Ad-FRNK and Ad-p53 Cooperate to Augment Drug-induced Death of a Transformed Cell Line

LORI KORNBERG

Department of Otolaryngology, University of Florida, Gainesville, FL 32610, U.S.A.

Abstract. *Background: Transformed cells have abnormalities in the survival pathways contributing to drug resistance. These abnormalities include p53 mutations and increased focal adhesion kinase (FAK) expression. Because FAK regulates cell survival via p53-dependent and -independent pathways, it was hypothesized that combined therapy using wild-type p53 and an inhibitor of FAK (FRNK) would sensitize cells to anticancer drugs. Materials and Methods: RPMI 2650 cells were infected with recombinant adenoviruses causing overexpression of p53 (Ad-p53) and FRNK (Ad-FRNK). Cell viability and apoptosis were measured using in vitro assays, whereas protein expression was determined by Western blotting. Results: Co-infection of cells with Ad-p53 and Ad-FRNK induced more cellular apoptosis than transfection with either agent alone. Likewise, the co-transfection of cells with Ad-FRNK and Ad-p53 improved the cytotoxic response to four commonly used anticancer drugs relative to cells transfected with Ad-FRNK alone, Ad-p53 alone, or the equivalent amount of control adenovirus. This effect was associated with loss of endogenous FAK protein.*

Focal adhesion kinase (FAK) is a tyrosine kinase, which serves as a point of convergence between extracellular matrix- and growth factor- mediated signaling pathways (reviewed in 1). FAK is overexpressed in several types of human cancer of epithelial origin, including those derived from the head and neck region (2-4), breast (5, 6), ovary (7), colon (5, 6) prostate (8) and brain (9). Although FAK is not an oncogene, studies using cultured cells demonstrated that FAK regulates cell cycle progression, cell migration, invasion and survival (reviewed in 1), whereas *in vivo* experiments suggested that FAK is involved in the early stages of metastasis (10, 11). Thus, there is interest in developing FAK inhibitors as anticancer drugs.

p53 is a tumor suppressor that regulates apoptosis or cell cycle arrest in response to DNA- damaging agents. Many human cancers lack functional p53, which contributes to drug resistance. When transformed cells are transfected with recombinant adenoviruses containing a wild-type p53 transgene (Ad-p53), they are sensitized to chemotherapeutic agents regardless of whether they have endogenous mutant or wild-type p53 (12). As such, Ad-p53 is being tested in clinical trials (12).

Head and neck cancers (SCCHN) contain numerous genetic abnormalities including p53 mutations and increased FAK expression. Cell survival is regulated by p53-dependent and -independent mechanisms and FAK can be involved in both processes (13). As such, transformed SCCHN cells, genetically engineered to have both increased wild-type p53 expression and reduced FAK activity, were hypothesized to be maximally sensitive to cytotoxic agents. Indeed, the results indicated that co-transfection of the SCCHN cell line, RPMI 2650, with Ad-p53 and Ad-FRNK induced more apoptosis than transfection with either agent alone. Moreover, co-transfection of RPMI 2650 cells with Ad-FRNK and Ad-p53 improved the cytotoxic response to four commonly used anticancer drugs relative to cells transfected with Ad-FRNK alone or Ad-p53 alone.

Materials and Methods

Cells. The cells were obtained from the American Type Culture Collection. The RPMI 2650 cells, which were derived from a metastatic pleural effusion originating from a squamous cell carcinoma of the nasal septum, were cultured in EMEM with L-glutamine and Earle's balanced salts containing 10 % FCS and antibiotics. The cells were cultured at 37˚C in a humidified atmosphere of 5% CO_2, 95% air.

Materials. Ad/CMV-p53 (14) was obtained from the M.D. Anderson Institutional Vector Core. Mouse anti-FAK (clone 4.47) and rabbit anti-FAK (# 06-543) were purchased from Upstate Biotechnology (Charlottesville, VA, USA). Rabbit anti-pY397 FAK (# 44-624G) was purchased from Biosource International

Correspondence to: Lori Kornberg, Ph.D., Department of Otolaryngology, 1600 SW Archer Road, Room M2-228, University of Florida, Gainesville, FL 32610, U.S.A. Tel: 352-273-5192, Fax: 352-392-6781, e-mail: kornblj@ent.ufl.edu

Key Words: p53, Ad-p53, FRNK, Fak-related non-kinase, FAK, focal adhesion kinase, adenovirus, apoptosis, 5-fluorouracil, etoposide, cisplatin, paclitaxel.

(Camerillo, CA, USA). Anti-pJNK (# 9255S), anti-pAKT (# 4051S), anti-AKT (# 9272), anti-p53 (# 2524) and anti-p21 (# 2946) were purchased from Cell Signaling Technology (Beverly, MA, USA). The anti- actin antibody and drugs were purchased from Sigma (St Louis, MO, USA).

