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SHORT COMMUNICATION

Mcl-1 is critical for survival in a subgroup of non-small-cell lung cancer cell lines

H Zhang, S Guttikonda, L Roberts, T Uziel, D Semizarov, SW Elmore, JD Leverson and LT Lam

Global Pharmaceutical Research and Development, Abbott Laboratories, Abbott Park, IL, USA

Non-small-cell lung cancer (NSCLC) is the most deadly type of cancer in the United States and worldwide. Although new therapy is available, the survival rate of NSCLC patients remains low. One hallmark of cancer cells is defects in the apoptotic cell death program. In this study, we investigate the role of B-cell lymphoma 2 (Bcl-2) family members Bcl-2, Bcl-x_L and Mcl-1, known to regulate cell survival and death, in a panel of fourteen NSCLC cell lines. NSCLC cell lines express high levels of Mcl-1 and Bcl-x_L, but not Bcl-2. Silencing the expression of Mcl-1 with small interfering RNA (siRNA) oligonucleotides potently killed a subgroup of NSCLC cell lines. In contrast, Bcl-x_L siRNA had no effect in these lines unless Mcl-1 siRNA was also introduced. Interestingly, high MCL1 to BCL-xl messenger RNA determines whether the cells depend on Mcl-1 for survival. We further investigated the role of Mcl-1 in NSCLC cells using a Mcl-1-dependent cell line, H23. The expression of a complementary DNA containing only the coding region of MCL1 rescued H23 cells from the toxicity of a 3′ untranslated region (UTR) targeting Mcl-1 siRNA but not a siRNA targeting the coding region of MCL1. Furthermore, we show that Mcl-1 sequesters the BH3-only protein Noxa and Bim and the apoptotic effector Bak. Not surprisingly, Noxa, Bim, or Bak knockdown partially rescued H23 cells from toxicity mediated by Mcl-1 siRNA to different degrees. Collectively, our results indicate that targeting Mcl-1 may improve therapy for a subset of NSCLC patients.
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Non-small-cell lung cancer (NSCLC) accounts for more than 80% of lung cancers and is the number one cause of cancer death in the United States and worldwide. Several mutations associated with this disease have been identified (Minna *et al*., 2002). For example, mutations in the K-ras proto-oncogene are found in 15–20% of NSCLC (Rodenhuis and Slebos, 1992). In addition, mutations and amplifications of the epidermal growth factor receptor are also very common in NSCLC (Linardou *et al*., 2009). These mutations provide the basis for the development of targeted therapies for this subtype of cancer (Sun *et al*., 2007). Although improved therapy is available, the median survival of late-stage disease is less than 1 year. Thus, new therapies are needed to improve the survival of NSCLC patients.

A hallmark of cancer cells is defects in the apoptotic cell death program (Hanahan and Weinberg, 2000). Apoptosis is a highly regulated cell suicide process that functions to remove unwanted cells. One means of inhibiting the apoptotic pathway is through upregulation of the anti-apoptotic B-cell lymphoma 2 (Bcl-2) family members (Cory *et al*., 2003). These proteins belong to a family of BH-domain-containing proteins (Reed *et al*., 2004). There are three classes of BH-domain-containing proteins: (1) multidomain anti-apoptotic (Bcl-2, Bcl-x_L, Bcl-w, Bfl-1/A1 and Mcl-1), (2) multidomain proapoptotic (Bax and Bak) and (3) BH3-only proapoptotic (Bid, Bim, Bad, Bik, Noxa, Puma, Bmf and Hrk). The BH3-only proteins contain a single BH3 domain that interacts with specific anti-apoptotic proteins (Chen *et al*., 2005). For example, Bcl-2 and Bcl-x_L bind and antagonize Bad but not Noxa. In contrast, Mcl-1 and A1 bind and antagonize Noxa but not Bad protein. Other BH3 domain proteins such as Bim and Puma are bound and antagonized by all anti-apoptotic proteins. Bax and Bak are the 'effectors'. Once activated, they form complexes and permeabilize the outer mitochondrial membrane, resulting in the release of cytochrome *c* and other pro-apoptogenic proteins to induce the cell death pathway. Currently, there are two models for activation of Bak/Bax. The direct activation model suggests that Bim, truncated Bid, and perhaps Puma can directly activate Bak and Bax (Letai *et al*., 2002). These BH3-only proteins are called activators. The indirect model suggests that Bak and Bax are suppressed by the multidomain anti-apoptotic proteins. Binding of the anti-apoptotic proteins by the BH3-only proteins causes the activation of Bak or Bax (Willis *et al*., 2007).

