Histol Histopathol (2014) 29: 1437-1443
DOI: 10.14670/HH-29.1437

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

INF-γ sensitizes head and neck squamous cell carcinoma cells to chemotherapy-induced apoptosis and necroptosis through up-regulation of Egr-1

Bei Xu[1], Yongqian Shu[1] and Peng Liu[2]
[1]Department of Medical Oncology and [2]Department of Hematology,
the First Affiliated Hospital of Nanjing Medical University, Nanjing, PR China

Summary. Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide. Acquired resistance to standard chemotherapy accounts for most of treatment failure. Here we demonstrate that Interferon-γ (INF-γ) may up-regulate Egr-1 gene expression in HNSCC cell line SCC-25. Forced expression of Egr-1 sensitizes SCC-25 cells to chemotherapy-induced apoptosis and necroptosis, a novel form of programmed cell death. Egr-1 up-regulation also significantly increases the production of Thrombospondin-1 (TSP-1), a matricellular glycoprotein which has been described to induce cell death in HNSCC. Moreover, INF-γ-induced sensitization of cells to chemotherapy-mediated cell death and TSP-1 production could be markedly abolished by Egr-1 silencing. The present investigation provides the first evidence that INF-γ may sensitize HNSCC cells to chemotherapy-induced apoptosis and necroptosis through up-regulation of Egr-1. These data support the combination use of INF-γ and cytotoxic drugs for HNSCC Therapy.

Key words: INF-γ, Egr-1, HNSCC, Necroptosis

Offprint requests to: Yongqian Shu or Peng Liu, Department of Medical Oncology (YS) or Department of Hematology (PL), The First Affiliated Hospital of Nanjing Medical University, 300 Guangzhou Rd, Nanjing 210029, PR China. e-mail: shuyongqian@csco.org.cn and liupeng9098@163.com

Introduction

Squamous cell carcinoma of the head and neck (HNSCC) is a relatively common human cancer with 600,000 new cases diagnosed per year worldwide. Therapeutic options include cytotoxic chemotherapy, radiation, and surgery. Clinical outcomes vary significantly according to different subsites within the head and neck region. Acquired resistance to standard chemotherapy has been noted as one of the hallmarks of HNSCC and accounts for most of treatment failure (Rothenberg and Elisen, 2012).

Interferon-γ (INF-γ) is a type II interferon and is produced predominantly by T lymphocytes and natural killer cells (Han et al., 2011). As a potent Immunomodifier, INF-γ regulates the expression of apoptosis-related genes and may overcome tumor cell apoptosis resistance, especially when used in combination with conventional chemotherapy (Ahn et al., 2002; Tekautz et al., 2006; Lissat et al., 2007; Zhou et al., 2008; Häcker et al., 2009; Liu et al., 2011). Moreover, INF-γ may enhance the anti-tumour immune response of dendritic cells against HNSCC through restoring the expression of antigen processing machinery genes, including Tap-1 and Tapasin (Wei et al., 2011).

Cytotoxic chemotherapy may lead to cancer cell death through the apoptotic pathway, which is well-characterized. Accumulating evidence suggests a novel kind of programmed necrosis, called necroptosis, could act as an alternative mechanism to kill cells, especially in apoptotic-deficient conditions (Hitomi et al., 2008; Declercq et al., 2009; He et al., 2009; Zhang et al., 2009). Several reports, including our recent

investigation, have shown that targeting the key regulators of both the apoptotic and necroptotic machine to restore the cell death pathway may represent a novel approach for cancer treatment (Horita et al., 2008; Liu et al., 2012). Much less is known with respect to the significance of necroptosis in HNSCC.

EGR1, a member of the immediate-early response gene family, is induced by diverse stimuli such as stress, injury, mitogens and differentiation factors. It regulates a number of physiological and pathological processes, including cell growth, differentiation and apoptosis (Shin et al., 2006; Liu et al., 2009). Furthermore, several studies, including our investigation, showed that Egr-1 is involved in regulating invasive behavior of cancer cells.

In the present study, we determined the effect of Egr-1 in INF-γ-induced chemosensitivity in HNSCC. We also described the detailed role of apoptotic and necroptotic mechanisms in cell death process in response to the combination of INF-γ and cytotoxic drugs.

Materials and methods

Cell culture and reagents

SCC-25 human head and neck squamous carcinoma (HNSCC) cell line was obtained from the American Type Culture Collection (ATCC, Manassas, VA). Cells were maintained in a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium containing 1.2 g/L sodium bicarbonate, 2.5 mM L-glutamine, 15 mM HEPES, 0.5 Mm sodium pyruvate, 4.5 g/L glucose, and 400 ng/mL hydrocortisone with 10% fetal bovine serum. Cell viability was determined by using a trypan blue dye exclusion assay. IFN-γ was obtained from R&D System (R&D Systems, Minneapolis, MN). Nec-1 and zVAD were purchased from Alexis Biochemicals (San Diego, CA, USA).