Construction of Ad-empty and Ad-FRNK. The construction of the adenoviruses using the BD Adeno-X-Expression Systems 2 kit (BD Biosciences, Palo Alto, CA, USA) has been previously described (15). Ad-FRNK contains the FRNK sequence under control of a CMV promotor, whereas Ad-Empty is a control adenovirus. The viruses were titered using the BD Adeno-X Rapid Titer Kit (BD Biosciences). The multiplicity of infection (m.o.i) is defined as the number of infectious particles divided by the number of cells.

Infection of SCCHN cells with recombinant adenovirus. The cells were trypsinized and known numbers of cells were replated in wells of the desired size. The cultures were incubated overnight. The adenoviruses were diluted in culture medium and the virus was added to the cells for the times indicated in the figure legends.

Western blotting. Cells were incubated at 37˚C for 48 h with viruses. The cell monolayers were rinsed in phosphate-buffered saline (PBS) and lysed in ice-cold lysis buffer (50 mM HEPES, pH 7.4; 150 mM NaCl; 1% NP40; 0.5% sodium deoxycholate; 1 mM sodium orthovanadate; 5 mM EDTA; 5 mM NaF) containing a 1:20 dilution of mammalian proteinase inhibitor cocktail (Sigma P 8340). Protein was assayed using the BCA reagent (Pierce, Rockford, IL, USA) and equal amounts of protein per lane were electrophoresed on a 10% polyacrylamide gel under denaturing and reducing conditions. The resolved proteins were electrophoretically transferred to PVDF membranes. Proteins were detected using the appropriate antibodies and the ECL system (Amersham, Piscataway, NJ, USA) as per the manufacturer's instructions. Where indicated, membranes were stripped by heating (50˚C/ 10 min) in 6 mM glycine; 1 % SDS, pH 2.0.

Cytotoxicity assay/ apoptosis assay. The cells were trypsinized, replated in 96-well plates ($2x10^4$ cells/ well) and allowed to attach overnight. Fresh medium containing adenoviruses and drugs were added to the cells, which were incubated for an additional 3 days. Cell viability was assessed using the MTT assay. MTT (10 μL of a 5 mg/ml solution) was added to the wells and the plates were incubated at 37˚C for 4 h. The reaction was stopped with 1% SDS in 0.1 N HCl, which solubilizes the cells and the blue reaction product. The plates were incubated overnight at 37˚C and the absorbance at 550-650 nm was determined using an ELISA plate reader. Each assay point was performed in triplicate and the results are presented as the mean±standard error. The assays were performed on at least three separate occasions with similar results.

Apoptosis was measured using the DNA stain, Hoechst 33342 (bisbenzimide). Cells, which were cultured in 96-well plates, were treated with adenoviruses for 48 h. The plates were centrifuged, the cells fixed for 10 min with 3.7% formaldehyde in PBS and were then stained with Hoechst 33342 (10 μg/ml in PBS). The stained nuclei were viewed under a fluorescent microscope equipped with a UV filter. The percent of apoptotic cells was determined by manually counting cells containing brightly staining condensed and/ or fragmented chromatin. This number was divided by the total number of cells in the microscopic field (13, 16). The results are the average of replicate wells.

Reverse transcription polymerase chain reaction. Cells ($1x10^6$ cells/ dish) were incubated at 37˚C for 72 h with viruses and RNA was then extracted using Trizol (Invitrogen, Carlsbad, CA, USA). Two micrograms of total RNA were reverse transcribed in a volume of 100 μL containing 10 mM Tris-HCl (pH 8.3), 200 μM dNTPs, 1.5 mM $MgCl_2$, 50 U/mL Rnasin, 2.5 mM random primers, 10 mM KCl, and 200 U Superscript (Invitrogen). The reactions were incubated at 25˚C for 10 min, 37˚C for 60 min and 92˚C for 5 min. DNA amplification was performed in a 50-μL reaction containing 5 μL of the RT reaction, 50 pmol of each primer, 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 200 μM dNTPs, and 0.25 U Taq polymerase. The primers were: FAK sense 5' CCAGGGATTATGAGATTC 3'; FAK antisense 5' GACAC CAGAACATTCCGAGCA 3'; Actin sense 5' GTGGGGCGCC CCAGGCACCA 3'; Actin antisense 5' CTCAATGTCACGC ACGATTTC 3'. The amplification reactions were performed in 35 cycles of 94˚C for 50 sec, 55˚C for 30 sec and 72˚C for 2 min. The products were electrophoresed on a 1% agarose gel containing ethidium bromide and the bands were photographed under UV light.

Statistical analysis. The data were analyzed using JMP (SAS Institute) Statistical Discovery Software Version 4. The difference between means was determined using a one-way analysis of variance (ANOVA) followed by the Student's *t*-test for each pair. Differences were considered significant where $p \leq 0.05$.