Expression of Bcl-2 family members has been reported in both NSCLC patient tissues and NSCLC cell lines (Borner *et al*., 1999; Berrieman *et al*., 2005).

Correspondence: Dr LT Lam, Cancer Research, Global Pharmaceutical Research and Development, AP10, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064, USA.
E-mail: Lloyd.Lam@abbott.com
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Whereas most studies have focused on Bcl-2 and Bcl-x_L, the role of Mcl-1 has not been extensively investigated. A few lines of evidence indicate that Mcl-1 may have an important role in NSCLC. First, Mcl-1 is expressed in 56% of the tumor samples from chemotherapy-naïve NSCLC patients as revealed by tissue microarray analysis (Wesarg *et al*., 2007) and in 58% of the specimens of patients with radically resected NSCLC (Borner *et al*., 1999). Protein expression analysis of several NSCLC cell lines also demonstrated the presence of Mcl-1 (Song *et al*., 2005). Second, the high level of Mcl-1 in a NSCLC cell line H1299 results in resistance to ABT-737, a specific inhibitor of Bcl-2 and Bcl-x_L but not Mcl-1. This resistance can be overcome by expressing Noxa to neutralize the effect of Mcl-1 (Wesarg *et al*., 2007). Third, modest cell killing was observed when antisense Mcl-1 oligonucleotide was introduced into A549 and H1299 (Borner *et al*., 1999). In this study, we sought to investigate the role of Bcl-2 family members in NSCLC cell lines systematically.

We first determined the protein expression of these Bcl-2 members in NSCLC cell lines. We collected cell lysates from 14 NSCLC cell lines and performed western blotting analysis (Figure 1a). We found that almost all NSCLC cell lines have high levels of Mcl-1 expression, whereas Bcl-x_L expression varies among these lines (Figure 1a). Low Bcl-2 expression was detected in only two NSCLC lines (data not shown), consistent with other studies (Li *et al*., 2008). Our data indicated that Mcl-1 and Bcl-x_L might have important roles in NSCLC.

To determine the role of Mcl-1 and Bcl-x_L in NSCLC cell lines, we silenced their expression by using small interfering RNA (siRNA) oligonucleotides specific to Mcl-1 and Bcl-x_L, respectively. Two Mcl-1 siRNAs and Bcl-x_L siRNA effectively knocked down Mcl-1 and Bcl-x_L protein expression in H1299 cells (Figure 1b). Viability of 14 NSCLC cell lines was assessed after transfecting Mcl-1 siRNA or Bcl-x_L siRNA alone or in combination. Greater than 50% growth inhibition was observed in four NSCLC cell lines (H23, H1568, H522 and H838) after 2 days of transfection with Mcl-1 siRNA (Figure 1c). There was minimal or modest effect on the viability of other NSCLC cell lines. In contrast, silencing the expression of Bcl-x_L or Bcl-2 had no effect on these NSCLC lines (Figure 1c and data not shown). Interestingly, the viability of many lines was greatly reduced (H358, H1299, H2405, HCC827 and H1975) when Mcl-1 and Bcl-x_L siRNAs were combined. Our data indicated that, although Mcl-1 is crucial for the survival of some NSCLC cell lines, most NSCLC cell lines utilize both Bcl-x_L and Mcl-1 for survival.

