IAPs as E3 ligases of Rac1
Shaping the move

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Inhibitors of Apoptosis Proteins (IAPs) are well-studied E3 ubiquitin ligases predominantly known for regulation of apoptosis. We uncovered that IAPs can function as a direct E3 ubiquitin ligase of RhoGTPase Rac1. cIAP1 and XIAP directly conjugate polyubiquitin chains to Lysine 147 of activated Rac1 and target it for proteasomal degradation. Consistently, loss of these IAPs by various strategies led to stabilization of Rac1 and mesenchymal mode of migration in tumor cells. IAPs also regulate Rac1 degradation upon RhoGDI1 depletion and CNF1 toxin treatment. Our observations revealed an evolutionarily conserved role of IAPs in regulating Rac1 stability shedding light on to the mechanisms behind ubiquitination-dependent inactivation of Rac1 signaling.

Keywords: IAPs, XIAP, cIAP1, Rac1, RhoA, RhoGTPases, ubiquitination, proteasome, E3 ligase, ubiquitin, cell migration, plasticity, amoeboid, mesenchymal

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Inhibitors of Apoptosis

Inhibitors of Apoptosis Proteins (IAPs) are a class of evolutionarily conserved, multifunctional proteins primarily characterized by the presence of a Baculoviral IAP repeat (BIR) domain.1,2 BIR domains are classical protein-protein interaction motifs with which IAPs directly bind to various proteins including caspases. Five IAPs including XIAP, cIAP1, cIAP2, ILP2 and ML-IAP possess a RING domain with E3 ubiquitin ligase activity.3 Upon apoptosis induction, natural IAP antagonists Smac/DIABLO, Omi/HtrA2 and ARTS translocate from the mitochondria to release IAP-mediated inhibition of caspases.4,5 XIAP is considered as a direct inhibitor of caspases and recent studies revealed an in vivo role for the RING domain of XIAP in regulating caspase activation.6,7 cIAPs are highly implied in regulating the activation of both canonical and non-canonical pathway of NFκB by functioning as the direct E3 ubiquitin ligases of RIPK1 and NIK respectively.8 IAPs also function as the E3 ubiquitin ligases of RIP2 to regulate innate immunity signaling.9 Further, IAPs are highly expressed in tumor cells and cIAP1 was recognized as an oncogene.10,11 Recent studies revealed that depletion of IAPs has led to sensitization of tumor cells to conventional chemotherapeutic drugs. These results have emboldened scientists to pursue for IAP antagonists to treat cancers and several of these drugs (antisense nucleotides and Smac mimetics) are already in clinical trials.2

IAPs Directly Regulate Rac1 and Cell Shape

We have recently uncovered that IAPs also control the RAF/MEK/ERK1/2 pathway and cell migration by directly regulating the stability of C-RAF kinase.11 These observations have raised an important question. Does treatment with IAP antagonists also lead to an increase in C-RAF levels and cell migration? To address this issue we have treated several tumor cell lines with various concentrations of IAP antagonist compound (BV6/IAC). Previous studies have shown that these compounds trigger the autoubiquitination of cIAPs leading to NIK stabilization and activation of the non-canonical NFκB pathway. This in turn leads to the production of TNFα and cell death in a cell type dependent manner.12,13 Treatment of HeLa cells with IACs promoted cell death...

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remained elusive. Ubiquitination is a most versatile form of posttranslational modification where proteins are covalently conjugated to ubiquitin in a three-step process. Proteins can be conjugated with single ubiquitin on one lysine or on multiple lysines (mono-or multi-mono ubiquitination) or as polyubiquitin chains of various kinds. Proteins conjugated with K-48 or K-29 linked ubiquitin chains are normally doomed for proteasomal degradation while the proteins tagged with K-63 linked or M1 linked chains lead to assemblage of protein complexes and signal transduction. E3 ubiquitin ligases constitute the third step of the ubiquitination process and play an obligatory role in catalyzing the conjugation of ubiquitin to a lysine residue in the target proteins. The chain initiation, elongation and topology are, however, primarily controlled by the E2 enzymes. RhoGTPases were initially observed to be degraded via proteasomes during host-pathogen interactions. Cytotoxic Necrotizing factor 1 (CNF1), a toxin secreted by uropathogenic E. coli, has been shown to constitutively activate RhoGTPases by deamidation leading to their proteasomal degradation. Smurf1 and Cullins (Cul3) were shown to regulate the polyubiquitination and degradation of RhoA, the E3 ligases driving Rac1 polyubiquitination were not known. Our observations unexpectedly revealed a role of IAPs in the inactivation of Rac1 by direct polyubiquitination and