Western blot

Whole-cell pellets were lysed in 50 mM Tris-HCl (pH 7.6) buffer containing 0.15 M NaCl, 1 mM EDTA, 10 mg/ml leupeptin and benzamidine, 1 mM PMSF and 1% Triton X-100. Proteins in cell lysates were resolved by SDS polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes (Bio-Rad, Hercules, CA, USA). Membranes were blocked in bovine serum albumin (3%) in Tris-buffered saline-Tween 20 (0.05%) and incubated with antibody against human Egr-1 (Santa Cruz Biotechnology, Santa Cruz, CA) at 1:1000 dilutions followed by horseradish peroxidase-conjugated secondary antibodies at 1:5000 dilutions. Detection was performed by using enhanced chemiluminescence.

Quantitative real-time reverse transcription-PCR

Total RNA was extracted from cells using Trizol (Invitrogen, San Diego, CA, USA) and treated with DNaseI (Ambion, Austin, TX, USA). In all, 1 mg of total RNA was used for first-strand DNA synthesis using iScript cDNA Synthesis system (Bio-Rad). Real-Time PCR was performed using iQ SYBR Green Supermix and the iCycler Real-Time PCR Detection System (Bio-Rad). Each sample was run in triplicate. The relative RNA amounts were calculated with the ΔΔCt method and normalized with an internal control, 18s rRNA. The following primers were used for Egr-1 mRNA amplification: forward 5'-AGCCCTACGAGC ACCTGAC-3' and reverse 5'-TGGGTTGGTCATGCTC ACTA-3'.

ELISA

TSP-1 protein secreted in cultured supernatant was measured by ELISA (Chemicon International, Temecula, CA) according to the recommendation by the manufacturer.

Plasmids

The expression plasmid of Egr-1 was constructed by cloning human Egr-1 cDNA into pcDNA3.1 (Invitrogen) with PCR primers containing NheI and XbaI sites. The nucleotide sequences were confirmed by restriction digestion and sequencing.

MTT assay

The MTT experiment was performed as described previously (Liu et al., 2009). Cells were incubated for 2 h with MTT by adding 20 mL of 5 mg/mL MTT into each well in a 96-well plate. Cells were then washed with phosphate-buffered saline and solubilised with 100 mL dimethyl sulfoxide. Absorbance was read at λ=570 nm and survival was calculated as a percentage of vehicle-treated controls.

siRNAs

siRNA transient transfection was performed using the HiPerfect Transfection Reagent (Qiagen, Valencia, CA, USA) according to the recommended procedure. The sequence of siRNA targeting Egr-1 was listed as follows: 5'-AGAGGCAUACCAAGAUCCATT-3'.

Statistical analysis

Data are presented as mean±s.d. of at least three independent experiments. Differences between groups were analyzed using two-tailed Student's t-test. $P<0.05$ was considered statistically significant.

Results

INF-γ regulates Egr-1 expression in SCC-25 cells

We first investigated the effect of INF-γ treatment

on Egr-1 expression in SCC-25 cells. SCC-25 cells were incubated with 1000 U/ml INF-γ for different time intervals following 12 h pretreatment with serum free medium. Immunoblot analysis of whole-cell lysates taken from SCC-25 cells indicated that Egr-1 protein level was up-regulated remarkably by INF-γ treatment, the maximal induction being observed following 3 h drug incubation and the up-regulation was sustained for at least 24 hours (Fig. 1A). SCC-25 cells were then treated with a series of concentrations of INF-γ (0-3000 U/ml) for the peak induction period (3h). As shown in Fig. 1B, the highest Egr-1 protein expression was induced at 1000 U/ml INF-γ treatment in SCC-25 cells. Also, as shown in Fig. 1C, among the different time intervals tested, 1h INF-γ treatment resulted in the most robust Egr-1 mRNA induction in SCC-25 cells.

Forced expression of Egr-1 sensitizes HNSCC cells to chemotherapy-induced apoptosis and necroptosis

To study the effect of Egr-1 expression on chemotherapy-mediated cytotoxicity, we transiently transfected Egr-1 expression construct into SCC-25 cells. Forty-eight hours following transfection, Egr-1 expression levels were verified by western blot (Fig. 1A). Measurement of cell survival was then performed. As shown in Fig. 2B, forced expression of Egr-1 led to a notable increase in 5-FU or cisplatin-induced cell death in SCC-25 cells.

A cell can die through the apoptosis or necrosis pathway. Defects in both cell death programs may result in chemotherapy resistance of cancer cells. In order to determine the specific mechanisms which modulate Egr-1-mediated cytotoxicity, Egr-1-transfected SCC-25 cells were treated with cytotoxic drugs plus specific cell death inhibitors [a pan-caspase inhibitor zVAD (20 mM), a chemical inhibitor of necroptosis Necrostatin-1 (Nec-1, 30 mM), or both]. Cell survival assay showed that both zVAD and Nec-1 protected SCC-25 cells against chemotherapy-induced cell death, indicating that not only apoptotic program but also necroptotic signaling contribute to Egr-1-mediated cytotoxicity (Fig. 2B).