Results

FRNK (fak-related non-kinase) is the non-catalytic carboxy-terminal portion of FAK (17). FRNK is autonomously expressed *in vivo* (17) and inhibits FAK activity, probably by binding to its intracellular "receptor" (18). As such, FRNK has been used to experimentally inhibit the activity of FAK (15, 19).

When the RPMI 2650 cells were infected with Ad-FRNK (Figure 1B), there was a small decrease in cell viability, as measured by the MTT assay, relative to both untreated cells and cells treated with the control adenovirus (Ad-Empty), which lacks a transgene. Although the cells treated with Ad-FRNK were consistently less viable than Ad-Empty-treated cells, this difference generally was not statistically significant at a multiplicity of infection (m.o.i) less than 100 (Figure 1B and ref. 15). However, when cells were treated with Ad-p53 (Figure 1A), there was clearly a concentration-dependent decrease in cell viability.

In order to minimize the cytotoxic effects of the adenovirus itself, subsequent experiments were performed using a relatively low concentration of virus. Moreover, the concentrations of Ad-p53 or Ad-FRNK causing minimal cytotoxity were chosen in order to maximize the observed responses caused by combining both viruses and adding drugs. Based on the results of pilot experiments, some experiments used Ad-p53 at m.o.i=5 and Ad-FRNK at m.o.i=10, whereas the control adenovirus, Ad-Empty, was used at an m.o.i=15. At this concentration, Ad-Empty induced a small increase (10-15%) in cytotoxicity (Figure 1)

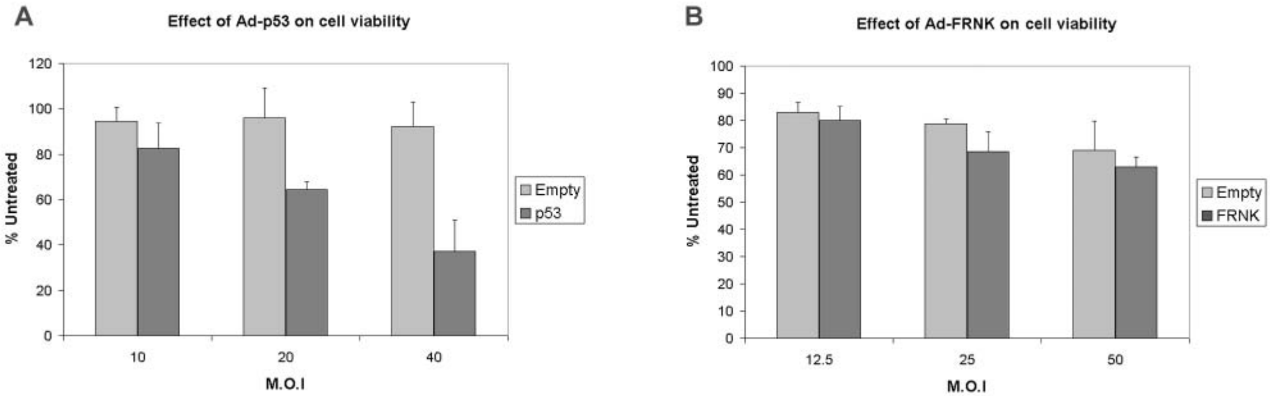


Figure 1. *Effect of Ad-p53 and Ad-FRNK on RPMI viability. RPMI 2650 cells were incubated with the indicated amount of Ad-p53 (A), Ad-FRNK (B), or Ad-Empty (control) for 72 h and cell viability was then assessed using MTT. The percent of viable cells relative to untreated cells was determined from the means of triplicate measurements for each treatment. Representative experiments are shown.*

and in apoptosis (Table I; 2.5- to 4.3-fold untreated controls). Table I shows that it is possible to titer Ad-p53 and Ad-FRNK so they cause no apoptosis (Table I, exp.1) or modest apoptosis (Table I, exp. 2) relative to Ad-Empty. However, when the viruses were combined, the amount of apoptosis appeared to be synergistic (exp. 1) or additive (exp. 2). This confirmed the hypothesis that overexpression of p53 in concert with inhibition of FAK would render cells more susceptible to death than either treatment alone.

