Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 319 (2004) 114–119
BBRC
www.elsevier.com/locate/ybbrc

Induction of apoptosis by ionizing radiation and CI-1033 in HuCCT-1 cells

Masateru Murakami,* Tamito Sasaki, Souichirou Yamasaki, Kenichi Kuwahara, Hideki Miyata, and Kazuaki Chayama

Department of Medicine and Molecular Science, Division of Frontier Medical Science, Programs for Biochemical Research, Graduate School of Biochemical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan

Received 20 April 2004
Available online 10 May 2004

Abstract

CI-1033 is a quinazoline-based HER family tyrosine kinase inhibitor that is currently being evaluated as a potential anticancer agent. The present study examines the molecular mechanism by which CI-1033 induces apoptosis either as a single agent or in combination with radiation. Although CI-1033 alone did not induce apoptosis, the simultaneous exposure of cells to CI-1033 and radiation induced significant levels of apoptosis. The sequential treatment of cells with CI-1033 followed by radiation induced an even greater effect with 62.6% of cells undergoing apoptosis but this enhanced effect was not seen if cells were treated first with radiation and then CI-1033. The combination treatment induces apoptosis of HuCCT-1 via upregulation of FasL and Bid cleavage. These data suggest that modulation of the Fas–FasL pathway and activation of Bid could be useful for increasing the anti-tumor effect of CI-1033 in this type of cancer.

Keywords: HuCCT-1; Ionizing radiation; CI-1033; FasL; Bid; Apoptosis

Cytotoxic drugs induce cell death in sensitive cells and this effect is, at least in part, mediated by the induction of apoptosis [1]. These drugs induce apoptosis through two major routes: the death receptor pathway and the mitochondrial pathway [2]. The mitochondrial pathway is dependent on the mitochondrial release of cytochrome *c*. The process is initiated by the interaction of the mitochondria with one or more of the pro-apoptotic members of the Bcl-2 family proteins. Bcl-2 family members are major regulators of the apoptotic process and comprise pro- as well as anti-apoptotic molecules.

Bid is a pro-apoptotic member of the Bcl-2 family member. Following death receptor ligation and activation, full-length cytoplasmic Bid (p22) is cleaved by caspase 8 [3,4], and the tBid; p15 translocates to the mitochondria where it induces the release of cytochrome *c*. Cytochrome *c*, in combination with Apaf-1, initially activates caspase 9 that then activates caspase 3 thereby resulting in apoptosis [5]. Bid is an essential component of the Fas death receptor pathway with Bid-deficient mice exhibiting resistance to the lethal effects of administration of anti-Fas antibody [6].

The HER family of receptors includes EGFR, HER-2, HER-3, and HER-4 with overexpression of these receptors being associated with anti-estrogen resistance and a poor prognosis in patients with breast cancer [7,8]. Co-expression of EGFR and HER-2 has also been associated with shortened survival of patients with carcinomas of the prostate, ovary, and upper gastrointestinal tract [9–11]. These observations have prompted extensive efforts to target HER family members using monoclonal antibodies [7,12] or small molecule inhibitors of receptor tyrosine kinases [13,14]. CI-1033 is a quinazoline-based HER family tyrosine kinase inhibitor that is currently being evaluated as a potential anticancer agent [15]. CI-1033 irreversibly binds to the ATP-binding site in the cytoplasmic domain of the receptor thereby preventing autophosphorylation and blocking

* Corresponding author. Fax: +81-82-257-5194.
E-mail address: muramura@hiroshima-u.ac.jp (M. Murakami).

0006-291X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.04.153

signaling. In vivo studies of orally administered CI-1033 have demonstrated significant tumor suppression at doses as low as 1 mg/kg for the A431 human epidermoid carcinoma with tumor regression occurring at higher doses. Although changes have taken place in cancer therapeutics, radiotherapy remains a key element for the treatment of a wide range of human malignancies. The role of Fas and FasL is potentially important in the response of epithelial tumors to ionizing radiation. Sbih-Lammali et al. [16] have demonstrated that NPC cells constitutively express high levels of functional Fas which is able to transduce a potent apoptosis signal.

The present study examines the effects of CI-1033 on apoptosis when administered either as a single agent or in combination with radiation together with the molecular mechanism involved.