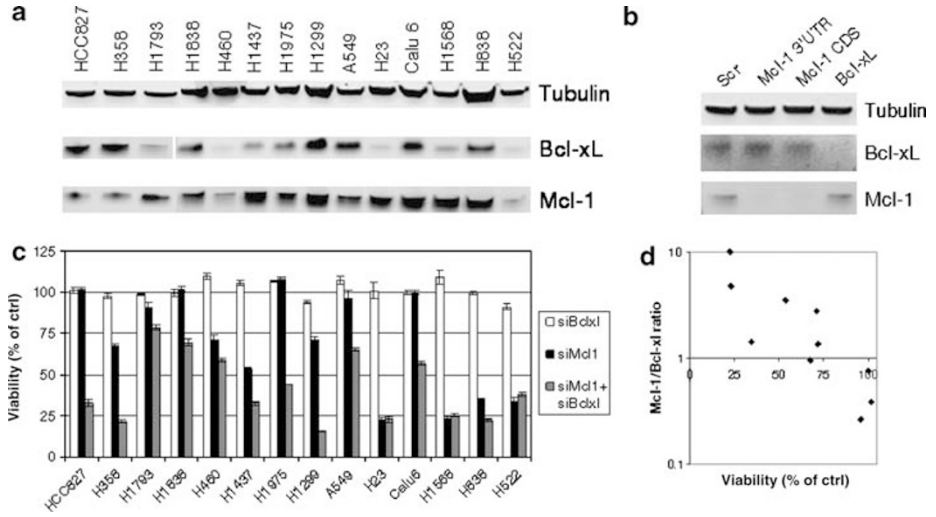

Figure 1 Silencing Mcl-1 decreases cell viability in a subset of NSCLC cell lines. (**a**) Protein expression of Bcl-2 family members in NSCLC cell lines. All NSCLC cell lines (ATCC) were cultured in RPMI (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Invitrogen), 1% sodium pyruvate, 4.5 g/l glucose and antibiotics (Invitrogen), and were maintained in a humidified chamber at 37 °C containing 5% CO_2. Cell lysates were prepared in RIPA buffer (Sigma, St Louis, MO, USA) plus protease inhibitor cocktail (Roche, Indianapolis, IN, USA). A measure of 20 μg of total protein was resolved on a 12% SDS polyacrylamide gel and probed with anti-Bcl-x_L (Epitomics, Burlingame, CA, USA) and anti-Mcl-1 (Epitomics). Antibody against tubulin (Santa Cruz, Santa Cruz, CA, USA) was used as a loading control. (**b**) Western blotting showing the degree of Mcl-1 and Bcl-x_L knock down by Mcl-1 and Bcl-x_L siRNAs in H1299 cells. H1299 cells were transfected in 6-well plates with siRNAs using Lipofectamine 2000 according to the manufacturer's instructions (Invitrogen). A final concentration of 20 nM siRNA was used in all cases. The sense sequences of the siRNAs used are: Mcl-1 CDS: 5′-GCATCGAACCATTAGCAGATT-3′; Mcl-1 3′UTR: 5′-CGAAGGAAGUAUCG AAUUU-3′; Bcl-2: 5′-GGGAGAUAGUGAUGAAGUAUU-3′; Bcl-x_L: 5′-ACAAGGAGAUGCAGGUAUUUU-3′. The cells were then grown in medium without antibiotic before harvesting for western blotting analysis. Bcl-2 was undetectable in these cells. (**c**) Silencing Mcl-1 decreases cell viability in a subset of NSCLC cell lines. NSCLC cell lines were transfected at 1.5–2.5 × 10^4 cells/100 μl in 96-well tissue culture plates with 20 nM Mcl-1, Bcl-x_L or both siRNAs. The cells were grown in medium without antibiotic before harvesting. Cells were assayed for viability after 48 h using the CellTiter-Glo luminescent cell viability assay according to the manufacturer's protocol (Promega, Madison, WI, USA). Results were normalized to cells transfected with scrambled siRNA control and presented as mean ± s.d. ($n = 3$). (**d**) High MCL1 to BCL-xl mRNA ratio correlates with effect of Mcl-1 silencing in NSCLC lines. MCL1 and BCL-xl mRNA levels were quantified with real-time quantitative PCR according to Tse *et al*. (Tse *et al*., 2008). The ratio of MCL1 to BCL-xl mRNA is plotted against viability of NSCLC cell lines after Mcl-1 siRNA transfection, as determined in (**c**).