As seen in Figure 2, RPMI 2650 cells, infected with Ad-FRNK (m.o.i=10) and/ or Ad-p53 (m.o.i=5), produced substantial amounts of FRNK and p53, respectively. Autophosphorylation of FAK on tyrosine 397 is a measure of its activity (1). As such, the RPMI 2650 cells contain active FAK as evidenced by the pY397 FAK immunoreactivity (Figure 2). This immunoreactivity decreased upon infection of cells with Ad-FRNK and Ad-FRNK/Ad-p53, indicating reduced FAK activity under these conditions (Figure 2). The reduction in FAK activity coincided with ectopic FRNK expression and a decrease in the immunoreactivity of endogenous full-length FAK in cells co-infected with Ad-FRNK and Ad-p53. The decrease in full-length FAK expression was not the result of electrophoresing unequal amounts of protein, as evidenced by the equivalent amounts of endogenous β-actin in the various cell extracts, nor was it the result of reduced expression of FAK mRNA (Figure 3). Therefore, co-infection of RPMI 2650 cells with Ad-FRNK and Ad-p53 may cause degradation of endogenous FAK protein.

Although anticancer drugs with varying mechanisms of action (etoposide, 5-fluorouracil and paclitaxel) caused cell death in a concentration-dependent manner, the RPMI 2650 cells were insensitive to cisplatin over a wide concentration range (1-100 μM) (Figure 4A). Infection of cells with Ad-p53 alone or Ad-FRNK alone augmented the cytotoxic effect of etoposide, paclitaxel and 5-fluorouracil, but not of cisplatin (Figure 4). Co-infection of cells with Ad-FRNK and Ad-p53 greatly potentiated the cytotoxicity of etoposide, paclitaxel and 5-fluoruracil with substantial amounts of cell killing occurring at the highest concentrations of the drugs (Figure 4). Interestingly, exposure to 100 μM cisplatin rendered the cells less sensitive to the cytotoxic effect of Ad-FRNK plus Ad-p53. It is possible that high concentrations of cisplatin compete with p53 for its DNA-binding site.

Table I. *Effect of Ad-FRNK and Ad-p53 on apoptosis. RPMI 2650 cells were incubated with the indicated concentration of virus for 48 h. Apoptosis was then determined using the Hoechst assay as described in Materials and Methods. The mean±standard error is shown for each treatment.*

	Experiment 1	Experiment 2
Ad-Empty (m.o.i=15)	4.3±0.5	2.5±0.3
Ad-p53 (m.o.i=5)	2.7±0.8	7.3±1.9**
Ad-FRNK (m.o.i=10)	2.7±0.8	4.9±0.2
Ad-p53 (m.o.i=5) and Ad-FRNK (m.o.i=10)	9.4±1.8*	12.4±0.8***

Less than 1% of untreated cells were apoptotic.
*Significantly different from Ad-Empty, Ad-FRNK, Ad-p53.
**Significantly different from Ad-Empty.
***Significantly different from Ad-Empty, Ad-FRNK, Ad-p53.

Discussion

Resistance to chemotherapeutic agents is a significant clinical problem in the treatment of SCCHN. This is, in part, caused by defects in drug transport, but clearly this is not the sole

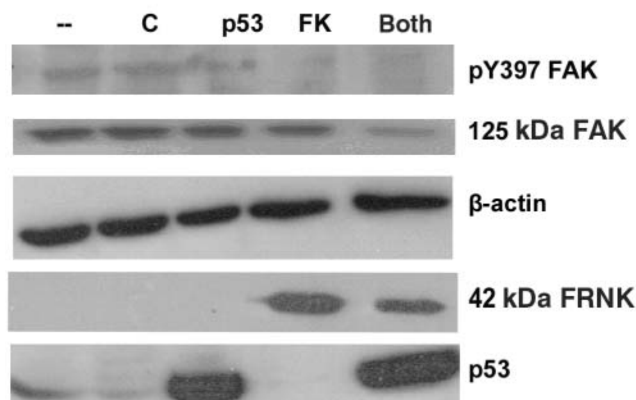


Figure 2. *Effect of Ad-FRNK and Ad-p53 on protein expression. RPMI 2650 cells (1x10^6) were incubated with nothing (--), Ad-Empty (C, m.o.i=15), Ad-p53 (p53, m.o.i=5), Ad-FRNK (FK, m.o.i=10), or Ad-p53 (m.o.i=5) + Ad-FRNK (m.o.i=10) (both) for 48 h. The detached cells were collected, washed and then pooled with the attached cells that had been lysed on the plates. Equal amounts of protein were electrophoresed in replicates on a 10% polyacrylamide gel. The proteins were transferred to PVDF and the indicated antigens were detected with antibodies, as per the manufacturers' instructions. The pY397 FAK blot (pY397 FAK) was stripped and then reprobed for endogenous 125 kDa FAK using an antibody directed against the amino-terminal domain of FAK. FRNK (42 kDa) was detected with an antibody directed against the carboxy-terminal portion of FAK. This antibody reacts with FRNK, which is contained in the carboxy-terminal portion of FAK.*