Materials and methods

Reagents. CI-1033 was obtained from Parke-Davis, formerly a division of Warner-Lambert and currently owned by Pfizer (Ann Arbor, MI). It was resuspended in dimethyl sulfoxide (DMSO) to give a 10 mM stock solution and further diluted with DMSO to a final concentration of 1 mM immediately prior to use in experiments.

Cell cultures and tumor tissues. A human bile duct carcinoma cell line (HuCCT-1) was purchased from the Japanese Cancer Research Resources Bank. Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (GIBCO Lab., Grand Island, NY, USA), pH 7.4, containing 100 U/ml penicillin–streptomycin (Sigma Chemical, St. Louis, MO) and 10% fetal bovine serum (FBS; Sigma Chemical, St. Louis, MO). Cells were grown in 25 cm^2 plastic flasks (Corning, Corning, NY) and placed in 5% CO_2 at 37 °C. Bile duct carcinoma tissues from specimens surgically resected at Hiroshima University Hospital between 1993 and 2002 underwent immunohistochemical staining and examination after obtaining informed consent from patients.

Immunohistochemistry. Consecutive 4 μm sections were cut from each tissue block and immunostained for EGFR. Immunohistochemistry (IHC) was performed using a Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA). Sections were incubated with a rabbit polyclonal anti-EGFR antibody (Santa Cruz Biotechnology, Santa Cruz, CA) at a 1:200 dilution.

Proliferation assay. The cell line was treated with various concentrations (0–1000 ng/ml) of CI-1033 for 48 h in RPMI supplemented with 0.1% FBS and cell proliferation assays were carried out using a Cell Counting Kit (Dojindo Labs, Kumamoto, Japan) according to the manufacturer's protocol. The absorbance at 450 nm of aliquots of cell supernatants was measured using an automatic plate analyzer (Toyo Sokki, Tokyo, Japan). Each experiment was performed twice in triplicate.

Western blot analysis. Cells were lysed with modified radioimmunoprecipitation assay (RIPA) lysis buffer (50 mM Tris–HCl (pH 7.4), 1% Nonidet P-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, and protease inhibitor mixture tablet (Roche Diagnostics, Indianapolis, IN), 1 μg/ml pepstatin, and 50 mM NaF). A volume of 30 μl from each sample was subjected to SDS–PAGE and the resolved proteins were transferred to a nitrocellulose membrane. After blocking with 5% non-fat dried milk, the blots were incubated with primary antibodies (1:1000 dilution), washed, and then incubated with horseradish peroxidase-conjugated anti-mouse or rabbit immunoglobulin G (Santa Cruz Biotechnology, Santa Cruz, CA). Blots were developed with enhanced chemiluminescence (Perkin–Elmer, Boston, MA) and exposed to Kodak X-Omat Blue film. The primary antibodies include: mouse anti-phosphotyrosine, clone 4G10 (Upstate Biotechnology, Lake Placid, NY), anti-EGFR, anti-Bax (Santa Cruz Biotechnology, Santa Cruz, CA), anti-Fas and anti-FasL (Transduction Laboratories, Lexington, KY), and anti-Bid (MBL, Nagoya, Japan).

Flow cytometry. FITC-phalloidin and propidium iodide (PI) were purchased from Sigma (Sigma Chemical, St. Louis, MO). Early apoptotic changes were detected by FITC–Annexin V that binds to phosphatidylserine exposed on the outer leaflet of apoptotic cell membranes. PI was used to discriminate between apoptotis and necrosis among the annexin–V-positive cells. HuCCT-1 cells (10^6) were washed and re-suspended in 490 μl binding buffer solution (Annexin–V-FITC Kit, Immunotech, Marseille, France). Annexin V-FITC (5 μl) and PI (5 μl) were then added to the cell suspension for a 10-min incubation period followed by FACS analysis (Becton–Dickinson, Franklin Lakes, NJ).

Treatment studies. In experiments involving treatment of HuCCT-1 cells, CI-1033 (100 nM in DMSO) and 6 Gy ionizing radiation given on a ^{60}Co unit were administered either as single agents or in combination for 3 days. The treatment protocols for the combination studies spanned 72 h and included (1) administration of radiation 64 h after CI-1033 and (2) addition of CI-1033 soon after radiation. All experiments were performed in duplicate.