Egr-1 up-regulates Thrombospondin-1 (TSP-1) expression in HNSCC cells.

Egr-1 has been reported to increase the expression levels of TSP-1 in human colon carcinoma cells (Zhao et al., 2008). Our previous study showed that TSP-1 up-regulation significantly enhances chemotherapy-induced cell death in HNSCCs (Xu a Liu, 2009; Xu et al., 2010). Therefore, we next explored the effect of Egr-1 forced expression on TSP-1 level in HNSCC cells. Since previous studies (Saumet et al., 2005; Xu ad Liu, 2009) showed that TSP-1 is mostly secreted into the extracellular culture medium upon induction by various stimuli, we detected its protein production using enzymelinked immunosorbent assay (ELISA) in this study. As shown in Fig. 2C, Egr-1 transfection markedly up-regulated TSP-1 protein production in SCC-25 cells.

INF-γ confers chemotherapy-mediated cytotoxicity in SCC-25 cells through Egr-1 induction

The Above-mentioned results indicated that INF-γ treatment may increase Egr-1 expression in SCC-25 cells

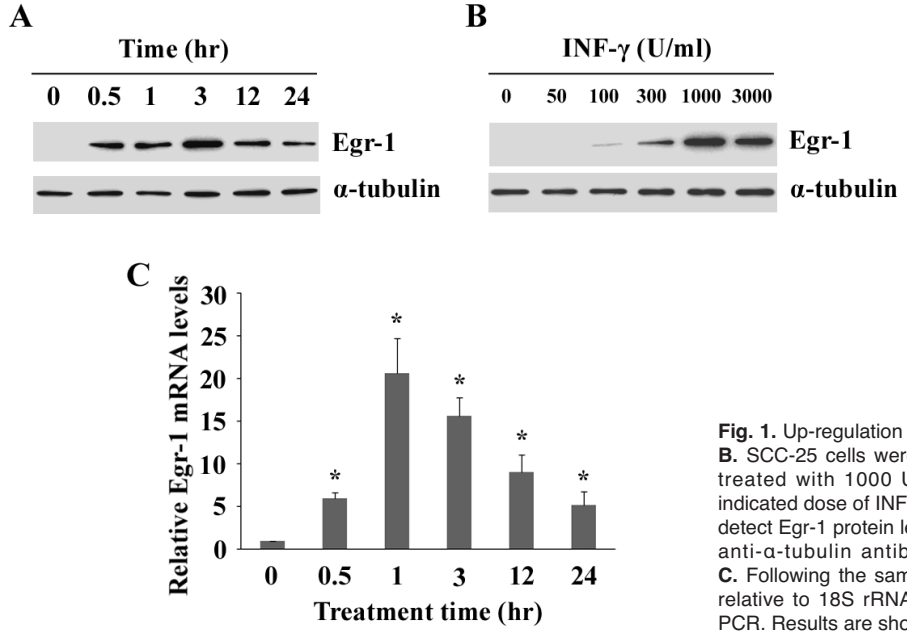

Fig. 1. Up-regulation of Egr-1 expression by INF-γ in SCC-25 cells. **A, B.** SCC-25 cells were incubated for 12 h in serum-free medium and treated with 1000 U/ml INF-γ for different time intervals **(A)** or indicated dose of INF-γ for 3 h **(B)**.Western blot was then performed to detect Egr-1 protein levels. The blots were stripped and re-probed with anti-α-tubulin antibody to show equal loading of total protein. **C.** Following the same treatment as Panel **A**, Egr-1 mRNA induction relative to 18S rRNA was determined by quantitative real time RT-PCR. Results are shown as mean+s.d. of triplicates samples. *P<0.05.

and that Egr-1 up-regulation could sensitize SCC-25cells to chemotherapy-induced cell death. To determine the specific contribution of Egr-1 in INF-γ-induced chemosensitivity, SCC-25 cells were transfected with Egr-1 siRNA for 24 h and then treated with INF-γ for 3 h. As shown in Fig. 3A, INF-γ incubation resulted in no more dramatic Egr-1 induction after Egr-1 siRNA transfection. Cell viability was then investigated by MTT assay following another 72 h. As shown in Fig. 3B, the INF-γ-induced sensitization of cells to chemotherapy-mediated cell death was markedly abolished by Egr-1 knockdown. These results suggest a specific contribution of Egr-1 in INF-γ-induced chemosensitivity.

We next tested the effect of INF-γ treatment on TSP-1 production and the role of Egr-1 in this process. As shown in Fig. 3C, INF-γ incubation led to a marked increase in TSP-1 production in SCC-25 cells, which could be significantly inhibited by Egr-1 silencing.

