Fibroblast Growth Factor-2 Induces Translational Regulation of Bcl-X<sub>L</sub> and Bcl-2 via a MEK-dependent Pathway

CORRELATION WITH RESISTANCE TO ETOPOSIDE-INDUCED APOPTOSIS

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The involvement of fibroblast growth factor-2 (FGF-2) in the biology of small cell lung cancer (SCLC) has not previously been investigated. Here we report that FGF-2 prevented etoposide-induced apoptosis in H-510 SCLC cells. Phosphatidylinositol 3-kinase/protein kinase B signaling did not mediate this effect because FGF-2 failed to activate phosphatidylinositol 3-kinase or protein kinase B. In contrast, the mitogen-activated extra-cellularly regulated kinase kinase (MEK) was crucial for this response because its inhibition abolished the pro-survival properties of FGF-2. Moreover, in H-69 SCLC cells, the failure of FGF-2 to prevent etoposide-induced apoptosis correlated with uncoupling from MEK signaling. However, the introduction of an activating MEK mutant rendered these cells resistant to etoposide-induced cell death. Rescue relied on de novo protein synthesis of anti-apoptotic proteins Bcl-X<sub>L</sub> and Bcl-2, and FGF-2 signaling, in a MEK-dependent fashion. Contrary to previous studies, up-regulation occurred at the translational rather than transcriptional level. Indeed, actinomycin D failed to prevent up-regulation of Bcl-X<sub>L</sub> and Bcl-2, and FGF-2 induced Bad by etoposide was also blocked by MEK kinase inhibitors. This MEK-dependent pathway. Thus, the MEK/protein kinase B signaling is critical for the coordinate modulation of both pro- and anti-apoptotic Bcl-2 family members by FGF-2.

The development of tumor cell resistance to chemotherapy is the most frequent reason for failing to cure patients with common cancers such as small cell lung cancer (SCLC). Consequently, it is of central importance to elucidate the molecular mechanisms involved in drug resistance. Previous preclinical studies have implicated the overexpression of efflux proteins as a major mechanism of chemotherapy-induced resistance (1). However, subsequent work using inhibitors of efflux proteins failed to demonstrate increased effectiveness of chemotherapy in patients (2). This suggested the presence of alternative chemotherapy resistance mechanisms. Other proteins involved in the ability of tumor cells to survive elevated in tumors. In particular, low levels of the pro-apoptotic protein Bak have been shown to correlate with resistance of SCLC tumors to chemotherapy (3). Bak has now been identified in B-cell lymphoma cell lines and is a growing family of death regulatory proteins that may be either death antagonists (e.g. Bax, Bad) or death agonists (e.g. Bak, Bim) (4). The balance between these two groups of proteins has been reported to partly determine cell survival (5). Overexpression of Bcl-2 (6) or Bcl-X<sub>L</sub> (7) prevents drug-induced apoptosis, whereas overexpression of Bcl-X<sub>L</sub> (8) induces cell death.

FGF-2 is a multifunctional cytokine involved in many biological processes including proliferation, differentiation, migration, neoangiogenesis, and cell survival (10–12). Recent work has shown that fibroblast growth factors (FGFs), including FGF-2, can promote resistance to multiple chemotherapeutic agents both in vitro and in vivo (12). This is of interest, because FGF-2 is frequently elevated in the serum of patients with various malignancies (13, 14). However, the molecular mechanisms by which FGF-2 promotes drug resistance in cancer cells have not been fully elucidated (15). FGF-2 is known to bind and activate specific split tyrosine kinase receptors (FGFRs), which couple to multiple intracellular signaling pathways (16, 17). The activation of tyrosine kinase receptors by other growth factors has been involved in cell survival through downstream signaling cascades such as the mitogen-activated Erk kinase (MEK)/extracellular regulated kinase (Erk) and the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB) pathways. These signals influence survival through several mechanisms including the regulation of Bcl-2 and its family members (15, 18–20). This regulation may be rapid or delayed as a consequence of phosphorylation (21) or increased transcription (18), respectively. However, translational regulation of Bcl-2 family members in response to growth factors has not been described.