Statistical analysis. Statistical analysis was performed with Scheffe's *F* test. Results are presented as the average ± SD of triplicate samples from at least two independent experiments. A p value <0.05 was considered statistically significant. The analyses were performed with STATISTICA statistical software (StatSoft, USA).

Results

Growth assay

We examined the effects of CI-1033 on the growth of HuCCT-1 cells. Cell proliferation was expressed as a percentage of the proliferation of cells cultured in the absence of CI-1033. CI-1033 significantly inhibited the

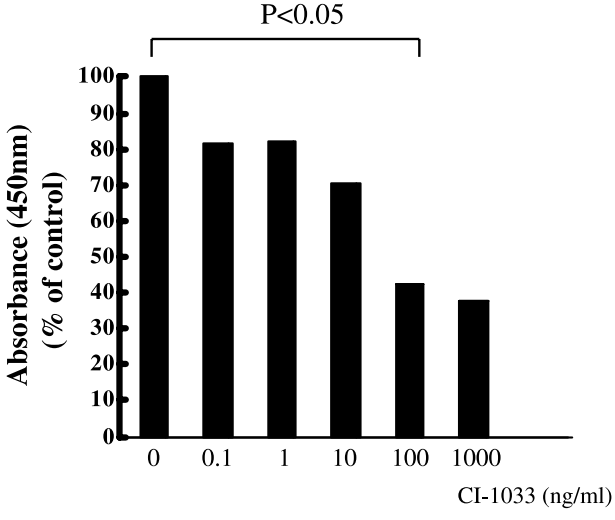

Fig. 1. Effect of CI-1033 on growth of HuCCT-1. Cells were exposed to different concentrations of CI-1033. After 48 h, the cell proliferation was determined using the Cell Counting kit described in Materials and methods. CI-1033 significantly inhibited the proliferation of HuCCT-1 cells in a dose-dependent manner. Cell proliferation was expressed as a percentage of the proliferation of cells cultured in the absence of CI-1033.

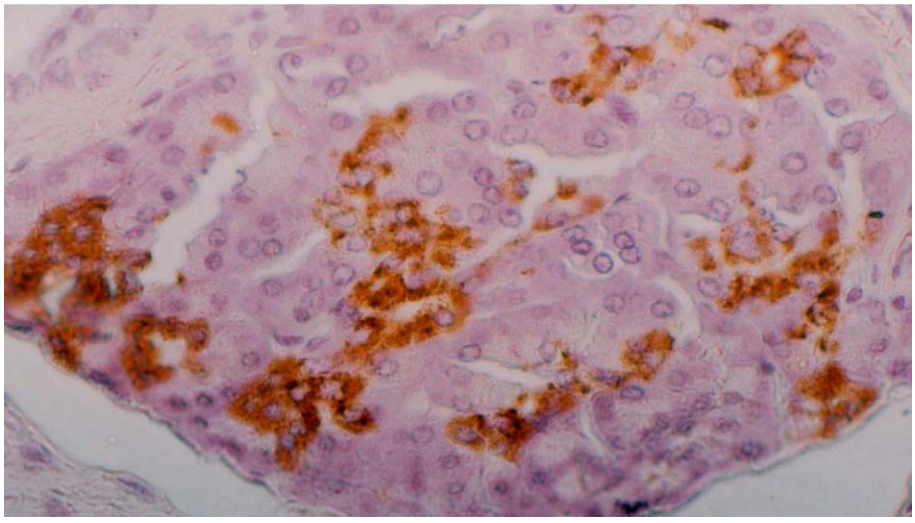

Fig. 2. Expression of EGFR in human cholangiocellular carcinoma tissue. Immunoreactivity for EGFR was detected in membrane and cytoplasm of tumor cells in surgical tissues (original magnification 400×).

proliferation of HuCCT-1 cells in a dose-dependent manner. Results from a representative experiment are shown in Fig. 1.

Expression of EGFR in human bile duct carcinoma tissues

The expression of EGFR in 35 human bile duct carcinoma tissues was examined. Immunohistochemical staining revealed that EGFR protein was expressed in 20 out of 35 patients (57%). Immunohistochemical expression of EGFR protein was found sporadically, primarily in the membrane and cytoplasm of cancer cells (Fig. 2).