Discussion

HNSCC is the sixth most frequent cancer worldwide, characterized by high morbidity and mortality (Rothenberg and Elisen, 2012). It encompasses a diverse group of malignancies originating in the oral cavity, oropharynx, larynx, and hypopharynx (Howard et al., 2012). Since the prognosis is globally poor with

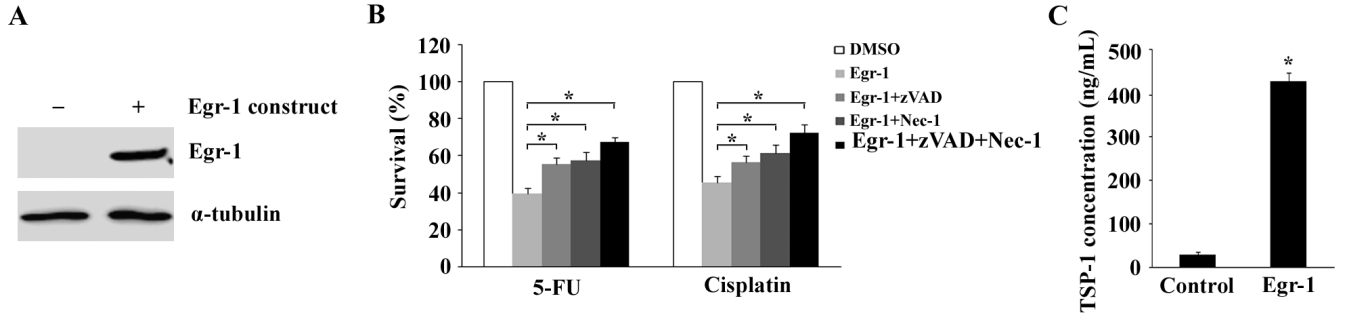

Fig. 2. Egr-1 regulates chemotherapy-induced apoptosis and necroptosis. **A.** SCC-25 cells were transfected with Egr-1 expression construct. Forty-eight hours later, Egr-1 protein levels were determined by immunoblot. **B.** Two days following Egr-1 plasmid transfection, SCC-25 cells were treated with 2 μM 5-FU or 10 μM cisplatin plus specific cell death inhibitors [a pan-caspase inhibitor zVAD (20 mM), a chemical inhibitor of necroptosis Nec-1 (30 mM), or both] for additional 72 h. The percentage of viable cells was then determined by MTT assay. Cell survival is presented as a percentage of viability of the cells treated with DMSO. **C.** Following 48 h Egr-1 construct transfection, the SCC-25 cell culture medium was collected and TSP-1 protein production was detected by ELISA. *P<0.05.

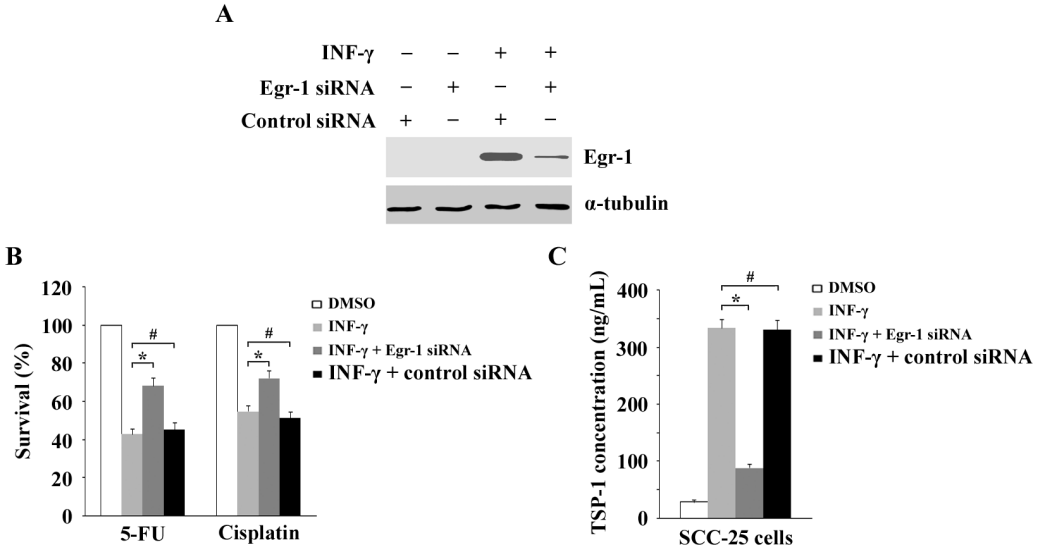

Fig. 3. Effect of Egr-1 on chemosensitivity and TSP-1 production conferred by INF-γ. **A.** SCC-25 cells were transfected with siEgr-1 for 24 h and then treated with 1000 U/ml INF-γ for additional 3 h. Egr-1 induction and resuppression were verified by western blot. **B.** SCC-25 cells were transfected with siEgr-1 for 24 h and then treated with 1000 U/ml INF-γ plus cytotoxic drugs (2 μM 5-FU or 10 μM cisplatin) for additional 72 h. Cell viability was determined using MTT assay. The percentage of viable cells was then determined by MTT assay and the cell survival is presented as a percentage of viability of the cells treated with DMSO. **C.** SCC-25 cells were transfected with siEgr-1 for 24 h and then treated with 1000 U/ml INF-γ for additional 24 h. Cell culture medium was collected and TSP-1 protein production was detected by ELISA. *P<0.05, # P>0.05.