Ubiquitin Dependent Regulation of Rac1: Role for IAPs and HACE1

Rac1 is a well-studied RhoGTPase, which controls numerous cellular functions. Apart from nucleotide binding and isoprenylation, Rho GTPases are also regulated by phosphorylation, SUMOylation and ubiquitination. The binding of nucleotides to Rac1 is regulated by several GEFs and GAPs and a recent study from Angeliki Malliri’s group has revealed a role for PIAS3 for SUMOylation of Rac1. Though Rac1 has been shown to be polyubiquitinated, the E3 ubiquitin ligases responsible for this process remained elusive. Ubiquitination is a most versatile form of posttranslational modification where proteins are covalently conjugated to ubiquitin in a three-step process. Proteins can be conjugated with single ubiquitin on one lysine or on multiple lysines (mono-or multi-mono ubiquitination) or as polyubiquitin chains of various kinds. Proteins conjugated with K-48 or K-29 linked ubiquitin chains are normally doomed for proteasomal degradation while the proteins tagged with K-63 linked or M1 linked chains lead to assemblage of protein complexes and signal transduction. E3 ubiquitin ligases constitute the third step of the ubiquitination process and play an obligatory role in catalyzing the conjugation of ubiquitin to a lysine residue in the target proteins. The chain initiation, elongation and topology are, however, primarily controlled by the E2 enzymes. RhoGTPases were initially observed to be degraded via proteasomes during host-pathogen interactions. Cytotoxic Necrotizing factor 1 (CNF1), a toxin secreted by uropathogenic E. coli, has been shown to constitutively activate RhoGTPases by deamidation leading to their proteasomal degradation. While Smurf1 and Cullins (Cul3) were shown to regulate the polyubiquitination and degradation of RhoA, the E3 ligases driving Rac1 polyubiquitination were not known. Our observations unexpectedly revealed a role of IAPs in the inactivation of Rac1 by direct polyubiquitination and
proteasomal degradation under various conditions including CNF1 toxin treatment. Apart from IAPs, elegant work from Emmanuel Lemichez and colleagues has also revealed a role for HACE1 for polyubiquitination of Rac1. HACE1, unlike IAPs carries a HECT domain to catalyze polyubiquitination of Rac1. Interestingly, HACE1 preferentially binds to the GTP-bound form of Rac1 while IAPs can bind to Rac1 irrespective of its activation status. These results suggest that IAPs are probably binding to the C-terminus of Rac1. Further IAPs can also bind to Rac2 and Rac3 though these isoforms are not regulated by IAP expression. In addition, IAP overexpression has failed to influence the protein levels of Rac1B, a tumor associated splice variant of Rac1. Binding of XIAP to Rac1 was independent of BIR3, UBA and RING domains and further studies are required to clarify if any of the three BIR domains exhibit preferential specificity for binding to Rac1. Though IAPs bind to Rac1Q61L and Rac1T17N, activated Rac1 seems to be a better substrate of cIAP1 at least in vitro in the presence of Ubc5H4 as an E2 enzyme. As XIAP can also ubiquitinate directly, it is plausible that XIAP-cIAP1 complex would function as a more efficient ubiquitin ligase in vivo and heteromerization between XIAP and cIAP1 has been demonstrated before. Depletion of either XIAP or cIAP1 or both has led to an identical increase in Rac1 levels. These results suggest that IAPs are functionally non-redundant in regulating Rac1 levels though we cannot completely rule out the possibility that these two IAPs might control two different subcellular pools of Rac1. Most of the Rac1 protein is localized to the cytosol in complex with RhoGDI and depletion of RhoGDI1 leads to a strong degradation of RhoGTPases including Rac1. Our observations suggest that XIAP is required for degradation of Rac1 upon RhoGDI1 depletion. As IAPs are predominantly cytosolic, it is tempting to propose that XIAP might primarily target the cytosolic pool of Rac1 (Fig. 2). Obviously there will be a significant contribution of other Rac1 effectors like caveolin in regulating the interaction between Rac1 and its E3 ligases. In these lines, it is interesting to point out that XIAP might primarily target the cytosolic pool of Rac1 in cells (Fig. 2). In these lines, it is interesting to point out that XIAP might primarily target the cytosolic pool of Rac1 in cells (Fig. 2).
Mammals express eight IAPs and mice deficient in individual IAPs lack any major developmental phenotypes. As IAPs directly regulate Rac1 levels, lack of any major migration phenotypes during development of mice is indeed intriguing. This could be attributed to the presence of other E3 ligases for Rac1 (like HACE1) and cross regulation between the IAPs. IAPs are constitutively organized into heteromeric protein complexes (IAPosomes) and loss of one IAP leads to an increase in the protein levels of another IAP. For instance, loss of cIAP1 led to an increase in the protein levels of cIAP2 and vice versa. Our unpublished observations further suggest that XIAP can also regulate the protein levels of cIAP1. This is evident in the experiments performed with MEFs derived from XIAP deficient mice as increased Rac1 levels observed in these early passage MEFs were cleared off at later passages possibly due to upregulation of cIAP1. Thus we would expect severe developmental defects in XIAP-cIAP1 double deficient mice. Further, it would be interesting to learn if there is any cross talk between IAPs and HACE1 and their relative contribution in regulating Rac1 levels under various conditions (Fig. 2). IAP mediated regulation of Rac1 is evolutionarily conserved as DIAP1, an IAP from Drosophila has been shown to bind to Rac1 in a nucleotide independent manner. We have extended on these observations and uncovered that Danio rerio XIAP can also bind to Rac1 in a nucleotide independent manner. Further, in collaboration with Reinhard Koster’s lab we have examined the role of Dr-XIAP in regulating cerebellar granule cell migration during zebrafish brain development. During vertebrate development, the cerebellar granule cells (CGNs) exhibit highly directional migration from the Upper Rhombic Lip to form the cerebellum. These cells primarily express Rac1 isoform and migrate collectively as chain-like structures in a Cadherin-2 dependent manner. Interestingly, increasing XIAP in these cells has led to the formation of rounded cells, which delaminate from the tissue architecture and are often found in the cerebrospinal fluid present in the fourth ventricle. These effects are clearly Rac1 dependent as co-expression of Rac1 with XIAP can partially rescue the phenotype. Further, depletion of XIAP using siRNAs has led to a strong increase in Rac1 levels in the immortalized cerebellar granule cells derived from mice. The delamination phenotype observed in XIAP overexpressing CGNs resembles medulloblastoma which originates from CGN cells. These observations reveal an in vivo role of XIAP in regulating progenitor cell migration by directly controlling Rac1 homeostasis during brain development of a vertebrate organism.

