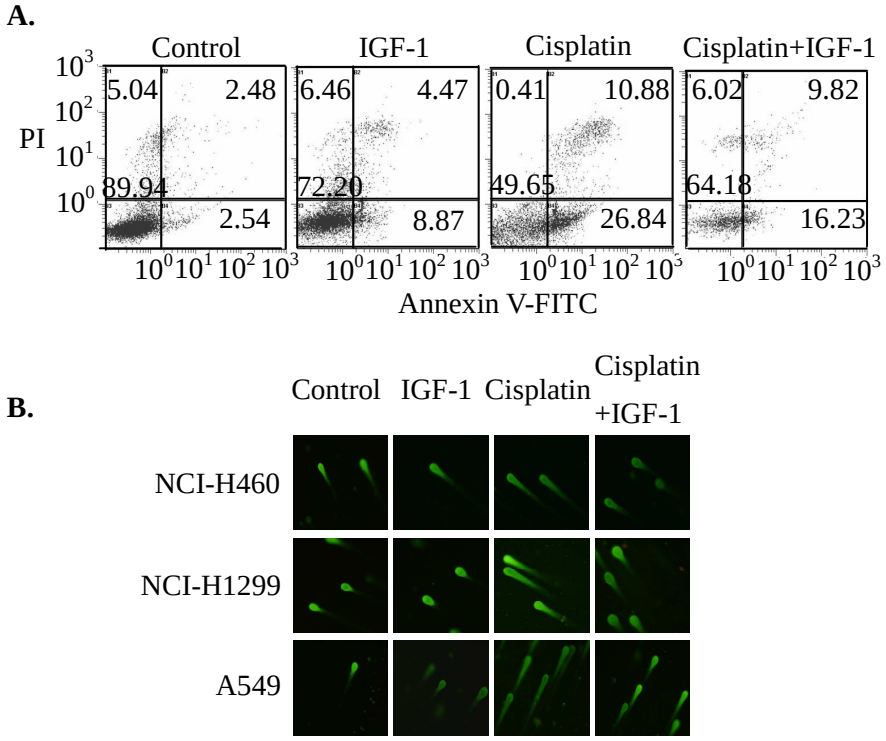

Fig. 2. IGF-1 protects from apoptosis and DNA damage induced by cisplatin. **A.** The cells treated with cisplatin and/or IGF-1 were estimated by FACS analysis. Cisplatin 100 μM triggered apoptosis in a quarter of H460 cells and cotreatment of IGF-1 50 ng/mL decreased by about 10% of cisplatin-induced apoptosis. **B.** Comet assay shows decreased DNA damage with cotreatment of IGF-1. In H1299 cells, overnight treatment with 35 μM cisplatin prolonged tail length and movement that were partially restored by IGF-1.

3. IGF-1 attenuates cisplatin-induced γH2AX formation.

Phosphorylation of the histone H2A variant H2AX at ser 139 is the marker of cellular response to DSBs resulting in discrete γH2AX (phosphorylated H2AX) foci at DNA damage sites. Cisplatin treatment showed bimodal expression of γH2AX, which showed early peak at 10 min and gradual increments of expression as time passed. Compared with cisplatin treatment alone, IGF-1 treatment markedly suppressed γH2AX formation at 10 min in cisplatin-treated cells followed by relatively decreased expression of γH2AX at other times as time passed. Phosphorylation of ATM at ser 1981, 1 of the molecules involved in the generation of γH2AX, took place at 10 min with cisplatin treatment and showed gradual increments until 24 hr. Cisplatin-induced $p^{ser1981}$ATM expression was also repressed by the addition of IGF-1 until 8 hr and peaked at 16 hr. Phosphorylated ATR at ser 428, another marker for activation of HRR against cisplatin-induced DSBs, was gradually expressed with time by cisplatin in 10 min. IGF-1 had little effect on the

degree and rate of its expression following cisplatin-induced DNA damage. Expression of DNA-PKcs, whose phosphorylation sites by DSBs have not been established, was confirmed by total protein, and its protein level was not affected by cisplatin and/or IGF-1 (Fig. 3).