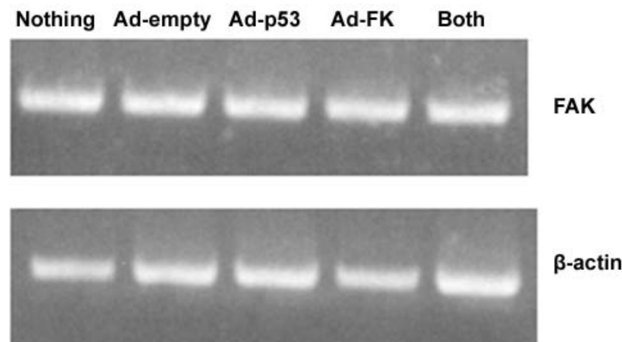


Figure 3. *Effect of Ad-FRNK and Ad-p53 on FAK expression. RPMI 2650 cells (1x10^6) were incubated with nothing, Ad-Empty (m.o.i=15), Ad-p53 (m.o.i=5), Ad-FRNK (m.o.i=10), or Ad-p53 (m.o.i=5) + Ad-FRNK (m.o.i=10) (both) for 48 h. Total RNA was prepared using Trizol and 2 μg of this were reverse transcribed. FAK and β-actin were amplified and detected as described in Materials and Methods. A photograph of an ethidium bromide-stained gel is shown.*

mechanism of drug resistance. Indeed, cancer cells have multiple abnormalities in the pathways regulating cell survival and apoptosis. Thus, one strategy for the development of new anticancer therapies involves using gene therapy to normalize the apoptotic response to cytotoxic agents.

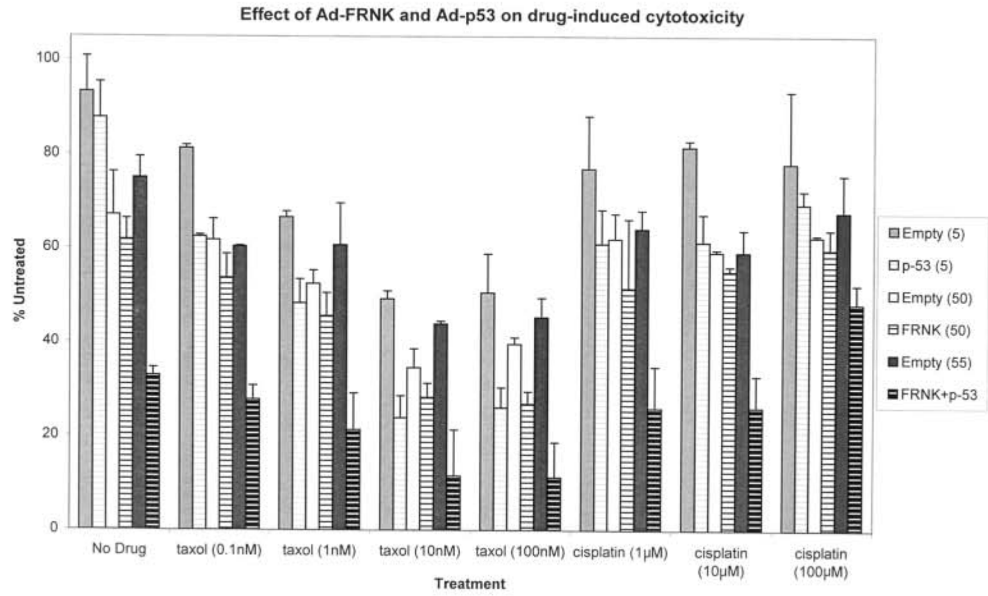
FAK is an intracellular tyrosine kinase, which is overexpressed in a variety of human tumors of epithelial origin (1-9). FAK regulates the cell cycle, survival, migration, and invasion *in vitro* and metastasis *in vivo*, so it appears to be an ideal target for cancer therapy (1, 10, 11, 20). Specific, small molecule inhibitors of FAK are not currently available, so investigators rely on genetic means, such as antisense oligonucleotides (21), siRNA (22) and dominant-negative constructs such as FRNK (15, 19, 23) to inhibit the expression or activity of FAK. Studies in cultured cells show that FAK inhibitors, themselves, induce cellular apoptosis under some conditions (19, 21). Inhibitors of FAK can also increase the action of cytotoxic agents (24). Indeed, we previously constructed HEK 293 cells, which inducibly overexpressed FRNK, thereby augmenting the cytotoxic effect of 5-fluorouracil (23). Moreover, when SCCHN cells were infected with Ad-FRNK, the cytotoxicity of anticancer drugs was enhanced by 20-30% (15), suggesting that multiple signaling pathways should be targeted for maximal drug-mediated cytotoxicity.