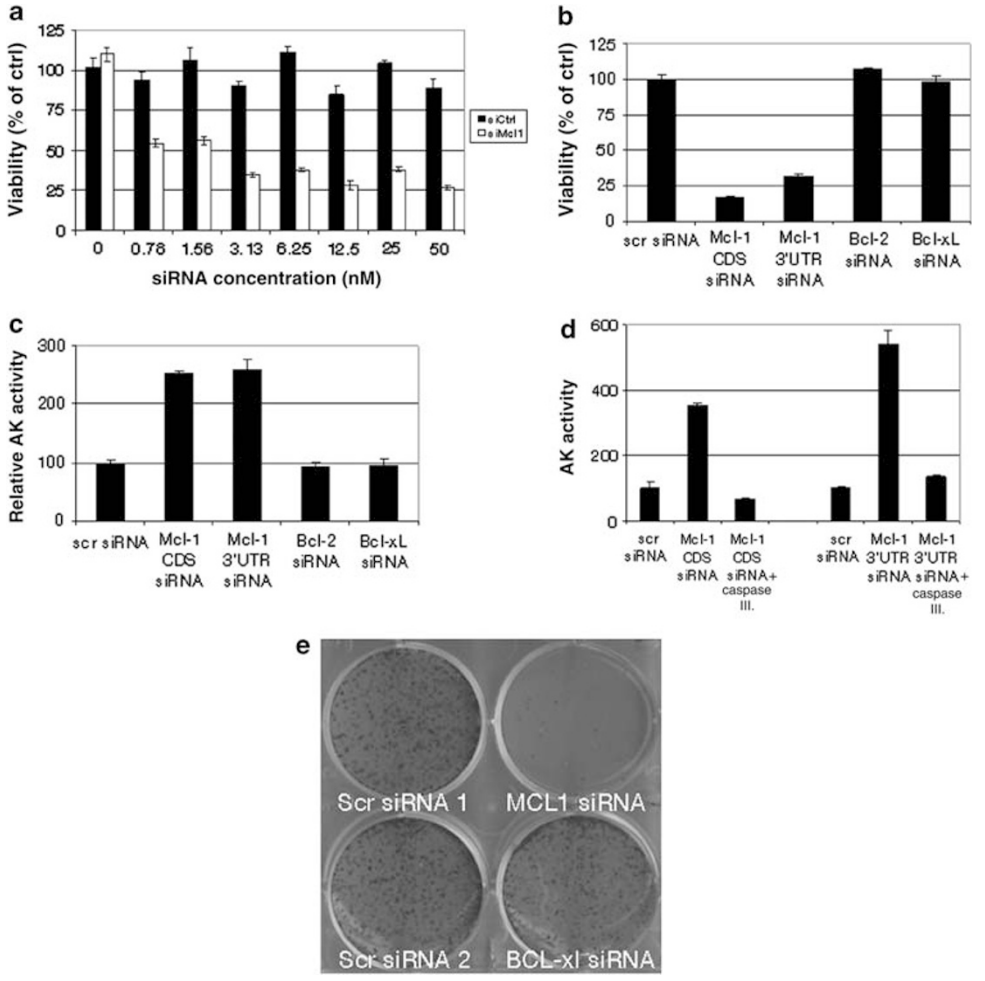

Figure 2 Mcl-1 is critical for the survival of H23 cells. (**a**) Increasing concentrations of Mcl-1 or control siRNA were transfected into H23 cells. Viability was determined after 48 h using the CellTiter-Glo luminescent cell viability assay (mean ± s.d., $n = 3$). (**b**, **c**) H23 cells were killed by Mcl-1 siRNAs. H23 cells were transfected with two different Mcl-1 siRNAs, scrambled control, Bcl-2 or Bcl-x_L siRNA. Cell viability was measured using CellTiter-Glo assays (mean ± s.d., $n = 3$) (**b**). Cell death was monitored by the release of adenylate kinase into cell culture medium using ToxiLight BioAssay kit (Lonza, Rockland, ME, USA) after 48 h (mean ± s.d., $n = 3$) (**c**). (**d**) H23 cells were rescued from the toxicity of Mcl-1 siRNAs with DEVD-fmk caspase inhibitor (Santa Cruz). H23 cells were transfected with two different Mcl-1 siRNAs in the presence or absence of DEVE-fmk (50 μM). Cell death was monitored by the release of adenylate kinase into cell culture medium after 48 h (mean ± s.d., $n = 3$). (**e**) Clonogenic survival of H23 cells transfected with control, Bcl-x_L or Mcl-1 siRNAs. At 1 day post-transfection, H23 cells were trypsinized and reseeded in 6-well plates. Colony formation was monitored over the following 10 days. Colonies were first washed with PBS, then stained with Coomassie brilliant blue R-250 staining solution (Bio-Rad, Hercules, CA, USA) for 30 min, washed with destaining solution (Bio-Rad) and water, air dried and scanned.