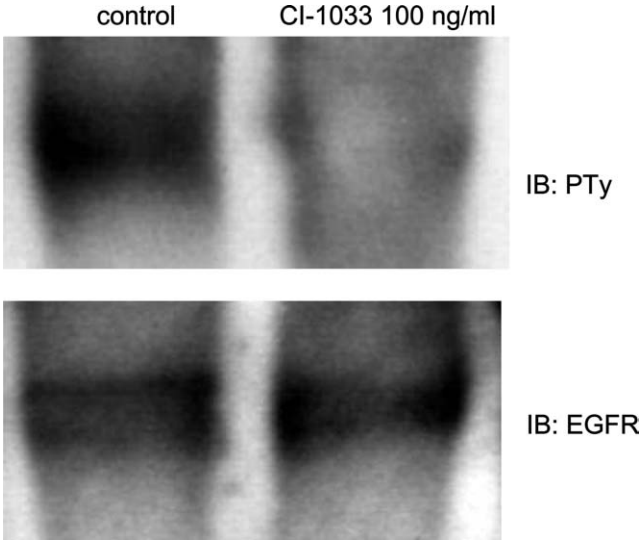

Fig. 3. EGFR phosphorylation is prohibited by CI-1033. HuCCT1 cells were cultured in the presence or absence of CI-1033, and equivalent amounts of protein were immunoblotted with antiphosphotyrosine antibody (Pty) and the anti-EGFR antibody.

Expression of EGFR in HuCCT-1

HuCCT-1 cells were cultured in the presence or absence of CI-1033 and phosphorylation of the EGFR was determined by immunoblotting using phosphorylation-specific antisera. Examination of whole cell lysates revealed that CI-1033 increased tyrosine phosphorylation of a protein with a molecular weight of 170–190 kDa, the approximate molecular weight of EGFR (Fig. 3).

Flow cytometry

The ability of CI-1033 to induce apoptosis either alone or in combination with radiation was examined by flow cytometric analysis (Fig. 4). 5.7% of the cell population was apoptotic in the presence of 100 nM CI-1033 whilst 16.4% was apoptotic following treatment with 6 Gy of radiation. The simultaneous exposure of cells to CI-1033 and radiation resulted in a significant enhancement of apoptosis with an even greater effect (62.6%) evident if the cells were treated first with CI-1033 and then radiation. However, this enhanced effect was not seen if cells were treated first with radiation and then CI-1033 (Table 1).

Western blot analysis for Fas, Bid, and FasL in HuCCT-1

To examine the mechanism underlying the apoptotic potential of CI-1033 and radiation, we performed Western blots for Fas, FasL, Bax, and Bid in HuCCT-1 cells. Fas, FasL, Bax, and Bid were detected in lysates of HuCCT-1. Representative Western blots demonstrate the cleavage of Bid to tBid (p15) together with and aggregation of FasL. The stability of Fas and Bax expression after the treatment was confirmed. Apoptosis was most prominent (62.6%) when radiation was administered 64 h after CI-1033 and this was associated