In the present study we show that FGF-2 prevents apoptosis induced by the chemotherapeutic agent etoposide in SCLC cells. This effect occurs via a MEK-dependent and PI3K/PKB-independent pathway. We show that FGF-2 signaling through MEK increases the levels of the anti-apoptotic members of the Bcl-2 family, Bcl-2 and Bcl-X<sub>L</sub>, and blocks etoposide-mediated induction of the pro-apoptotic protein Bad. These effects were
selective because Bax protein expression was unchanged. Surprisingly, the increase in Bcl-X<sub>l</sub> and Bcl-2 was not due to increased mRNA transcription but instead resulted from enhanced translation. This represents a new mechanism by which growth factor activation of MEK increases cell survival and resistance to chemotherapy.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—The H-510 and H-69 SCLC cell lines were maintained as previously described (22). For experimental purposes, the cells were grown in serum-free medium (SMFM, RPMI 1640 supplemented with 5 ng/ml transferrin, 100 ng/ml insulin, 10 mM sodium selenite, 0.25% bovine serum albumin) and used after 3–7 days.

**Cell Death Assay**—H-510 cells (5 × 10<sup>4</sup> cells/ml SFM) were pretreated with or without various inhibitors: 25 μM PD98059, 20 μM U0126 (New England Biolabs), 10 ng/ml rapamycin, 10 μM LY294002 (Calbiochem), or 100 μM cycloheximide (Sigma). The cells were then stimulated with or without 0.1 ng/ml FGF-2 (Amersham Biosciences) for 4 h prior to treatment with 0.1 μM etoposide (Sigma) and incubated at 37°C for 96 h. A single cell suspension was generated by passing the samples four times through a 19-G needle, and live cell number was determined using trypan blue exclusion.

**Annexin V/Propidium Iodide Flow Cytometry Analysis**—H-510 cells (2 × 10<sup>5</sup> cells) were incubated with or without 0.1 ng/ml FGF-2 for 4 h prior to addition of 0.1 μM etoposide for 4 h. The cells were then resuspended at 1 × 10<sup>6</sup> cells/ml binding buffer (10 mM HEPES, pH 7.4, 140 mM NaCl, 2.5 mM CaCl<sub>2</sub>). 100 μl of this suspension were mixed with 5 μl of fluorescein isothiocyanate-conjugated annexin V reagent ( Molecular Probes) and 10 μl of propidium iodide solution ( Molecular Probes). The samples were left for 15 min at room temperature in the dark before the addition of a further 400 μl of binding buffer. The samples were analyzed by flow cytometry using a Becton Dickinson FACS Vantage S.E. turbo.

**Caspase 3 Activity Assay**—H-510 cells (2 × 10<sup>5</sup> cells) were incubated with or without 0.1 ng/ml FGF-2 for 4 h, resuspended in 1 ml of 0.1 μM etoposide for 10 h. The cells were homogenized in 1 ml of 10 mM Tris, pH 7.5, 10 mM NaCl, 1% Triton X-100, 10 mM NaF, and 10 mM NaCl, 1% Triton X-100, 10 mM NaF, respectively. The lystate s were centrifuged at 4°C for 10 min at 300 g. The supernatants were mixed with 200 μl of 0.5% sodium Deoxycholate (0.1% CHAPS, 10% glycerol, 10 mM ethylenediamine tetraacetic acid (EDTA), 10 mM sodium fluoride, 10% (v/v) glycerol, 1 mM phenylmethylsulfonyl fluoride, 20 μM leupeptin, 18 μM pepstatin, 20 μM aprotinin, 1 μM dithiothreitol, 2 mM Na<sub>3</sub>VO<sub>4</sub> and 10 μM sodium fluoride). Immunoprecipitation was performed for 2 h using a murine antibody or an irrelevant mouse IgG. Protein G-Sepharose CL-4B (Amersham Biosciences) was added at 37°C for 30 min and incubated at 37°C for 90 min. The immune complexes were collected, washed twice in lysis buffer and three times in kinase buffer (20 mM Tris, 10 mM MgCl<sub>2</sub>, 1 mM dithiothreitol, 10 μM poly[ADP-ribose] polymerase, Lamin B, and resistance to chemotherapy.