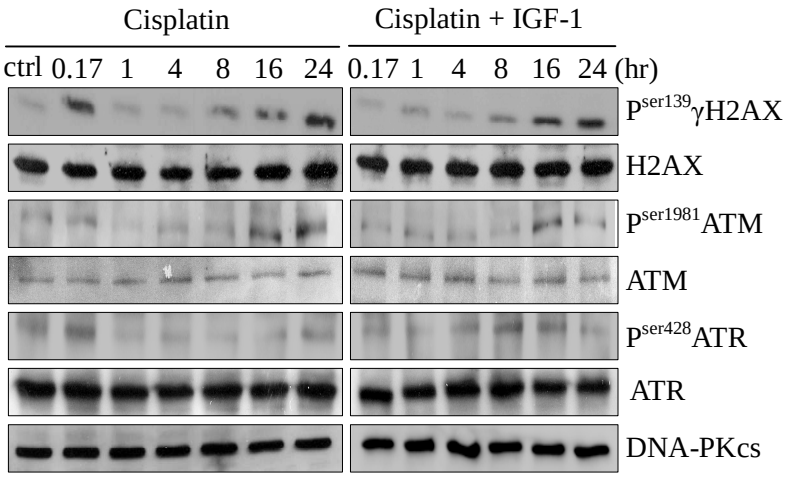

Fig. 3. IGF-1 blocks cisplatin induced γH2AX formation. IGF-1 treatment suppressed bimodal expression of γH2AX and subsequent HRR pathway. Time sequences of $p^{ser1981}$ATM and p^{ser428}ATR expression corresponded to that of γH2AX. The active form of DNA-PKcs was not available and was not included in this study.

4. IGF-1 represses cisplatin-mediated HRR pathway activation.

Activation of ATM, ATR, and DNA-PKcs influences phosphorylation of downstream molecules chk2, chk1, and p53, respectively, and were appraised by Western blotting (Fig. 4). Phosphorylation of chk2 at thr 68, which is mediated by ATM activation, appeared as early as 10 min after cisplatin treatment and became stronger as time passed whereas cotreatment with IGF-1 suppressed expression of p^{thr68}chk2. Cisplatin treatment also changed expression of p^{ser317}chk1, a target molecule of ATR. It appeared as a weak band in 10 min and became evident in 8 hr of exposure to cisplatin but IGF-1 treatment delayed and weakened p^{ser317}chk1 expression in 16 hr exposure to cisplatin. In DSBs, phosphorylation of p53 at ser 37 was mediated by DNA-PKcs, which mainly involved in NHEJ. P^{ser37}p53 had a weak but similar expression pattern to γH2AX showing bimodal expression that was suppressed by IGF-1 treatment.

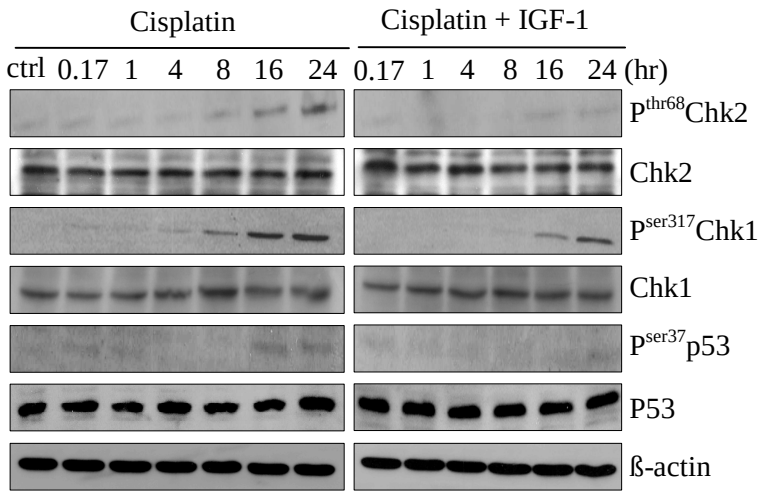

Fig. 4. Phospholation of chk1 and chk2 is affected by IGF-1 in cisplatin treated NSCLC cells. Chk2, chk1, and p53, which are downstream molecules of ATM, ATR, and DNA-PKcs, respectively, were phosphorylated by cisplatin treatment. Although phosphorylation of all downstream molecules was also derived in IGF-1-cotreated cells, the level of expression was weaker and delayed than that of cisplatin alone. Equal loading was confirmed using ß-actin.