It is interesting to note that the Mcl-1-dependent lines have higher protein expression of Mcl-1 than Bcl-x_L. In order to determine whether this Mcl-1 dependency could be predicted at the messenger RNA (mRNA) level, we measured the mRNA levels of MCL1 and BCL-xl using quantitative real-time PCR assays. As expected, the ratio of MCL1 to BCL-xl mRNA expression is inversely correlated (−0.75) with the dependency of these lines on Mcl-1 (as determined by Mcl-1 knockdown; Figure 1d).

H23 cells were found to have the most dramatic response when Mcl-1 was silenced, and so we used this cell line to further investigate the role of Mcl-1 in NSCLC. First, we determined the Mcl-1 dependence of H23 cells. Viability of H23 cells was assessed after transfection with increasing concentrations of control or Mcl-1 siRNA for 48 h. H23 cells were killed at less than 1 nM concentration of Mcl-1 siRNA (Figure 2a), confirming that Mcl-1 is a critical survival factor for H23 cells. To further assess the role of Bcl-2 anti-apoptotic proteins in H23 cells, we evaluated the effect of transfecting Bcl-2, Bcl-x_L and two Mcl-1 siRNAs, one targeting the coding sequence (CDS) and one targeting the 3′ untranslated region of MCL1 (Mcl-1 3′UTR). Cell viability was measured using CellTiter-Glo assay and cell death was quantified by the release of adenylate kinase into cell culture medium from damaged cells. Only transfection of the two Mcl-1 siRNAs led to H23 cell death (Figures 2b and c). Both Mcl-1 siRNAs effectively knocked down Mcl-1 protein expression (data not shown), suggesting that the cell killing is Mcl-1 specific. Transfection of Bcl-x_L siRNA had no effect on H23 cell viability and cell death despite

effective silencing of Bcl-x_L protein in these cells (Figures 2b and c). Similarly, Bcl-2 siRNA had no effect on cell viability or cell death (Figures 2b and c), possibly because of undetectable Bcl-2 protein expression in these cells (data not shown). We also determined whether apoptosis was induced with Mcl-1 siRNAs by measuring adenylate kinase activity in the presence or absence of 50 μM caspase 3/7 inhibitor DEVE-fmk. Adenylate kinase activity was strongly increased upon Mcl-1 silencing with both Mcl-1 siRNAs, but was completely blocked by co-treatment with caspase 3/7 inhibitor (Figure 2d). These data support the view that H23 cells utilize Mcl-1 to resist apoptosis.

An independent method was used to validate these findings. Clonogenic survival assay was performed in H23 cells following transfection with two control, Bcl-x_L or Mcl-1 siRNAs. Consistent with the viability data, colony formation was significantly reduced with Mcl-1 siRNA as compared with control and Bcl-x_L siRNAs (Figure 2e).

To ascertain whether Mcl-1 is specific and crucial for H23 cell survival, we expressed a complementary DNA containing only the CDS of MCL1 with a Flag tag to perform rescue experiments in these cells. As shown in Figure 3a, viability of control vector-transfected H23 cells was greatly reduced by Mcl-1 siRNAs targeting the CDS or the 3′UTR. As expected, endogenous Mcl-1 was knocked down by both siRNAs (data not shown). In contrast, Mcl-1 siRNA targeting the 3′UTR had no effect on the viability of Flag-MCL1-transfected H23 cells, whereas viability was greatly reduced by Mcl-1 siRNA targeting the CDS (Figure 3a). Immunoblotting showed that Mcl-1 CDS siRNA knocked down both endogenous and exogenous Mcl-1 in Flag-MCL1-transfected H23 cells (Figure 3b). On the other hand, Mcl-1 siRNA targeting the 3′UTR knocked down endogenous Mcl-1 but had no effect on the exogenously expressed Flag-Mcl-1, as shown by western blotting using anti-Flag antibody (Figure 3b). These data again confirm the essential role of Mcl-1 for H23 cell survival.