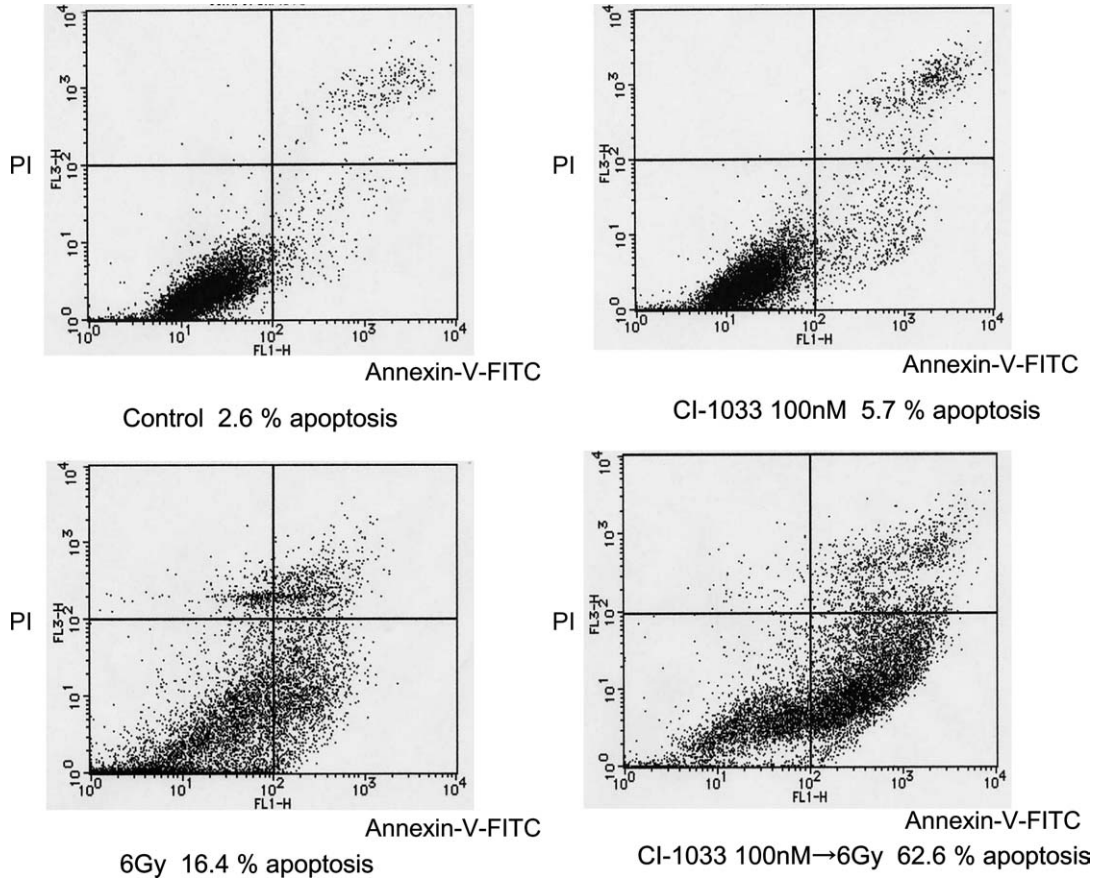

Fig. 4. Apoptosis induced by CI-1033 and radiation in HuCCT-1 cells. 5.7% of the cell population was apoptotic in the presence of 100 nM CI-1033 whilst 16.4% was apoptotic following treatment with 6 Gy of radiation. The simultaneous exposure of cells to CI-1033 and radiation resulted in a significant enhancement of apoptosis with an even greater effect (62.6%) evident if the cells were treated first with CI-1033 and then radiation.

Table 1
Analysis of the apoptotic fraction in HuCCT-1 cells treated with CI-1033 and radiation

	Apoptosis (%)
Controls	2.6
CI-1033 100 nM	5.7
6 Gy	16.4
6 Gy → CI-1033 100 nM	18.9
CI-1033 100 nM → 6 Gy	62.6

Enhanced effect was not seen if cells were treated first with radiation and then CI-1033.

with activation of FasL and Bid (Fig. 5). When the treatment order was reversed such that CI-1033 was added soon after radiation, the fraction of cells exhibiting the features of apoptosis was only 18.9%. Furthermore, this schedule did not result in the activation of FasL and Bid.

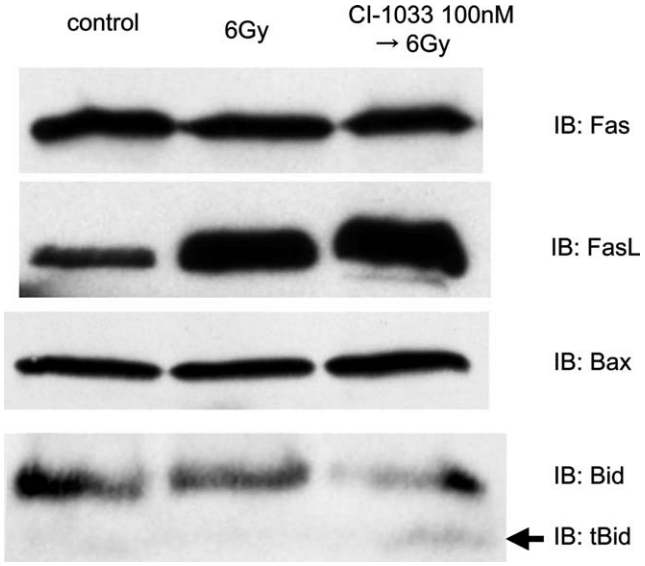

Fig. 5. Radiation after CI1033 induces FasL aggregation and cleavage of Bid. HuCCT-1 cells were treated in Materials and methods. Cell lysates were prepared and used for Western blot analysis. Representative Western blots show the cleavage of Bid to tBid (p15), and aggregation of FasL.