5. IGF-axis inhibitors enhance cisplatin-induced γH2AX formation.

To scrutinize the role of the IGF system on cisplatin-induced DSBs, the effect of AG 1024 (IGF-1R inhibitor) and *si*RNA against IRS-1, a major docking molecule for IGF signaling, were investigated on DSB repair molecules by Western blotting. Treatment during 24 hr with custom and pre-designed *si*RNA against IRS-1 successfully suppressed IRS-1 expressin in the range of 40-60 nM in the cells used in this study (Fig. 5A). Under the influence of cisplatin, 40 nM of IRS-1 *si*RNA and 0.4 μM of AG 1024 promoted p^{ser139}γH2AX compared to cisplain treatment (Fig. 5B).

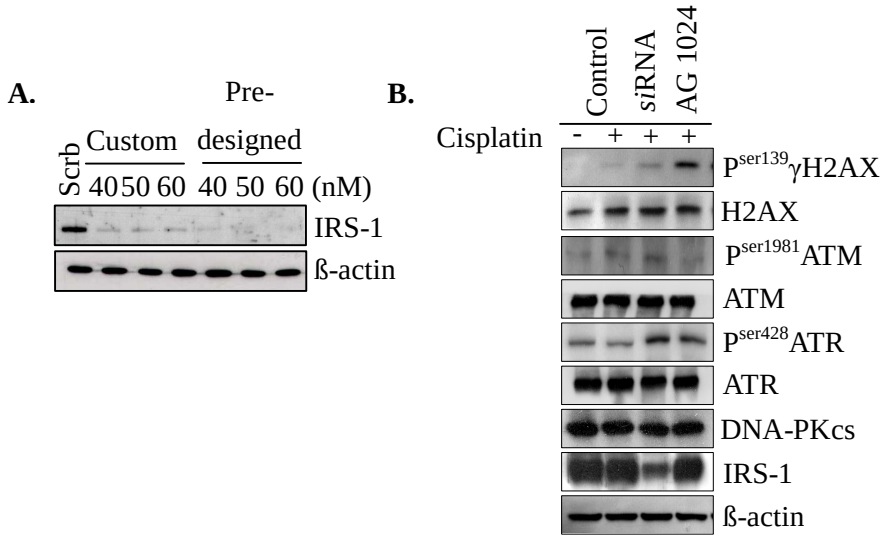

Fig. 5. Inhibitors of IGF-axis load abundant γH2AX formation and activate **A.** To assess the effect of *si*RNA against IRS-1 formation, cells were transfected with 40-60 nM pre-designed and custom *si*RNA for 24 hr. Pre-designed and custom *si*RNA 40nM was enough to repress IRS-1 expression compared with RNAi control. **B**. H1299 cells treated with *si*RNA for 24 hr or 0.4 μM of AG 1024 for 30 min and expression of γH2AX, $p^{ser1981}$ATM, and p^{ser428}ATR was assessed by Western blotting. Compared with cisplatin-treated and untreated cells, cells treated with *si*RNA or AG 1024 expressed increased level of γH2AX and $p^{ser1981}$ATM and p^{ser428}ATR.

6. IGF-1 supresses activation of ATM and IRS-1 induced by cisplatin

Interactions between DSBs and IRS-1 were evaluated using Immunocytochemistry and Immunoprecipitation. Resting state cells showed perinuclear localization of $p^{ser1981}$ATM and cytoplasmic expression of IRS-1. IGF-1 treatment had little influence on their expression and location. Cisplatin-induced strong $p^{ser1981}$ATM expression in the nucleus with colocalization of IRS-1 was interrupted by IGF-1 (Fig. 6A). Immunopreciptation showed interaction with IRS-1 and ATM, ATR, and DNA-PKcs with treatment of cisplatin, which was enhanced by IGF-1R inhibitors. The interaction of IRS-1 with ATR and DNA-PKcs was also prompted by IRS-1 *si*RNA treatment compared to cisplatin treatment alone. When the cells were treated with IGF-1 and cisplatin, ATM, ATR, and DNA-PKcs did not bind with IRS-1, but IGF-1 alone influenced interaction with IRS-1/ATR and DNA-PKcs (Fig. 6B).