**Ribosomal S6 Kinase (S6K) Immunocomplex Kinase Assay**—SCLC cells grown in SFM were washed three times in RPMI 1640, and 2 × 10<sup>6</sup> cell aliquots were incubated in this medium for 30 min at 37°C. The cells were then stimulated with FGF-2 for 10 min and lysed at 4°C in 1 ml of lysis buffer. Immunoprecipitation was performed for 2 h using a polyclonal anti-S6K1 antibody (Santa Cruz). Protein A-agarose beads (40 μl, 1:1 slurry) were added for a further 1 h. Immune complexes were washed twice in lysis buffer and three times in kinase buffer (20 mM Tris, 10 mM MgCl<sub>2</sub>, 1 mM dithiothreitol, 10 μM poly[ADP-ribose] polymerase). The kinase reaction was initiated by resuspending the beads in 25 μl of kinase buffer supplemented with 100 μM ATP, 100 μCi/ml [γ<sup>32</sup>P]ATP, 200 μM microcystin LR, and 1 mg/ml S6 peptide (RRRLSSLRA). The reactions were incubated at 30°C for 10 min (linear assay conditions) and terminated on ice by the addition of 10 μl of ice-cold 0.5% orthophosphoric acid. The samples were centrifuged (15,000 × g for 30 s), and 30 μl of the supernatant was spotted onto P81 chromatography paper (Whatman). The filters were washed four times with 2 ml of 50% ethanol and dried. Radioactivity of two blank samples containing beads (40 μl, 1:1 slurry) but no immune complex was used as a result of each sample. The specific activity of 5 × 10<sup>4</sup>–1200 cpm/mmol.

**mRNA Quantification by Real Time Quantitative PCR**—H-510 cells (4 × 10<sup>5</sup> cells/ml SFM) were incubated for 4 h with or without 0.1 ng/ml FGF-2. The total mRNA was extracted with RNAzol (Biogenesis), and reverse transcription was performed using the avian myeloblastosis virus first strand reverse transcription-PCR kit (Roche Molecular Biochemicals). Equal amounts of cDNA were then introduced in a TaqMan real time quantitative PCR. For Bcl-X<sub>l</sub>, primers with the sequences 5'-TCTTTGTTCACTGCTTTCCAG-3' and 5'-GGTCGAGATTGCTGCCTTT-5' were used together with a 5'-ACAGTGCCCAGGCGGAAGA-3' and 5'-ACAGTGCCCAGGCGGAAGA-3' Taqman probe. The probes were labeled at the 5' end with the reporter 6-carboxy-fluorescein and at the 3' end with the quencher molecule 6-carboxy-tetramethyl-rhodamine. Real time PCR amplification was performed according to the TaqMan Universal PCR Master Mix protocol. Relative quantification of gene expression was obtained as described in the manual using glyceraldehyde-3-phosphate dehydrogenase mRNA as an internal standard.
Etoposide (Eto) FGF-2. Etoposide (Eto) was then added, and cell survival was determined after 96 h. The results shown are the means ± S.E. of three independent experiments performed in quadruplicate. B, H-510 cells were incubated with or without FGF-2 or fetal bovine serum (FBS). PI3K activity was assessed in vitro on anti-p85α immunoprecipitates (upper panel). The amounts of p85α immunoprecipitated per condition were equivalent as shown by Western blot (lower panel). C, COS-1 and H-510 cells were treated with or without 100 ng/ml EGF or 0.1 ng/ml FGF-2, respectively. The levels of total (upper panel) or phospho-Ser-473-PKB (lower panel) were determined by Western blot.