standard radiation and cytotoxic chemotherapy, a novel therapeutic strategy is needed for this disease (Lallemant et al., 2010; Rothenberg and Elisen, 2012).

Egr-1 gene encodes a transcription factor containing three tandem zinc finger motifs that bind to GC-rich DNA elements in the promoters of a range of target genes to activate their transcription (Baek et al., 2005). Through activation of downstream regulators such as p53, Egr-1 is involved in the apoptotic process very early upon diverse cell death stimuli (Nair et al., 1997). Egr-1 up-regulation and nuclear translocation may lead to transcriptional activation of proapoptotic target genes and induce cell death in prostate cancer (Kang et al., 2013). Up-regulation of Egr-1 via the CXCR3 receptor induces reactive oxygen species (ROS) and inhibits Na+/K+-ATPase activity in an immortalized human proximal tubule cell line (Bek et al., 2003).

Consistent with our previous report in another cancer cell model, Egr-1 is up-regulated very shortly following stimuli treatment. As shown in Fig. 1A and B, upon INF-γ incubation, the time period needed to reach Egr-1 protein peak induction was only 3 hours. Among the series of INF-γ concentrations (0-3000 U/ml), 1000 U/ml INF-γ induced the most robust Egr-1 at protein level in SCC-25 cells. Subsequent experiments demonstrated that 3h INF-γ treatment at 1000 U/ml is capable of exerting its synergy effect with chemotherapeutic drugs (Fig. 3B).

Several findings from this study indicate a regulatory role for Egr-1 in the control of INF-γ-induced chemosensitivity. Transient enhanced Egr-1 expression resulted in a significant increase in 5-FU or cisplatin-induced cell death in SCC-25 cells (Fig. 2B). INF-γ treatment could sensitize SCC-25cells to chemotherapy-induced cell death. More importantly, this sensitization of cells to chemotherapy was mediated by Egr-1, which was confirmed by siRNA-mediated knockdown experiments (Fig. 3B). Our data support a combination of the use of INF-γ with routine chemotherapy in HNSCC treatment.

Additional studies indicate that Egr-1 sensitizes HNSCC cells to chemotherapy-induced cell death through both the caspase-dependent and caspase-independent mechanism. Recently, the term necroptosis has been used to designate one novel form of programmed necrosis mediated by stimulating death receptors with agonists such as TNFα, FasL and TRAIL (Hitomi et al., 2008; Declercq et al., 2009; Liu et al., 2012; Wu et al., 2012). The necroptotic signaling pathway requires the involvement of receptor interaction protein kinase 1 and 3 (RIP1 and RIP3) and a recently-characterized mixed lineage kinase domain-like protein (MLKL) (Liu et al., 2012; Sun et al., 2012; Wu et al., 2012), Moreover, Necrostatin-1 (Nec-1), the specific chemical inhibitor of necroptotic pathway, has been identified as a powerful tool to distinguish necroptosis from apoptotic cell death (Degterev et al., 2005). As shown in Fig. 2B, we pre-treated Egr-1-transfected SCC-25 cells with cytotoxic drugs plus the caspase inhibitor zVAD and NEC-1 separately, and together in cell survival assay. Our results showed that chemotherapy-induced cell death was more effectively inhibited by the combination of these two inhibitors, suggesting that necroptosis also contributes to Egr-1-mediated cytotoxicity. This data is consistent with a previous study showing that Egr-1 is implicated in glucose deprivation-induced necrosis and tumour progression (Jeon et al., 2013).

TSP-1 is a homotrimeric matricellular glycoprotein that participates in the regulation of cell invasion, proliferation, and cell death in a number of physiological and pathological conditions (Chen et al., 2000; Liu et al., 2003; Carlson et al., 2008). TSP-1 is the first protein to be recognized as a endogenous suppressor of capillary morphogenesis and has been shown to inhibit angiogenesis in multiple in vitro and in vivo assays (Good et al., 1990; Liu et al., 2003). For cell death regulation, TSP-1 has been shown to be capable of triggering both caspase-dependent and caspase-independent cell death in cancer cells (Mateo et al., 2002; Li et al., 2003; Roué et al., 2003; Saumet et al., 2005). Through high-throughput protein microarray techniques, TSP-1 was found to be significantly down-regulated in HNSCC patient specimens (Weber et al., 2007). Our previous investigations demonstrated that up-regulation of TSP-1 could markedly potentiate chemotherapy-induced cell death in HNSCC cells (Xu and Liu, 2009; Xu et al., 2010). In the current study, we showed that Egr-1 overexpression may induce TSP-1 production in HNSCC cells, which is consistent with a previous investigation by Zhao et al. (2008).