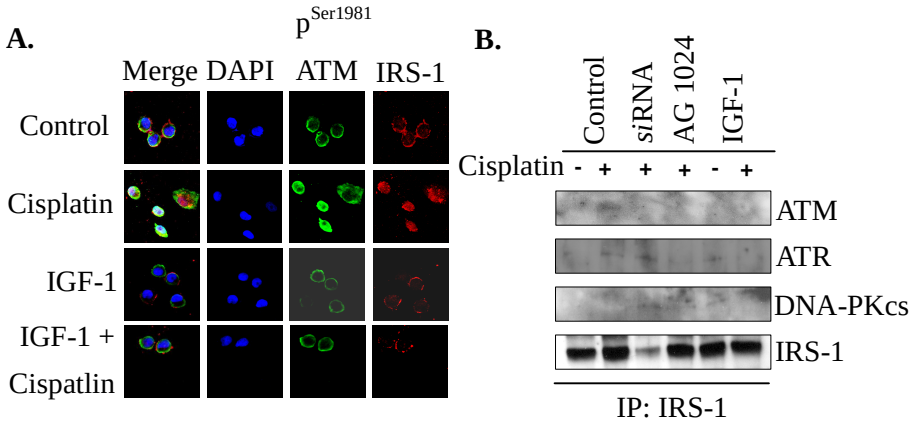

Fig. 6. IGF-1 represses nuclear translocation and interaction. **A**. For 8 hr double immunostaining with $p^{ser1981}$ATM and IRS-1 were performed by treatment with 35 μM of cisplatin and/or 50 ng/mL of IGF-1. There were peculiar colocalizations of IRS-1 and $p^{ser1981}$ATM within the nucleus in cisplatin-treated H1299 cells that were suppressed by cotreatment of IGF-1. **B.** The cells were treated with 35 μM of cisplatin, 40nM siRNA, 0.4 μM AG 1024, and/or 50 ng/mL of IGF-1. ATM interacted IRS-1 in cells treated by cisplatin. DNA-PKcs indicated interation with IRS-1 on IGF-1 and *si*RNA/cisplatin cotreatment, similar to ATR, which also showed interactions in cisplatin treatment.

IV. DISCUSSION

Although cisplatin-based chemotherapy is widely used for standard first-line treatment in NSCLC cells [21], the effect of cisplatin is reduced by decreased intracellular accumulation, drug inactivation, and activation of damaged DNA repair system [22, 23]. The IGF system promotes not only mitosis, cancer cell survival and growth but also confers antiapoptotic properties in cellular stressful conditions. Therefore, this report aimed to investigate the role of the IGF system in cisplatin chemoresistance in NSCLC cells.

Our data reaffirmed that IGF-1 protected apoptosis and recovered cell proliferation in cisplatin-treated NSCLC cells. We also detected that IGF-1 decreased DNA damage from cisplatin in individual cells using comet assay performed in neutral conditions, showing decreased tail length and movement. These findings suggest that IGF-1 may play a role in resistance against chemotherapeutic effects and the DNA damage response pathway of cisplatin. Among the several platinum-DNA adducts, the one generated from interstrand

G-G cross-links induces DSBs, which is critical to cell fate determination and leads expression of p^{ser139}γH2AX [24]. Kinetic studies on γH2AX clearance after DNA damaging agents showed a strong correlation between increased γH2AX expression and unrepaired DNA damage and cell death [25]. Unlike earlier reports showing gradual accumulation of platinum-DNA adducts in cells with the passage of time [26], γH2AX showed peculiar bimodal expression with cisplatin treatment. Overall expression of γH2AX within 24 hr was inhibited by IGF-1 whereas inhibitors of the IGF system amplified γH2AX expression and activated the HRR system.

Furthermore, the early peak of γH2AX at 10 min was strongly suppressed by IGF-1. These findings are incongruous with earlier reports showing that PP2A inhibitors such as insulin, IGF-1, epithelial growth factor, and okadaic acid [27, 28] hinder dephosphorylation of p^{ser139}γH2AX through the blocking of PP2A activity. Our data revealed that IGF-1 had an antiapoptotic effect and suppressed γH2AX formation induced by cisplatin, especially in the early

time favor the findings from Adam *et al*. that its function might originate from activation of lipid raft resident myristoylated Akt [29]. Cells confronted with DSBs activate of ATM/chk2 pathway while those faced with bulky DNA damage and replication folk collapse during S phage activate ATR/chk1 cascade [30]. Our Western blotting results coincided with earlier findings that DSBs acquired from cisplatin treatment triggered activation of response molecules γH2AX, ATM, and ATM-dependent chk2 for repair signaling cascade. ATM plays an important role in histone loss, which occurs in nucleosomal change during DSBs repair at DNA damage sites [31]**.** We, therefore, regard accumulated platinum-DNA adducts more strongly activate the ATM pathway than that of ATR and/or DNA-PKcs. Cisplatin treatment phosphorylated downstream molecules chk2, chk1, and P53, which was delayed or suppressed by IGF-1. Together with other findings, different time sequences in the appearance of p^{thr68}chk2 and p^{ser317}chk1 between IGF-1-treated and untreated cells leaded us to investigate another interface between