We next investigated how Mcl-1 prevents apoptosis in H23 cells. In the direct activation model, anti-apoptotic proteins oppose cell death by binding and sequestering BH3-only activators such as Bim, truncated Bid, and perhaps Puma to prevent their activation of effectors Bax and Bak. Immunoprecipitations with anti-Mcl-1 in H23 cells showed that Mcl-1 interacted with activator Bim, but not with Puma (Figure 4a). Mcl-1 also interacted with Noxa. Immunoprecipitations with anti-Bcl-x_L did not pull down these proteins (Figure 4b). In contrast, Bim was pulled down by both anti-Mcl-1 and anti-Bcl-x_L in H1975 cells, which depend on both Mcl-1 and Bcl-x_L for survival (data not shown). These data indicated that H23 cells utilize Mcl-1 to neutralize the activity of activator BH3-only protein Bim for cell survival. It is likely that silencing Mcl-1 expression allows the release of these activators to induce apoptosis.

The activity of the apoptosis effectors Bak and Bax can be suppressed by the multidomain anti-apoptotic proteins such as Mcl-1 and Bcl-x_L, as suggested by the indirect activation model (Willis *et al*., 2007). We thus asked whether Mcl-1 and Bcl-x_L interact with Bak and/or Bax in H23 cells. Immunoprecipitations with anti-Mcl-1 showed that Mcl-1 interacted with Bak but not Bax (Figure 4a). In contrast, immunoprecipitations with anti-Bcl-x_L did not pull down either of these proteins (Figure 4b). Our data thus indicated that Mcl-1 could also neutralize the activity of the effector protein Bak for cell survival.

Finally, to determine the roles of the Mcl-1 binding partners in Mcl-1 siRNA-induced cell death, we asked whether silencing these Mcl-1-binding proteins would rescue H23 cells from Mcl-1 knockdown. Silencing either Bim or Noxa rescued H23 cells from Mcl-1 knockdown to a lesser extent than silencing both Bim and Noxa together (Figure 4c). Bim and Noxa siRNAs knocked down their respective target proteins effectively (Figure 4d). These data further suggested that Mcl-1 binds and sequesters BH3-only proteins Bim and Noxa to resist cell death. We have also determined the role of apoptosis effector Bak and Bax in inducing cell death due to Mcl-1 knockdown. Silencing Bak rescued H23 cells from Mcl-1 knockdown to a much greater extent than by silencing Bax (data not shown). The partial rescue could be explained by incomplete knockdown of Bak in the experiment (data not shown). Our data

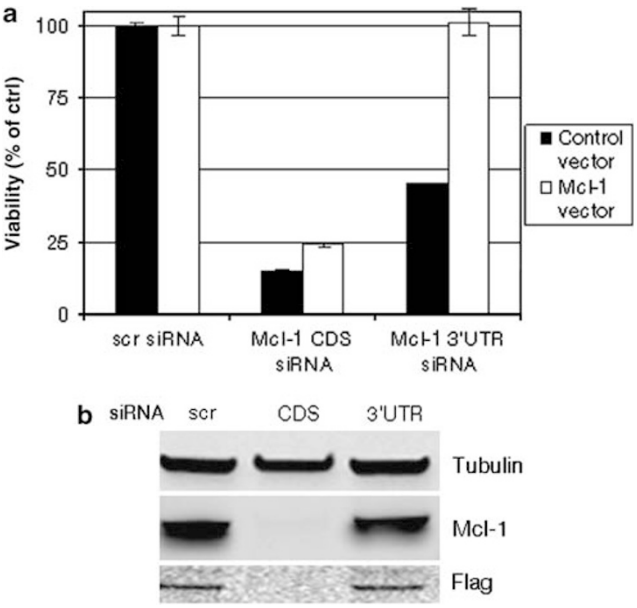

Figure 3 Exogenous expression of Mcl-1 rescues H23 cells from Mcl-1 silencing. (**a**) The coding region of MCL1 with a Flag tag was subcloned into the pHLCX vector (Invitrogen). H23 cells were stably transfected with empty pHLCX or pHLCX-Flag-MCL1 coding region and selected with 100 μg/ml of hygromycin. Individual clones were obtained and used in the Mcl-1 siRNA rescue experiments. H23 cells transfected with pHLCX and pHLCX-Flag-MCL1 were tranfected with 20 nM of Mcl-1 CDS or 3′UTR siRNAs for 48 h, and cell viability was analyzed using CellTiter Glo (mean ± s.d., $n = 3$). (**b**) Western blotting showing Mcl-1 knock down by Mcl-1 siRNAs in H23 cells stably transfected with pHLCX-Flag-MCL1 coding region. Antibody against Flag-tag (Sigma) was used to detect the exogenous form of Mcl-1 in these cells.