PI3K/PKB Signaling Does Not Mediate the Prosurvival Activity of FGF-2—PI3K/PKB signaling plays a key role in survival induced by other growth factors in many cell systems (30–32). Moreover, in H-510 cells, we have found that S6K, which frequently lies downstream of PI3K, is activated in response to FGF-2 (see Fig. 4B). Therefore, we examined the effect of the PI3K inhibitor LY294002 on the ability of FGF-2 to prevent apoptosis in H-510 cells. When used at concentrations previously shown to block PI3K activity in SCLC cells (30), the addition of LY294002 alone resulted in reduced cell survival to an extent similar to that seen with etoposide (Fig. 2A). Nevertheless, LY294002 did not prevent FGF-2 induced rescue of etoposide killing. Similarly, inactivation of S6K with rapamycin (22) also failed to prevent FGF-2 induced rescue (Fig. 2A). Further evidence that PI3K was not involved in FGF-2-induced resistance to etoposide was provided by the failure of FGF-2 to stimulate PI3K in anti-p85α immunoprecipitates, although p85α was present, and PI3K activity could be induced by serum in H-510 SCLC cells (Fig. 2B). FGF-2 similarly failed to induce PI3K activity in anti-phosphotyrosine immunoprecipitates (data not shown). The activation of PKB relies on the phosphorylation of Thr-308 and Ser-473, and phospho-specific antibodies to the latter site have frequently been used as a simple and sensitive readout for any changes in PI3K activity. Strikingly,
we were unable to detect Ser-473 phosphorylation in response to FGF-2 stimulation. The levels of biphospho-Erk1/2 were determined by Western blot. Immunodetection of Lamin B was used as a control for protein loading. B, H-510 cells were pretreated with or without PD098059 and incubated in the absence or presence of FGF-2, as indicated, for 4 h. Etoposide (Eto) was then added, and cell survival was determined 96 h later. The results shown are the means ± S.E. of three independent experiments. Each condition was performed in quadruplicate.

FIG. 3. The MEK/Erk pathway mediates the anti-apoptotic effect of FGF-2 in H-510 cells. A, H-510 cells were treated with increasing concentrations of PD098059 prior to FGF-2 stimulation. The levels of biphospho-Erk1/2 were determined by Western blot. Stimulation with 400 nM phorbol 12,13-dibutyrate (PDB) was used as a positive control. Immunodetection of Lamin B was used as a control for protein loading. B, H-510 cells were stimulated with FGF-2 (1 ng/ml) for 10 min, and S6K was immunoprecipitated prior to immune complex kinase assay. The MEK/Erk Pathway Mediates the Anti-apoptotic Effect of FGF-2—Increasing evidence implicates the MEK/Erk pathway as an alternative mechanism capable of providing survival signals (15, 18, 19, 21, 34). PD098059 is a well-documented MEK1/2 inhibitor that has been extensively used to inhibit various biological processes (35). Dose response experiments indicated that addition of 25 μM of PD098059 was sufficient to maximally inhibit FGF-2-induced activation of Erk1/2 in H-510 cells (Fig. 3A). At this concentration, PD098059 induced only a modest reduction in SCLC cell survival. However, this inhibitor completely prevented FGF-2-induced rescue of H-510 cells from etoposide killing (Fig. 3B). To substantiate the notion that MEK could mediate FGF-2-induced resistance to etoposide, we performed similar experiments in the H-69 SCLC cell line. In these cells, FGF-2 failed to activate MEK/Erk signaling at all concentrations and times tested (Fig. 4A). This was not due to a dysfunctional MEK/Erk pathway because Erk phosphorylation could be induced using phorbol esters (Fig. 4A). Moreover, as in H-510 cells, FGF-2 was still able to induce other signaling events such as S6K activation (Fig. 4B). Nevertheless, the addition of FGF-2 failed to rescue etoposide-induced cell death in H-69 cells (Fig. 4C). Furthermore, introduction of an activated version of MEK into H-69 cells induced phosphorylation of Erk1/2 and complete rescue from etoposide killing (see Fig. 6). Taken together, these results indicate that MEK/Erk signaling is the principal mediator of FGF-2-induced rescue from etoposide killing in SCLC cells.