the IGF system and damaged DNA repair system. IRS-1 is a major adaptor molecule in the IGF signaling pathway and possesses multiple tyrosine and serine/threonine phosphorylation sites that control cellular response against environmental stress [32, 33]. Its total cellular expression as well as its translocation into the nucleus is influenced by various factors, including osmotic stress, hyperglycemia, high IGF-1, and cytotoxic agents. Through Immunoprecipitation and Immunocytochemical studies, we could confirm interactions between IRS-1 and molecules in the HRR system. Binding between IRS-1 and ATM/ATR that coincide with γH2AX expression was reinforced by the treatment of IGF-1 inhibitors. Further studies are needed to investigate the effect of inhibition on the interaction between the IGF and HRR systems on cell fate that confronts genotoxic stress.

V. CONCLUSION

Taken together, IGF-1 overcomed antineoplastic and proapoptotic effects of cisplatin in NSCLC cells. It also decreased DNA damage and attenuated damaged DNA repair system activation. Cisplatin-induced interactions between IRS-1 and HRR molecules were inhibited by IGF-1 treatment. Currently, numerous IGF system inhibitors, DNA repair pathway system inhibitors, and chk inhibitors are being developed. This study may help to the application of these agents to treat NSCLC. Elucidating the underlying mechanism of decreased interaction between the 2 systems after IGF-1 treatment may also be of help for the development of further treatment modalities and therapeutic targets.

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<ABSTRACT (IN KOREAN)>

비소세포성 폐암에서 *cis*-diamminedichloroplatinum II 에 의해 유도된 gamma H2AX 에 미치는 인슐린양 성장인자-1 의 영향

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전 정 희

인슐린양 성장인자는 *cis*-diamminedichloroplatinum II (cisplatin)에 의한 DNA-백금계 부가 생성물이 DNA 손상과 세포사를 감쇠시키는 기능이 있어 cisplatin 으로 유도된 유전자 수복 기전에서 인슐린양 성장인자의 영향에 대해 알아보았다. 비소세포성 폐암 세포주 H1299, H460, A549 와 Cos7 에 인슐린양 성장인자의 처리는 cisplatin 에 의해 억제된 세포 증식력과 세포사멸을 회복시킴을 확인하였고, Comet assay 로 부터 cisplatin 에 의한 손상이 회복되었음을 관찰할 수 있었다. Cisplatin 단독 처리에 비해 인슐린양 성장인자와 병행 처리시

$p^{ser1981}$ataxia telangiectasia mutated (ATM), p^{ser427}ATM-rad3 related (ATR)의 감소된 발현에 따라 p^{ser139}gammaH2AX (γH2AX)도 감소되었으며, ATM 과 ATR 의 하위분자인 p^{ser428}chk2 와 p^{ser317}chk1 발현도 감소되었다. DNA-dependent protein kinase catalytic sybunit (DNA-PKcs)의 하위분자 p^{ser37}p53 는 약제 처리시 p^{ser428}chk2 와 p^{ser317}chk1 과 발현 양상이 비슷하였으나, 그 정도는 미미 하였다. 또한 IGF-1R 억제제인 AG 1024 와 Insulin receptor substrate-1 (IRS-1)의 *si*RNA 을 cispaltin 과 동시에 처리하였을 때, cisplatin 만 처리하였을 때에 비해 p^{ser139}γH2AX 의 활성화가 증가되었다. 면역 염색법과 면역 침강법으로 cisplatin 을 처리하였을 때, ATM 과 IRS-1 은 상호작용하고, 핵 내 이동이 촉진되나, 인슐린양 성장인자의 처리는 이를 억제함을 관찰할 수 있었다.

즉, cisplatin 은 γH2AX 의 형성을 유도하나 인슐린양 성장인자에 의해 억제되고, 인슐린양 성장인자 체계 억제에 의해

다시 활성화됨을 확인할 수 있었다. 이 결과들로부터, 인슐린양 성장인자 체계가 비소세포성 폐암의 치료에 기본적 자료로 사용될 수 있을 것으로 예상된다.

핵심되는 말: 비소세포성 폐암, cisplatin, 인슐린양 성장 인자, 유전자 수복, Ataxia-telangiectasia mutated, ATM-Rad3-related, DNA-dependent protein kinase catalytic subunit, γH2AX