Several studies indicated that FAK mediates cell survival through p53-dependent and -independent pathways (1, 13), suggesting that co-expression of FRNK and wild-type p53 could improve the response of cultured SCCHN cells to cytotoxic agents. The results described herein show that infection of RPMI 2650 cells with either Ad-FRNK alone or Ad-p53 alone improved the response to cytotoxic drugs. The results obtained with Ad-p53 are consistent with many previously described studies showing that Ad-p53 infection of transformed cells potentiated the cytotoxicity of anticancer drugs regardless of whether the cells had endogenous wild-type or mutant p53 (12), whereas the results obtained with Ad-FRNK are consistent with the previously described work by our group (15) and others (22, 25). The novel finding of

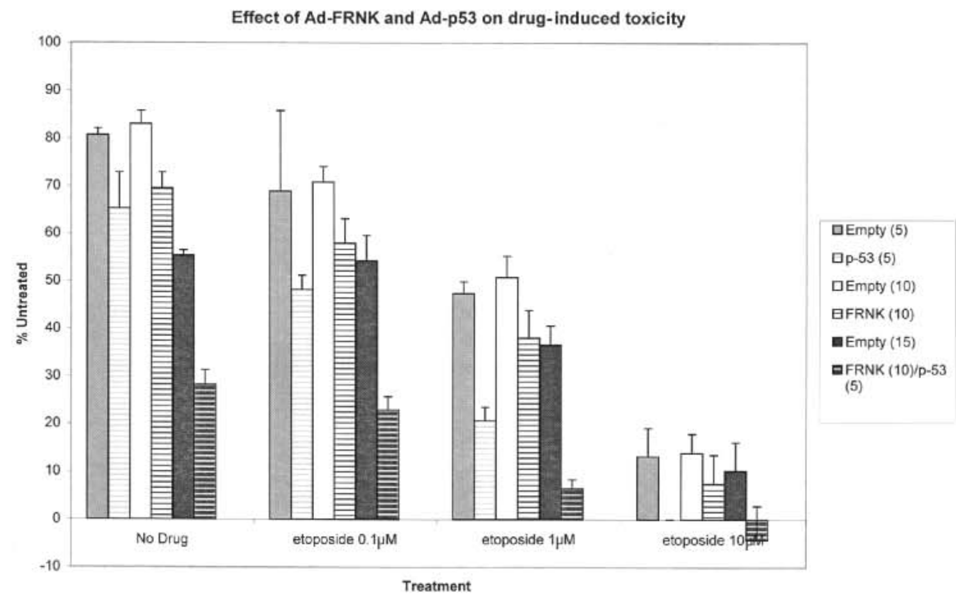
→

Figure 4. *Effect of Ad-FRNK and Ad-p53 on drug-induced cytotoxicity. Cells were trypsinized, replated in 96-well plates (2x10^4 per well), and then incubated overnight. The indicated amounts of drugs (50 μL) and viruses (50 μL) were added to triplicate wells and the plates were incubated for 72 hours prior to performing the MTT assay as described in the Materials and Methods. The mean±standard error was calculated for each treatment and percent control viability relative to untreated cells (no drug, no virus) was determined. The control consisted of Ad-empty, which was used in the same concentration as Ad-p53, Ad-FRNK, and Ad-p53 + Ad-FRNK. The numbers in the figure key refer to the multiplicity of infection (m.o.i) of virus. A) Paclitaxel (taxol) and cisplatin. At all drug concentrations, cells treated with Ad-FRNK and Ad-p53 were significantly less viable than cells subjected to any other treatment. B) Etoposide. At all drug concentrations, cells treated with Ad-FRNK and Ad-p53 were significantly less viable than cells subjected to any other treatment. C) 5-Fluorouracil. Cells treated with Ad-p53 and Ad-FRNK were significantly less viable than any other treatment except at 0 μg/ml 5-fluorouracil (5-FU).*

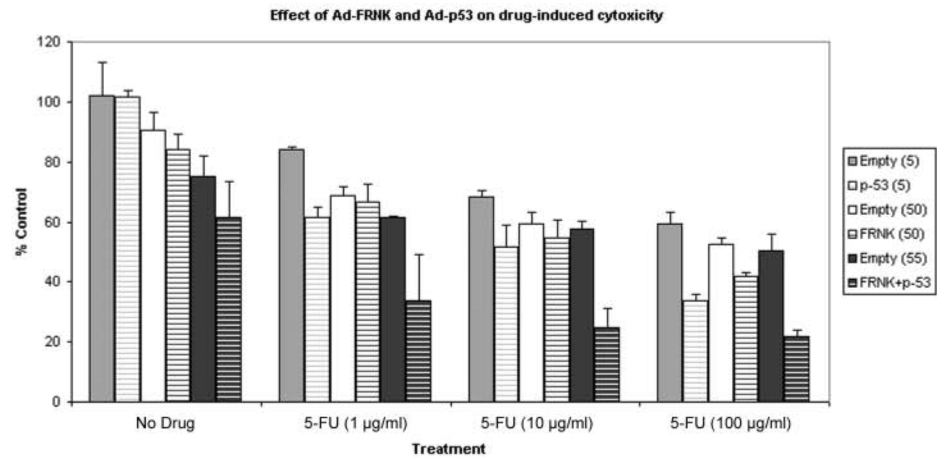
A



B



C



this work, however, is the demonstration that adenovirus-mediated FRNK gene transfer cooperates with Ad-p53 to augment drug-induced death of a SCCHN cell line. RPMI 2650 cells, which were co-infected with Ad-FRNK and Ad-p53, showed considerably more cell death in the presence of cytotoxic drugs than cells transfected with either virus alone or equivalent amounts of control virus (Figure 4).