Discussion

The balance between cell survival and cell death is a complex issue and considerable effort has been made in

an attempt to understand how tumor cells regulate the decision points between these critical pathways. A significant number of clinically useful anticancer agents have been shown to induce apoptosis in tumor cells [17], and this process is believed to be a major component of their therapeutic action. The reported pathways through which these agents trigger apoptosis in cancer cells have varied and tend to depend on both the nature of the drug and the genetic background and origin of the tumor [2].

Because of the role of the HER family in a variety of neoplasms including adenocarcinoma of the breast, prostate, and ovary as well as high-grade gliomas, there has been considerable interest in combining HER family kinase inhibitors with agents that have been demonstrated to be active in these diseases. The present study provides evidence that modulation of the stress-activated pathway plays a role in the strong synergistic interaction between CI-1033 and radiation in terms of enhanced apoptosis in HuCCT-1 cells that express high levels of EGFR. CI-1033 is a specific inhibitor of the HER family of receptor tyrosine kinases that have been shown to be a useful target in cancer chemotherapy [18]. Inhibitors of both the kinase activity and neutralizing antibodies to the extracellular domain are currently in clinical trials with encouraging results [13,18].

Nelson and Fry [19] reported that CI-1033 significantly reduced the constitutively activated components of both pathways (activation of Akt and the MAP kinases) in the MDA-MB-453 breast tumor cell line but that this effect alone did not induce cell death. In this present study, the addition of CI-1033 to cells treated with radiation resulted in the induction of maximum levels of apoptosis that were markedly greater than the level of cell death evident following the use of each agent in isolation. Indeed, treatment with CI-1033 alone did not induce apoptosis, but the simultaneous exposure of cells to CI-1033 and radiation resulted in significant apoptosis with an even greater effect (62.6%) evident if cells were treated initially treated with CI-1033 followed by radiation. This enhanced effect was not seen if cells were treated with radiation followed by CI-1033.

The biochemical mechanism by which CI-1033 in combination with radiation-induced cells to undergo apoptosis was explored. Our data suggest that FasL and Bid activation is an essential component of the apoptotic response by CI-1033 and is responsible for maximal cell death. It is reported that cells expressing FasL can potentially kill themselves ("suicide") and/or induce apoptosis in nearby Fas-expressing cells ("fratricide cell death") [20,21]. With regard to irradiated HuCCT-1 cells, our Western-blot experiments indicate that there is a true increase of FasL protein content in HuCCT-1 cells treated by CI-1033 and radiation. We do not yet know the mechanism whereby ionizing radiation induces FasL expression in HuCCT-1 cells, but this mechanism might involve ceramide as a signaling intermediate [22].

Cell death initiators or repressors such as Bid, Bax, and Bcl-2 have been shown to regulate this event and suggest that this is a critical step in the death signal cascade [3,23]. Bid is a substrate of caspase 8 in the pathway of apoptosis triggered by ligation of cell surface death receptors such as Fas, tumor necrosis factor-α, and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) [3,4,24]. The active form of Bid is tBid and is a 15 kDa C-terminal fragment resulting from caspase cleavage. tBid localizes to mitochondria and acts to promote the release of cytochrome *c* [25].

Caspase 8 is activated following Fas ligation as it is directly recruited into the Fas signaling complex that is assembled following Fas receptor oligomerization [26,27]. Also, recent reports suggest that caspase 8 activation, when triggered downstream of the mitochondrial pathway of apoptosis, may amplify caspase 9 activation through the cleavage of the pro-apoptotic protein Bid as this elicits a further efflux of cytochrome *c* from mitochondria [28,29]. Modulation of these pathways was investigated with regard to the molecular mechanism by which CI-1033 and radiation synergize to induce cell death.

In conclusion, our findings demonstrate that, in addition to CI-1033 inhibiting proliferation of HuCCT-1 cells, CI-1033 sensitizes cells to ionizing radiation. The combination of CI-1033 and radiation induces apoptosis of HuCCT-1 via upregulation of FasL and Bid cleavage. These data suggest that modulation of the Fas–FasL pathway could be useful for increasing the anti-tumor effect of ionizing radiation in this type of cancer. Indeed, FasL induced by ionizing radiation and CI-1033 could mediate a differential effect and lead to apoptosis in tumor cells expressing Fas.