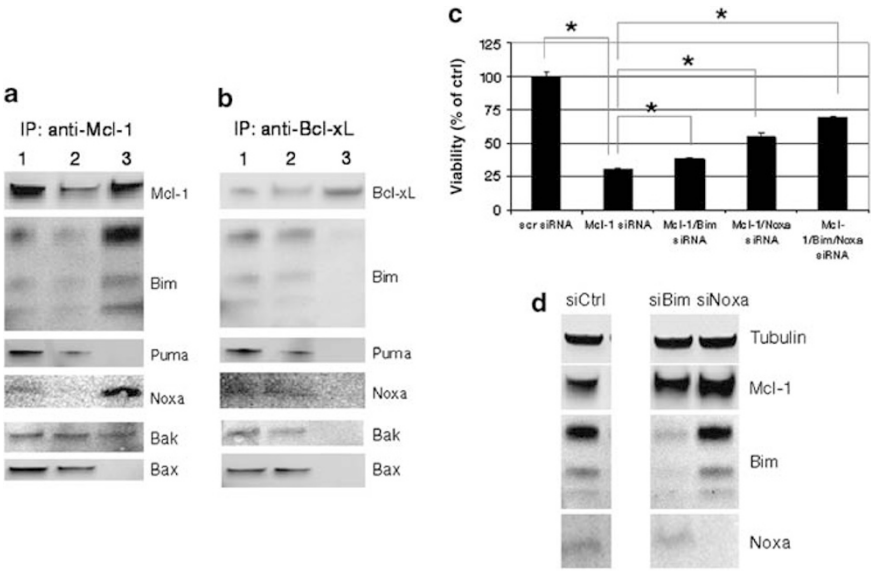

Figure 4 Sequestration of Bim, Noxa and Bak by Mcl-1 in H23 cells. Immunoprecipitation (IP)/immunoblot of Bim, Noxa and Bak binding to (**a**) Mcl-1 and (**b**) Bcl-x_L in H23 cells. Cell lysates were prepared from H23 cells in a CHAPS buffer (1% (w/v) CHAPS, 10 mM HEPES pH 7.4, 150 mM NaCl) plus protease inhibitor cocktail (Roche). A measure of 1 mg of cell lysate was incubated with 4 µg of biotin-conjugated anti-Bcl-x_L (Abcam, Cambridge, MA, USA) or anti-Mcl-1 (Thermo Scientific, Rockford, IL, USA) overnight at 4 °C. Streptavidin MagneSphere paramagnetic particles (Promega) were added and lysates were incubated for an additional 1 h at room temperature. The supernatant from the first separation was collected. Particles were washed 5 × in 1000 µl lysis buffer and finally suspended in 50 µl SDS-sample buffer. A volume of 20 µl were used for western blotting. Immunoblotting were performed and probed with anti-Bim (Epitomics), anti-Noxa (Abcam), anti-Puma (Abcam), anti-Bak (Epitomics) and anti-Bax (Epitomics). Lane 1: cell lysate, lane 2: supernatant after IP, lane 3: IP. (**c**) Protection of Mcl-1 knockdown-mediated cell death by silencing both Noxa and Bim. H23 cells were transfected with control siRNA, Mcl-1 siRNA alone, Mcl-1 siRNA and Bim siRNA (Dharmacon siGenome smart pool (M004383-02; Dharmacon, Lafayetta, CO, USA)), Mcl-1 siRNA and Noxa siRNA (Dharmacon onTargetPlus smart pool (L005275-00)), or Mcl-1, Bim and Noxa siRNAs. Cells were assayed for viability after 48 h (mean ± s.d., $n = 3$). Student's *t*-test was performed and *P*-values of <0.05 were considered significant. (**d**) Western blotting showing the levels of Bim and Noxa silencing by Bim and Noxa siRNAs in H23 cells.

suggested that Mcl-1 could neutralize the activity of the effector protein Bak for cell survival.