In this work, three distinct SCCHN cell lines (RPMI 2650, SCC25, FaDu) were tested. However, under the described experimental conditions, the cooperation between Ad-FRNK and Ad-p53 was only observed in RPMI 2650 cells. The cause of this disappointing result is not known, but it is possible that the appropriate experimental conditions have yet to be found. Indeed, preliminary data show that Ad-FRNK and Ad-p53 together inhibit the cloning efficiency of cells (RPMI 2650, FaDu, SCC25) relative to cells treated with Ad-p53 alone and Ad-FRNK alone. It is also possible that SCC25 and FaDu cells contain abnormalities downstream of FAK and/ or p53, which interfere with the apoptotic response. Curiously, SCC25 and FaDu cells have mutant p53 (26), whereas RPMI 2650 cells contain functional p53 (27), so the situation is more complicated than merely restoring wild-type p53 to deficient cells.

The mechanism of the cooperative response between Ad-FRNK and Ad-p53 in RPMI 2650 cells is not known. FAK can mediate cell survival through ERK, AKT and JUN (1), but changes in phosphorylation of these proteins were not observed on Western blots (data not shown). It is possible that changes in the phosphorylation state of these molecules exist, but were undetectable under the described experimental conditions. Interestingly, FAK was lost from cells which were co-transfected with Ad-FRNK and Ad-p53. Because FAK can reduce the transcriptional activity of p53 (28), this suggests a mechanism whereby Ad-FRNK may cooperate with Ad-53 to increase apoptosis.

Conclusion

Adenovirus-mediated gene transfer of FRNK cooperates with Ad-p53 to augment drug-induced death under some circumstances. Additional work is needed to discern the mechanism of this interaction.

Acknowledgements

I would like to acknowledge the technical support of Justin Jacobs, BS. Lori Kornberg is supported by the Flight Attendant Medical Research Institute, the Grader Foundation and the Andrew J. Semesco Foundation, U.S.A.

References

1 Gabarra-Nieko V, Schaller MD and Dunty JM: FAK regulates biological processes important for the pathogenesis of cancer. Cancer Metastasis Rev *22*: 359-374, 2003.
2 Kornberg LJ: Focal adhesion kinase expression in oral cancers. Head Neck *20*: 634-639, 1998.
3 Aronsohn MS, Brown HM, Hauptman G and Kornberg LJ: Expression of focal adhesion kinase and phosphorylated focal adhesion kinase in squamous cell carcinoma of the larynx. Laryngoscope *113*: 1944-1948, 2003.
4 Agochiva M, Brunton VG, Owens DW, Parkinson EK, Paraskeva C, Keith WN and Frame MC: Increased dosage and amplification of the focal adhesion kinase gene in human cancer cells. Oncogene *18*: 5646-5653, 1999.
5 Owens LV, Xu LH, Craven RJ, Dent GA, Weiner TM, Kornberg LJ, Lui ET and Cance WG: Overexpression of the focal adhesion kinase (p125fak) in invasive human tumors. Cancer Res *55*: 2752-2755, 1995.
6 Cance WG, Harris JE, Iacocca MV, Roche E, Yang X, Chang J, Simpkins S and Xu L: Immunohistochemical analyses of focal adhesion kinase expression in benign and malignant human breast and colon tissues: correlation with preinvasive and invasive phenotypes. Clin Cancer Res *6*: 2417-2423, 2000.
7 Judson PL, He X, Cance WG and Van LEL: Overexpression of focal adhesion kinase, a protein tyrosine kinase, in ovarian carcinoma. Cancer *86*: 1551-1556, 1999.
8 Tremblay L, Hauck W, Aprikian AG, Begin LR, Chapelaine A and Chevalier S: Focal adhesion kinase (pp125FAK) expression, activation and association with paxillin and p50csk in human metastatic prostate carcinoma. Int J Cancer *68*: 164-171, 1996.
9 Hecker TP, Grammer JR, Gillespie GY and Stewert J Jr: Focal adhesion kinase enhances signaling through the Shc/ extracellular signal-regulated kinase pathway in anaplastic astrocytoma tumor biopsy samples.Cancer Res *62*: 2699-2707, 2002.
10 McLean GW, Korniyarna NH, Serrels B, Asano H, Reynolds L, Conti F, Hodala-Dilke K, Metzger D, Chambon P, Grant SG and Frame MC: Specific deletion of focal adhesion kinase suppresses tumor formation and blocks malignant progression.Genes Dev *18*: 2998-3003, 2004.
11 van Nimwegen MJ, Verkoeijen S, van Buran L, Burg D and van de Water B: Requirement for focal adhesion kinase in the early phase of mammary adenocarcinoma lung metastasis formation. Cancer Res *65*: 4698-4706, 2005.
12 Horowitz J: Adenovirus-mediated p53 gene therapy: overview of preclinical studies and potential clinical applications. Curr Opin Mol Ther *1*: 500-509, 1999.
13 Ilic D, Almeida EAC, Schlaepfer DD, Dazin P, Aizawa S and Damsky CH: Extracellular matrix survival signals transduced by focal adhesion kinase suppress p53-mediated apoptosis. J Cell Biol *143*: 547-560, 1998.
14 Zhang WW, Fang X, Mazur W, French BA, Georges RN and Roth JA: High-efficiency gene transfer and high-level expression of wild-type p53 in human lung cancer cells mediated by recombinant adenovirus Cancer Gene Therapy *1*: 5-13, 1994.
15 Kornberg LJ: Adenovirus-mediated transfer of FRNK augments drug-induced cytotoxicity in cultured SCCHN cells. Anticancer Res *25*: 4349-4356, 2005.
16 Allen S, Sotos J, Sylte MJ and Czuprynski CJ: Use of Hoechst 33342 staining to detect apoptotic changes in bovine mononuclear phagocytes infected with mycobacterium avium subsp. Paratuberculosi. Clin Diagn Lab Immunol *8*: 460-464, 2001.