As a powerful antiproliferative and immune modulatory cytokine, INF-γ was shown to increase the anti-tumour immune response of dendritic cells against HNSCC cells through stimulation of the expression of various antigen processing genes, such as Tap-1 and Tapasin (Wei et al., 2011). Since INF-γ treatment may activate the PI3K/AKT signaling pathway, some authors suggested that combined use of LY294002 (a specific inhibitor of AKT signaling) and INF-γ could not only preserve Tap1 and tapasin expression but also induce apoptosis of tumor cells (Han et al., 2011).

INF-γ also regulates cell death directly. Fulda et al demonstrated that 5-Aza-2'-deoxycytidine and INF-γ, at relatively low individual concentrations, cooperate to restore caspase-8 expression and sensitize resistant neuroblastoma and medulloblastoma cells to TRAIL-induced apoptosis (Fulda and Debatin, 2006). INF-γ exerts its synergistic effect on Fas-induced apoptosis in A549 human non-small cell lung cancer cells by Jak1 activation (Kurdi and Booz, 2007). For the necrotic process, inhibition of NF-κB signaling allows INF-γ to trigger RIP1 kinase-dependent necroptosis in resistant cancer cells (Balachandran and Adams, 2013).

Collectively, our study demonstrates that INF-γ may increase Egr-1 expression level in HNSCC SCC-25 cells. Forced expression of Egr-1 sensitizes SCC-25 cells to chemotherapy-induced apoptosis and necroptosis.

Egr-1 up-regulation also markedly enhances the production of TSP-1 in HNSCC cells. Moreover, INF-γ-induced sensitization of cells to chemotherapy-mediated cell death and TSP-1 production could be significantly inhibited by Egr-1 silencing. These data provide the first evidence that INF-γ may sensitize HNSCC cells to chemotherapy-induced apoptosis and necroptosis through up-regulation of Egr-1. Our findings have important implications for novel strategies targeting both apoptotic and necroptotic pathways and support the combination of the use of INF-γ and cytotoxic drugs for HNSCC Therapy.

Acknowledgements. This work was supported by National Natural Science Foundation of China (81170486, 81101498), Natural Science Fund for Distinguished Young Scholars of Jiangsu Province (BK20130049), Scientific Research Foundation from the Ministry of Education of China (43-NJYKDX-3), Jiangsu Province's Medical Elite Program (RC2011168), Key Project of Jiangsu Province Health Agency (K201107), "Liu Da Ren Cai Gao Feng" Project (2012-WS-017), A Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institute (JX10231801).

Conflict of interest disclosures. The authors declare no conflict of interest.

References

Ahn E.Y., Pan G., Vickers S.M. and McDonald J.M. (2002). IFN-gamma upregulates apoptosis-related molecules and enhances Fas-mediated apoptosis in human cholangiocarcinoma. Int. J. Cancer 100, 445-451.

Baek S.J., Kim J.S., Moore S.M., Lee S.H., Martinez J. and Eling T.E. (2005). Cyclooxygenase inhibitors induce the expression of the tumor suppressor gene EGR-1, which results in the up-regulation of NAG-1, an antitumorigenic protein. Mol. Pharmacol. 67, 356-364.

Balachandran S. and Adams G.P. (2013). Interferon-γ-induced necrosis: an antitumor biotherapeutic perspective. J. Interferon. Cytokine Res. 33, 171-180.

Bek M.J., Reinhardt H.C., Fischer K.G., Hirsch J.R., Hupfer C., Dayal E. and Pavenstädt H. (2003). Up-regulation of early growth response gene-1 via the CXCR3 receptor induces reactive oxygen species and inhibits Na+/K+-ATPase activity in an immortalized human proximal tubule cell line. J. Immunol. 170, 931-940.

Carlson C.B., Lawler J. and Mosher D.F. (2008). Structures of thrombospondins. Cell. Mol. Life. Sci. 65, 672-886.

Chen H., Herndon M.E. and Lawler J. (2000). The cell biology of thrombospondin-1. Matrix. Biol. 19, 597-614.

Declercq W., Vanden Berghe T. and Vandenabeele P. (2009). RIP kinases at the crossroads of cell death and survival. Cell 138, 229-232.

Degterev A., Huang Z., Boyce M., Li Y., Jagtap P., Mizushima N., Cuny G.D., Mitchison T.J., Moskowitz M.A. and Yuan J. (2005). Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury. Nat. Chem. Biol. 1, 112-119.