References

[1] D.E. Fisher, Apoptosis in cancer therapy: crossing the threshold, Cell 78 (1994) 539–542.
[2] S.H. Kaufmann, W.C. Earnshaw, Induction of apoptosis by cancer chemotherapy, Exp. Cell Res. 256 (2000) 42–49.
[3] X. Luo, I. Budihardjo, H. Zou, C. Slaughter, X. Wang, Bid, a Bcl2 interacting protein, mediates cytochrome *c* release from mitochondria in response to activation of cell death receptors, Cell 94 (1998) 481–490.
[4] A. Gross, X.M. Yin, K. Wang, M.C. Wei, J. Jockel, C. Milliman, H. Erdjument-Bromage, P. Tempst, S.J. Korsmeyer, Caspase cleaved BID targets mitochondria and is required for cytochrome *c* release, while BCL-XL prevents this release but not tumor necrosis factor-R1/Fas death, J. Biol. Chem. 274 (1999) 1156–1163.
[5] X. Liu, C.N. Kim, J. Yang, R. Jemmerson, X. Wang, Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome *c*, Cell 86 (1996) 147–157.

[6] X.M. Yin, K. Wang, A. Gross, Y. Zhao, S. Zinkel, B. Klocke, K.A. Roth, S.J. Korsmeyer, Bid-deficient mice are resistant to Fas-induced hepatocellular apoptosis, Nature 400 (1999) 886–891.
[7] J.S. Ross, J.A. Fletcher, The HER-2/neu oncogene: prognostic factor, predictive factor and target for therapy, Semin. Cancer Biol. 9 (1999) 125–138.
[8] H. Kim, W.J. Muller, The role of the epidermal growth factor receptor family in mammary tumorigenesis and metastasis, Exp. Cell Res. 253 (1999) 78–87.
[9] J.G. Klijn, P.M. Berns, P.I. Schmitz, J.A. Foekens, The clinical significance of epidermal growth factor (EGF-R) in human breast cancer: a review on 5232 patients, Endocr. Rev. 13 (1992) 3–17.
[10] J.M. Bartlett, S.P. Langdon, B.J. Simpson, M. Stewart, D. Katsaros, P. Sismondi, S. Love, W.N. Scott, A.R. Williams, A.M. Lessells, K.G. Macleod, J.F. Smyth, W.R. Miller, The prognostic value of epidermal growth factor receptor mRNA expression in primary ovarian cancer, Br. J. Cancer 73 (1996) 301–306.
[11] M. Maurizi, G. Ferrandina, G. Almadori, G. Scambia, G. Cadoni, G. D'Agostino, F.G. Serra, M. Piantelli, S. Mancuso, F.O. Ranelletti, Prognostic significance of methyl-*p*-hydroxyphenyllactate-esterase activity in laryngeal squamous cell carcinoma, Br. J. Cancer 77 (1998) 1253–1259.
[12] Z. Fan, J. Mendelsohn, Therapeutic application of anti-growth factor receptor antibodies, Curr. Opin. Oncol. 10 (1998) 67–73.
[13] D.W. Fry, Inhibition of the epidermal growth factor receptor family of transferase kinases as an approach to cancer chemotherapy: progression from reversible to irreversible inhibitors, Pharmacol. Ther. 82 (1999) 207–218.
[14] M.L. Levitt, P.O. Koty, Tyrosine kinase inhibitors in preclinical development, Invest. New Drugs 17 (1999) 213–226.
[15] J.B. Smaill, B.D. Palmer, G.W. Rewcastle, W.A. Denny, D.J. McNamara, E.M. Dobrusin, A.J. Bridges, H. Zhou, H.D. Showalter, R.T. Winters, W.R. Leopold, D.W. Fry, J.M. Nelson, V. Slintak, W.L. Elliot, B.J. Roberts, P.W. Vincent, S.J. Patmore, Tyrosine kinase inhibitors 15.4-(phenylamino) quinazoline and 4-(phenylamino) pyrido[d]pyrimidine acrylamides as irreversible inhibitors of the ATP binding site of the epidermal growth factor receptor, J. Med. Chem. 42 (1999) 1803–1815.