Previous reports suggested that Mcl-1 has a role in modulating survival and sensitivity to diverse apoptotic stimuli, and overcoming drug resistance in NSCLC (Song *et al*., 2005; Wesarg *et al*., 2007). In this study, we systematically evaluated the role of Mcl-1 by RNA interference and show that Mcl-1 is critical for survival in a subset of NSCLC cell lines, suggesting that different treatment regimes may be required for NSCLC subgroups. Various lines of evidence support our findings. First, Mcl-1-dependent NSCLC cell lines express high levels of MCL1 mRNA and protein, and low levels of BCL-xl mRNA and protein (Figures 1a and d). Second, silencing Mcl-1 with siRNA killed these Mcl-1-dependent NSCLC cell lines effectively even after 2 days of transfection (Figure 1c). Third, exogenous expression of a complementary DNA containing only the coding region of MCL1 rescued H23 cells from the toxicity of the 3′UTR-directed Mcl-1 siRNA (Figure 3a). Fourth, we have shown by immunoprecipitations that Mcl-1 binds BH3-only proteins Bim and Noxa (Figure 4a). As expected, silencing Bim and Noxa almost completely rescued H23 cells from Mcl-1 knockdown (Figure 4c). One potential explanation for the incomplete rescue is Mcl-1 could also bind effector Bak and prevents apoptosis (Leu *et al*., 2004; Zhai *et al*., 2008). Indeed, we have also shown that Mcl-1 interacts with Bak directly (Figure 4a). In addition, silencing Bak rescued H23 cells from Mcl-1 knockdown (data not shown). Taken together, these data indicate that a subset of NSCLC cell lines is Mcl-1 dependent and could be very valuable for studying the function of Mcl-1.

Mcl-1 overexpression has been reported in a variety of human hematopoietic cancers, lymphoid cancers and solid tumors (Akgul, 2009). Although Mcl-1 has been shown to be a critical survival factor in multiple myeloma (Zhang *et al*., 2002), Mcl-1 overexpression was generally believed to be a resistance mechanism to conventional cancer therapies in solid tumors (Akgul, 2009). Our results in NSCLC cell lines suggested that Mcl-1 may be the primary survival factor in a subset of NSCLC patients. Interestingly, all these Mcl-1-dependent cell lines express high levels of Mcl-1 and low levels of Bcl-x_L (Figures 1a and d). The combination of high Mcl-1 and low Bcl-x_L expression might serve as a stratification marker for these patients. Indeed, the ratio of MCL1 to BCL-xl mRNA levels was 2- to 10-fold higher in Mcl-1-dependent cell lines (Figure 1d).

In light of our findings, a working model of the two NSCLC subgroups is presented. The first subgroup of NSCLC expresses high levels of Mcl-1 relative to Bcl-x_L. Mcl-1 could bind activators such as Bim and also inhibit the activity of effector such as Bak. When Mcl-1 is silenced, these activators and effectors are released and

cells undergo apoptosis. The second subgroup of NSCLC has expression of both Mcl-1 and Bcl-x_L. In this case, activators and effectors are bound by both of these anti-apoptotic proteins. When Mcl-1 is silenced, only a portion of activators and effectors are released and, thus, only a small portion of cells undergo apoptosis. It is also possible that Bcl-x_L could compensate for the function of Mcl-1 when Mcl-1 is silenced, as suggested by previous work (Willis *et al*., 2005). Indeed, the viability of a portion of NSCLC lines was greatly reduced only when combining both Mcl-1 and Bcl-x_L siRNAs (Figure 1c). In this regard, it was shown that Mcl-1 could contribute to the overall resistance of SCLC cell lines and other cancer lines to a novel and potent Bcl-2 and Bcl-x_L antagonist, ABT-737 (Chen *et al*., 2007; Tahir *et al*., 2007; Tagscherer *et al*., 2008; Keuling *et al*., 2009). Thus, combining agents that target Mcl-1 with ABT-737 might restore the apoptotic program in cells expressing high levels of Bcl-x_L and Mcl-1 (Lin *et al*., 2007). Our observations might have broader implications for patients with other cancer subtypes.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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