De Novo Protein Synthesis Is Required for FGF-2-mediated Rescue; Coordinate and Selective Regulation of Bcl-2 Family Members through a MEK-dependent Pathway—Time course studies demonstrated that preincubation periods shorter than 3 h with FGF-2 were insufficient to rescue H-69 cells from etoposide killing. In contrast, the activation of Erk1/2 in response to FGF-2 was rapid and transient (peak, 5 min; control levels, 10 min) in these cells (data not shown). This discrepancy between transient Erk1/2 activation and the prolonged preincubation with FGF-2 necessary to prevent etoposide killing suggested the need for protein synthesis downstream of the MEK/Erk and/or additional pathways. We therefore tested whether the protein synthesis inhibitor cycloheximide could block FGF-2-mediated resistance to etoposide. We initially determined that 100 μM cycloheximide was the minimum concentration required to inhibit protein synthesis by >99% in [35S]Met/Cys-labeled H-510 cells (data not shown). At this concentration, cycloheximide prevented FGF-2-stimulated rescue
of etoposide-treated H-510 cells (Fig. 5A). Thus, de novo protein synthesis is required for FGF-2 to induce resistance to etoposide killing in H-510 SCLC cells.

We next wished to identify proteins induced by FGF-2 that might mediate resistance to etoposide killing of H-510 SCLC cells. P-glycoprotein is an efflux protein known to regulate the in vitro sensitivity of many cancer cell lines to chemotherapeutic drugs (1). However, no increase was seen in either mRNA and protein expression or pump activity of P-glycoprotein in FGF-2-stimulated H-510 cells (data not shown).

The Bcl-2 protein family also controls the sensitivity of cancer cells to chemotherapeutic agents (3). Thus, the anti-apoptotic members, including Bcl-XL and Bcl-2, induce resistance, whereas the pro-apoptotic Bcl-2 members such as Bad and Bax enhance sensitivity. Fig. 5B demonstrates that a 4-h treatment with FGF-2 increased Bcl-XL and Bcl-2 protein expression by 3.6- and 2.8-fold (means ± S.E., n = 8), respectively. This effect was blocked by PD098059, indicating that the MEK/Erk pathway mediates the increased expression of these proteins by FGF-2 (Fig. 5B). Similar results were obtained using the structurally distinct MEK inhibitor U0126 (Fig. 5C).

However, in H-69 cells, which do not activate MEK/Erk signaling in response to FGF-2, no increase in Bcl-XL or Bcl-2 proteins was observed (Fig. 5C). Nevertheless, introduction of an activated version of MEK into H-69 cells induced an increase in Bcl-XL and Bcl-2 protein expression (Fig. 6A). Collectively, these results demonstrate that FGF-2 induces enhanced expression of the anti-apoptotic proteins Bcl-XL and Bcl-2 via the MEK/Erk signaling pathway.

In contrast to Bcl-XL and Bcl-2, which were easily detected (Fig. 5, B and C), the pro-apoptotic protein Bad was only weakly expressed in untreated SCLC cells (Fig. 5D and data not shown). The addition of etoposide to H-510 cells resulted in a marked increase in expression of Bad. Strikingly, FGF-2 almost completely prevented the induction of Bad by etoposide in H-510 cells, an effect that could be blocked by MEK inhibition (Fig. 5D). However, in H-69 cells FGF-2 failed to block the induction of Bad by etoposide (data not shown). Intriguingly,
stability is unaffected by FGF-2. Nevertheless, higher levels of FGF-2 did not significantly increase the levels of Bcl-XL mRNA. Similarly, FGF-2 time quantitative PCR, and, as demonstrated in Fig. 7A, Bcl-XL mRNA levels. This hypothesis was tested using Taqman real translational mechanism. The increase in protein expression levels of another pro-apoptotic protein Bad was unaffected by FGF-2 or etoposide treatment (Fig. 5A and data not shown). Thus, FGF-2 selectively regulates some but not all Bcl-2 family members in a MEK/Erk-dependent fashion.