Fulda S. and Debatin K.M. (2006). 5-Aza-2'-deoxycytidine and IFN-gamma cooperate to sensitize for TRAIL-induced apoptosis by upregulating caspase-8. Oncogene 25, 5125-5133.

Good D.J., Polverini P.J., Rastinejad F., Le Beau M.M., Lemons R.S., Frazier W.A. and Bouck N.P. (1990). A tumor suppressor-dependent inhibitor of angiogenesis is immunologically and functionally indistinguishable from a fragment of thrombospondin. Proc. Natl. Acad. Sci. USA 87, 6624-6628.

Häcker S., Dittrich A., Mohr A., Schweitzer T., Rutkowski S., Krauss J., Debatin K.M. and Fulda S. (2009). Histone deacetylase inhibitors cooperate with IFN-gamma to restore caspase-8 expression and overcome TRAIL resistance in cancers with silencing of caspase-8. Oncogene 28, 3097-3110.

Han W., Pan H., Jiang L., Wei K., Zou D. and Zhang Z. (2011). A novel approach to rescue immune escape in oral squamous cell carcinoma: Combined use of interferon-γ and LY294002. Oncol. Rep. 25, 181-187.

He S., Wang L., Miao L., Wang T., Du F., Zhao L. and Wang X. (2009). Receptor interacting protein kinase-3 determines cellular necrotic response to TNF-alpha. Cell 137, 1100-1111.

Hitomi J., Christofferson D.E., Ng A., Yao J., Degterev A., Xavier R.J. and Yuan J. (2008). Identification of a molecular signaling network that regulates a cellular necrotic cell death pathway. Cell 135, 1311-1323.

Horita H., Frankel A.E. and Thorburn A. (2008). Acute myeloid leukemia-targeted toxin activates both apoptotic and necroptotic death mechanisms. PLoS. One 3, e3909.

Howard J.D., Lu B. and Chung C.H. (2012). Therapeutic targets in head and neck squamous cell carcinoma: identification, evaluation, and clinical translation. Oral. Oncol. 48, 10-17.

Jeon H.M., Lee S.Y., Ju M.K., Kim C.H., Park H.G. and Kang H.S. (2013). Early growth response 1 regulates glucose deprivation-induced necrosis. Oncol. Rep. 29, 669-675.

Kang H.S., Ock J., Lee H.J., Lee Y.J., Kwon B.M. and Hong S.H. (2013). Early growth response protein 1 upregulation and nuclear translocation by 2'-benzoyloxycinnamaldehyde induces prostate cancer cell death. Cancer Lett. 329, 217-227.

Kurdi M. and Booz G.W. (2007). Jak inhibition, but not Stat1 knockdown, blocks the synergistic effect of IFN-gamma on Fas-induced apoptosis of A549 human non-small cell lung cancer cells. J. Interferon Cytokine Res. 27, 23-31.

Lallemant B., Evrard A., Chambon G., Sabra O., Kacha S., Lallemant J.G., Lumbroso S. and Brouillet J.P. (2010). Gene expression profiling in head and neck squamous cell carcinoma: Clinical perspectives. Head Neck 32, 1712-1719.

Li K., Yang M., Yuen P.M., Chik K.W., Li C.K., Shing M.M., Lam H.K. and Fok T.F. (2003). Thrombospondin-1 induces apoptosis in primary leukemia and cell lines mediated by CD36 and Caspase-3. Int. J. Mol. Med. 12, 995-1001.

Lissat A., Vraetz T., Tsokos M., Klein R., Braun M., Koutelia N., Fisch P., Romero M.E., Long L., Noellke P., Mackall C.L., Niemeyer C.M. and Kontny U. (2007). Interferon-gamma sensitizes resistant Ewing's sarcoma cells to tumor necrosis factor apoptosis-inducing ligand-induced apoptosis by up-regulation of caspase-8 without altering chemosensitivity. Am. J. Pathol. 170, 1917-1930.

Liu P., Wang Y., Li Y.H., Yang C., Zhou Y.L., Li B., Lu S.H., Yang R.C., Cai Y.L., Tobelem G., Caen J. and Han Z.C. (2003). Adenovirus-mediated gene therapy with an antiangiogenic fragment of thrombospondin-1 inhibits human leukemia xenograft growth in nude mice. Leuk. Res. 27, 701-708.

Liu P., Li J., Lu H. and Xu B. (2009). Thalidomide inhibits leukemia cell invasion and migration by upregulation of early growth response gene 1. Leuk. Lymphoma 50, 109-113.

Liu F., Hu X., Zimmerman M., Waller J.L., Wu P., Hayes-Jordan A., Lev D. and Liu K. (2011). TNFα cooperates with IFN-γ to repress Bcl-xL expression to sensitize metastatic colon carcinoma cells to TRAIL-mediated apoptosis. PLoS. One 6, e16241.