[16] F. Sbih-Lammali, B. Clausse, H. Ardila-Osorio, R. Guerry, M. Talbot, S. Havouis, L. Ferradini, J. Bosq, T. Tursz, P. Busson, Control of apoptosis in Epstein–Barr-virus-positive nasopharyngeal-carcinoma cells: opposite effects of CD95 and CD40 stimulation, Cancer Res. 59 (1999) 924–930.
[17] S. Kasibhatla, T. Brunner, L. Genestier, F. Echeverri, A. Mahboubi, D.R. Green, DNA damaging agents induce expression of Fas ligand and subsequent apoptosis in T lymphocytes via the activation of NF-kappa B and AP-1, Mol. Cell 1 (1998) 543–551.
[18] D.W. Fry, Inhibition of the epidermal growth factor receptor family of tyrosine kinases as an approach to cancer chemotherapy: progression from reversible to irreversible inhibitors, Pharmacol. Ther. 82 (1999) 207–218.
[19] J.M. Nelson, D.W. Fry, Akt, MAPK (Erk1/2), and p38 act in concert to promote apoptosis in response to ErbB receptor family inhibition, J. Biol. Chem. 276 (2001) 14842–14847.
[20] S. Strand, W.J. Hofmann, H. Hug, M. Muller, G. Otto, D. Strand, S.M. Mariani, W. Tremmel, P.H. Krammer, P.R. Galle, Lymphocyte apoptosis induced by CD95 (APO-1/Fas)-ligand-expressing tumors cells: a mechanism of immune evasion?, Nat. Med. 2 (1996) 1331–1336.
[21] J. O'Connell, J.C. O'Sullivan, J.K. Collins, F. Shanahan, The Fas counterattack: Fas-mediated T-cell by colon cancer expressing Fas-ligand, J. Exp. Med. 184 (1996) 1075–1081.
[22] I. Herr, D. Wilhelm, T. Bohler, P. Angel, K.M. Debatin, Activation of CD95 (APO-1/Fas) signaling by ceramide mediates cancer-therapy-induced apoptosis, EMBO J. 16 (1997) 6200–6208.
[23] S.J. Korsmeyer, M.C. Wei, M. Saito, S. Weiler, J. Oh, P.H. Schlesinger, Proapoptotic cascade activates BID, which oligomerizes BAK or BAX into pores that result in the release of cytochrome *c*, Cell Death Differ. 7 (2000) 1166–1173.
[24] H. Yamada, S.T. Tada-Oikawa, A. Uchida, S. Kawanishi, TRAIL causes cleavage of Bid by caspase-8 and loss of mitochondrial membrane potential resulting in apoptosis in BJAB cells, Biochem. Biophys. Res. Commun. 265 (1999) 130–133.
[25] M.D. Esposti, The roles of Bid, Apoptosis 7 (2002) 433–440.
[26] F.C. Kischkel, S. Hellbardt, I. Behrmann, M. Germer, M. Pawlita, P.H. Krammer, M.E. Peter, Cytotoxicity-dependent APO-1 (Fas/CD95)-associated proteins form a death-inducing signaling complex (DISC) with the receptor, EMBO J. 14 (1995) 5579–5588.
[27] M. Muzio, A.M. Chinnaiyan, F.C. Kischkel, K. O'Rourke, A. Shevchenko, J. Ni, C. Scaffidi, J.D. Bretz, M. Zhang, R. Gentz, M. Mann, P.H. Krammer, M.E. Peter, V.M. Dixit, FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death-inducing signaling complex, Cell 85 (1996) 817–827.
[28] C.L. Perkins, G. Fang, C.N. Kim, K.N. Bhalla, The role of Apaf-1, caspase-9 and BID proteins in etoposide-or paclitaxel-induced mitochondrial events during apoptosis, Cancer Res. 60 (2000) 1645–1653.
[29] V. Viswanath, Y. Wu, R. Boonplueang, S. Chen, F.F. Stevenson, F. Yantiri, L. Yang, M.F. Beal, J.K. Anderson, Caspase-9 activation results in downstream caspase-8 activation and BID cleavage in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinson's disease, J. Neurosci. 21 (2001) 9519–9528.