FGF-2 Increases Bcl-XL—and Bcl-2 expression by a translational mechanism. The increase in protein expression levels of Bcl-XL and Bcl-2 induced by FGF-2 could result from several mechanisms including an increase in protein stability. To assess this, pulse-chase experiments were performed in H-510 cells labeled with [35S]Met/Cys for 4 h in the absence or presence of FGF-2. In unstimulated cells, the t1/2 of Bcl-2 protein was 45 min, whereas the t1/2 of Bcl-XL was longer than the 4-h time course of the experiment (Fig. 7A). The addition of FGF-2 did not alter these t1/2 data. Thus, Bcl-XL and Bcl-2 protein stability is unaffected by FGF-2. Nevertheless, higher levels of [35S]Met/Cys-labeled Bcl-XL and Bcl-2 were observed after 4 h of exposure with FGF-2 as compared with unstimulated cells (data not shown), confirming that this growth factor increases the rate of synthesis of both proteins.

The stimulation of Bcl-XL and Bcl-2 protein synthesis by FGF-2 could be explained by an increase in the corresponding mRNA levels. This hypothesis was tested using Taqman real-time quantitative PCR, and, as demonstrated in Fig. 7B, FGF-2 did not significantly increase the levels of Bcl-XL mRNA. Similar results were seen for Bcl-2 (data not shown). However, this approach cannot exclude a <2-fold increase in mRNA levels for these proteins. Consequently, we utilized actinomycin D to block mRNA synthesis and examined whether FGF-2 could still up-regulate Bcl-XL and Bcl-2 proteins under these conditions. Strikingly, at actinomycin D concentrations that completely blocked de novo mRNA synthesis, FGF-2 was still able to induce a 3.4 ± 0.1- and 2.3 ± 0.2-fold (means ± S.E.; n = 3) increase in Bcl-XL and Bcl-2 protein levels, respectively (Fig. 7C and data not shown). Therefore, despite the ability of actinomycin D to partly block FGF-2-mediated Bcl-XL and Bcl-2 up-regulation, our results show that this induction is largely achieved through a translational mechanism.

DISCUSSION

The polypeptide growth factor FGF-2 has been shown to prevent cell death induced by several chemotherapeutic agents including paclitaxel, doxorubicin, and 5-fluorouracil in human prostate cancer cells and rat tumors (12). However, the mechanism by which this protection is achieved including the early signaling events and their interaction with the apoptotic machinery has not been previously elucidated.

Here we show that FGF-2, used at a concentration frequently found in the serum of patients with cancer (14), protects H-510 SCLC cells from apoptosis induced by etoposide. PI3K/PKB signaling is known to mediate survival by polypeptide growth factors (30–32), which involves the phosphorylation of the putative PKB substrate directly by PKB or the related kinase Akt (36, 37). Phosphorylation of PKB/Akt, preventing it from being rapidly degraded, is a common strategy used by growth factors to protect cells from death. In agreement with this, we found that etoposide was able to induce rapid phosphorylation of both PKB/Akt and BAD in H-510 cells, consistent with the ability of FGF-2 to partially protect cells from apoptosis induced by these agents (data not shown). However, we were able to show that the phosphorylation of BAD induced by FGF-2 was not followed by apoptosis in these cells, whereas etoposide treatment induced apoptosis in approximately 50% of the cells (data not shown). These results suggest that BAD phosphorylation is unlikely to play a critical role in FGF-2-mediated rescue of SCLC cells.