Liu P., Xu B., Shen W., Zhu H., Wu W., Fu Y., Chen H., Dong H., Zhu Y., Miao K., Xu W. and Li J. (2012). Dysregulation of TNFα-induced necroptotic signaling in chronic lymphocytic leukemia: suppression of CYLD gene by LEF1. Leukemia 26, 1293-1300.

Mateo V., Brown E.J., Biron G., Rubio M., Fischer A., Deist F.L. and Sarfati M. (2002). Mechanisms of CD47-induced caspase-independent cell death in normal and leukemic cells: link between phosphatidylserine exposure and cytoskeleton organization. Blood 100, 2882-2890.

Nair P., Muthukkumar S., Sells S.F., Han S.S., Sukhatme V.P. and Rangnekar VM. (1997). Early growth response-1-dependent apoptosis is mediated by p53. J. Biol. Chem. 272, 20131-20138.

Rothenberg S.M. and Ellisen L.W. (2012). The molecular pathogenesis of head and neck squamous cell carcinoma. J. Clin. Invest. 122, 1951-1957.

Roué G., Bitton N., Yuste V.J., Montange T., Rubio M., Dessauge F., Delettre C., Merle-Béral H., Sarfati M. and Susin S.A. (2003). Mitochondrial dysfunction in CD47-mediated caspase-independent cell death: ROS production in the absence of cytochrome c and AIF release. Biochimie 85, 741-746.

Saumet A., Slimane M.B., Lanotte M., Lawler J. and Dubernard V. (2005). Type 3 repeat/C-terminal domain of thrombospondin-1 triggers caspase-independent cell death through CD47/alphavbeta3 in promyelocytic leukemia NB4 cells. Blood 106, 658-667.

Shin S.Y., Bahk Y.Y., Ko J., Chung I.Y., Lee Y.S., Downward J., Eibel H., Sharma P.M., Olefsky J.M., Kim Y.H., Lee B. and Lee Y.H. (2006). Suppression of Egr-1 transcription through targeting of the serum response factor by oncogenic H-Ras. EMBO J. 25, 1093-1103.

Sun L., Wang H., Wang Z., He S., Chen S., Liao D., Wang L., Yan J., Liu W., Lei X. and Wang X. (2012). Mixed lineage kinase domain-like protein mediates necrosis signaling downstream of RIP3 kinase. Cell 148, 213-227.

Tekautz T.M., Zhu K., Grenet J., Kaushal D., Kidd V.J. and Lahti JM. (2006). Evaluation of IFN-gamma effects on apoptosis and gene expression in neuroblastoma--preclinical studies. Biochim. Biophys. Acta 1763, 1000-1010.

Weber A., Hengge U.R., Stricker I., Tischoff I., Markwart A., Anhalt K., Dietz A., Wittekind C. and Tannapfel A. (2007). Protein microarrays for the detection of biomarkers in head and neck squamous cell carcinomas. Hum. Pathol. 38, 228-238.

Wei H., Hongya P., Linlin J., Mujiang A., Kuijie W., Duohong Z., Qingang H. and Zhiyuan Z. (2011). IFN-γ enhances the anti-tumour immune response of dendritic cells against oral squamous cell carcinoma. Arch. Oral. Biol. 56, 891-898.

Wu W., Liu P. and Li J. (2012). Necroptosis: an emerging form of programmed cell death. Crit. Rev. Oncol. Hematol. 82, 249-258.

Xu B. and Liu P. (2009). 5-FU pretreatment potentiates cisplatin-induced apoptosis through up-regulation of thrombospondin-1 in head and neck squamous cell carcinomas. Cancer. Chemother. Pharmacol. 63, 1181-1183.

Xu B., Liu P., Li J. and Lu H. (2010). c-MYC depletion potentiates cisplatin-induced apoptosis in head and neck squamous cell carcinoma: involvement of TSP-1 up-regulation. Ann. Oncol. 21, 670-672.

Zhang D.W., Shao J., Lin J., Zhang N., Lu B.J., Lin S.C., Dong M.Q. and Han J. (2009). RIP3, an energy metabolism regulator that switches TNF-induced cell death from apoptosis to necrosis. Science 325, 332-336.

Zhao H.Y., Ooyama A., Yamamoto M., Ikeda R., Haraguchi M., Tabata S., Furukawa T., Che X.F., Zhang S., Oka T., Fukushima M., Nakagawa M., Ono M., Kuwano M. and Akiyama S. (2008). Molecular basis for the induction of an angiogenesis inhibitor, thrombospondin-1, by 5-fluorouracil. Cancer Res. 68, 7035-7041.

Zhou Y., Weyman C.M., Liu H., Almasan A. and Zhou A. (2008). IFN-gamma induces apoptosis in HL-60 cells through decreased Bcl-2 and increased Bak expression. J. Interferon Cytokine Res. 28, 65-72.

Accepted May 20, 2014