17 Schaller MD, Borgman CA and Parsons JT: Autonomous expression of a non-catalytic domain of focal adhesion-associated protein tyrosine kinase pp125FAK. Molec Cell Biol *13*: 785-791, 1993.
18 Richardson A and Parsons JT: A mechanism for regulation of the adhesion-associated protein tyrosine kinase pp125FAK. Nature *380*: 538-540, 1996.
19 Xu LH, Yang X, Craven RJ and Cance WG: The COOH-terminal domain of the focal adhesion kinase induces loss of adhesion and cell death in human tumor cells. Cell Growth Diff *9*: 999-10005, 1998.
20 Zhao JH, Reiske H and Guan JL: Regulation of the cell cycle by focal adhesion kinase. J Cell Biol *143*: 1997-2008, 1998.
21 Xu LH, Owens LV, Sturge GC, Yaang X, Liu ET, Craven RJ and Cance WG: Attenuation of the expression of the focal adhesion kinase induces apoptosis in tumor cells. Cell Growth Differ *7*: 413-418, 1996.
22 Duxbury MS, Ito H, Benoit E, Zinner MJ, Ashley SW and Whang EE: RNA interference targeting focal adhesion kinase enhances pancreatic adenocarcinoma gemcitabine chemosensitivity. Biochem Biophys Res Commun *311*: 786-792, 2003.
23 Kornberg LJ and Fleigel J: The effects of inducible overexpression of FAK-related non-kinase (FRNK) on a transformed epithelial cell line. Anticancer Res *23*: 91-97, 2003.
24 Van Water B, Houtepen F, Huigsloot M *et al*: Suppression of chemically induced apoptosis but not necrosis of renal proximal tubular epithelial (LLC-PK1) cells by focal adhesion kinase (FAK). J Biol Chem *276*: 36183-36193, 2001.
25 Satoh TH, Surmacz TA, Nyormoi O and Whitacre CM: Inhibition of focal adhesion kinase by antisense oligonucleotides enhances the sensitivity of breast cancer cells to camptothecins. Biocell *27*: 47-55, 2003.
26 Min BM,. Baek JH, Shin KH, Gujuluva CN, Cherrick HM and Park NH: Inactivation of the p53 gene by either mutation or HPV infection is extremely frequent in human oral squamous cell carcinoma cell lines. Eur J Cancer B Oral Oncol *5*: 338-345, 1994.
27 Heiliez C, Baricault L, Barboule N and Valett A: Paclitaxel increases p21 synthesis and accumulation of its AKT-phosphorylated form in the cytoplasm of cancer cells. Oncogene *22*: 3260-3268, 2003.
28 Golubovskaya VM, Finch R and Cance WG: Direct interaction of the N-terminal domain of focal adhesion kinase with the N-terminal transactivation domain of p53. J Biol Chem *280*: 25008-25021, 2005.

Received April 17, 2006
Accepted May 30, 2006