MEK signaling provides an alternative pathway by which growth factors prevent apoptosis (18, 39). Indeed, pharmacological inhibition of MEK blocked FGF-2-induced rescue from etoposide killing in H-510 cells. The involvement of MEK in this rescue was supported by results obtained in H-69 SCLC cells. In these cells FGF-2 did not prevent etoposide killing, and this correlated with failure to activate the MEK/Erk pathway. The latter effect was not a result of the absence of functional FGFRs or MEK/Erk, because FGF-2 induced the activation of FGFR1, FGFR2, and S6K, whereas phosphor esters stimulated MEK/Erk signaling, respectively. Furthermore, the expression levels of MEK and Erk1/2, as well as FGFRs 1 and 2, which principally mediate the actions of FGF-2, are comparable in the H-510 and H-69 SCLC cell lines.3 Taken together, our results point to a crucial role for MEK/Erk signaling in FGF-2-mediated resistance to etoposide. Moreover, confirmation of these observations was provided by the resistance of H-69 cells to etoposide killing upon overexpression of a constitutively activated form of MEK.

The MEK/Erk pathway has previously been implicated in cellular protection from various apoptotic signals (15, 18, 19, 21, 34). To date, only two mechanisms were reported to mediate this effect: (i) the phosphorylation of Bcl-2 family members and (ii) the transcriptional up-regulation of Bcl-XL and Bcl-2. The selective up-regulation of Bcl-2 or Bcl-XL has been shown to shift the balance between pro- and anti-apoptotic Bcl-2 family members toward increased cell survival and resistance to various cellular insults. Indeed, resistance of tumor cells to chemotherapy was found to correlate with cellular Bcl-2 levels (3). Here, we found that Bcl-XL and Bcl-2 were up-regulated in a

3 O. E. Pardo, A. Arcaro, G. Salerno, S. Raguz, J. Downward, and M. J. Seckl, unpublished observations.
MEK-dependent fashion in response to FGF-2 treatment. Surprisingly, this occurred via a novel mechanism involving the increased translation of both proteins.

The translational regulation of Bcl-2 family members is poorly understood. Recent work has demonstrated that Mcl-1 could be translationally regulated through a PI3K pathway (40). To our knowledge, there are no previous reports concerning the translational regulation of Bcl-X<sub>L</sub>, and there is only one report that has shown that a short sequence in the 5′-untranslated region of Bcl-2 mRNA can repress its translation (41). However, the upstream signaling pathway(s) that might regulate this site, or an equivalent site as yet unidentified in the 5′-untranslated regions of Bcl-X<sub>L</sub>, remains to be elucidated. It is tempting to speculate that there may be a common mechanism leading to the enhanced translation of these two proteins in response to FGF-2, because the process depends on a common signaling pathway involving MEK/Erk activation. This hypothesis, together with a more detailed analysis of the mechanism(s) coupling MEK/Erk to the translational machinery regulating Bcl-X<sub>L</sub> and Bcl-2, now warrants further investigation.

The present study has shown a correlation between the up-regulation of Bcl-X<sub>L</sub> and Bcl-2 with FGF-2-mediated resistance to etoposide killing. To directly prove this association, it would be necessary to specifically inhibit the increase in Bcl-X<sub>L</sub> and Bcl-2 expression induced by FGF-2. However, antisense molecules directed against Bcl-X<sub>L</sub> and Bcl-2 rapidly down-regulate endogenous levels of these proteins leading to loss of viability of H-510 and H-69 cells (42, 43). Therefore, this approach is unlikely to clarify the issue. Nevertheless, these findings provide evidence toward the crucial role of Bcl-X<sub>L</sub> and Bcl-2 in the survival of SCLC cells.

Our data revealed one additional point. While we have induced the expression of the pro-survival proteins selectively, this too could be blocked by FGF-2. Collectively, these results show that FGF-2 can coordinately up-regulate Bcl-X<sub>L</sub> and Bcl-2 while blocking a pro-apoptotic fashion, which likely explains the synergistic killing. Furthermore, this coordinate regulation of Bcl-2 family members was selective because neither Bax expression was unaffected by either FGF-2 or etoposide.

In summary we have presented evidence of a novel epigenetic mechanism by which a growth factor can regulate the translation of selective mRNAs. Inhibition of the translational regulation of Bcl-2 family members or the selective targeting of growth factor signaling responsible for this effect may provide new therapeutic strategies for cancer treatment.

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