Role of IAPs in Regulating Tumor Cell Migration and Metastases

The role of IAPs in regulating cell migration remains controversial, as two other labs have detected a role for IAPs in promoting cell migration. The apparent discrepancy can be attributed to the difference in the cell types employed or on the strategy adopted to downregulate IAPs. While our studies are primarily based on HeLa cells, Lopez et al. employed MCF-7 cells in their analysis. Similarly, Liu et al. primarily employed shRNAs or genetic deletion in human tumor cell lines to downregulate XIAP in contrast to our loss of function studies, which are primarily based on transient downregulation of IAPs by employing siRNAs and IAC. However, this issue needs to be reconciled as we have detected an increase in Rac1 levels upon IAP depletion in multiple cell types including MCF-7 cells and SV40 transformed MEFs derived from XIAP deficient mice have also exhibited enhanced migration and wound healing under our experimental settings. Further, by creating a transgenic adenocarcinoma of the mouse prostate (TRAMP) mice that lack XIAP, Collin Duckett and colleagues have shown that loss of XIAP has actually led to an aggressive form of disease. In a more recent study, Dario Altieri and colleagues have shown that XIAP survivin complex can promote metastases in a NFκB dependent manner. Whether our current results have any relevance to these observed phenotypes in mice deserves further investigations. As downregulation of IAPs enhanced cell migration, one obvious concern is that treatment with IAP antagonists might unexpectedly promote the metastases of surviving tumor cells. However, this may not necessarily be the case as increased Rac1 levels can be compensated by co-regulation of GAPs and GEFS. Further, Rac1 activity has been shown to both promote and inhibit tumor cell invasion in a cell type and dependent manner. For instance, ectopic expression of Tiam1, a Rac1 GEF or activated Rac1 actually led to an increase in E-cadherin mediated cell adhesion and loss of invasiveness in RAS-transformed MDCK cells. However, Rac isoforms are known to be highly expressed in tumor cells and overexpression of Rac1 GEFS like P-Rex is often detected in invasive breast cancer cells. Rac1 is also shown to be required for K-Ras-mediated lung tumor formation. Thus, IAP-mediated Rac1 regulation might regulate tumor cell migration in a tissue/cell type dependent manner. As IAP antagonists are primarily pursued in combination with other chemotherapeutics in clinical trials, one
would expect sensitization of tumor cells to apoptosis. However, if the cells survive there may be a risk of promoting tumor cell invasion by loss of IAPs through an increase in CRAF and Rac1 levels (Fig. 3). Indeed dual targeting of IAPs and Rac1 effector Pak1 has been shown to be effective in inducing effector caspase activation and apoptosis in NSCLC tumor cells. A better understanding of the role of IAPs in regulating tumor cell migration and invasion will assist in patient selection as well as to adroitly administer IAP antagonist compounds in treating a complex disease like cancer. The discovery of IAPs and HACE1 as the E3 ubiquitin ligases of Rac1 has revealed the molecular mechanisms behind polyubiquitination of Rac1, a thus far uncharacterized layer of regulation apart from GEFs, GAPs and RhoGDIs.

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**References**

1. Sriravasula SM, Ashwell JD. IAPs: what’s in a name? Mol Cell 2008; 30:123-35; PMID:18439892; http://dx.doi.org/10.1016/j.molcel.2008.03.008.

2. Gyrd-Hansen M, Mester P. IAPs: from caspase inhibitors to modulators of NFκB, inflammation and cancer. Nat Rev Cancer 2010; 10:561-74; PMID:20651737; http://dx.doi.org/10.1038/nrc2889.

3. Vaux DL, Silke J. IAPs, RINGs and ubiquitination. Nat Rev Mol Cell Biol 2005; 6:287-97; PMID:15803136; http://dx.doi.org/10.1038/nrm1621.

4. Vaux DL, Silke J. Mammalian mitochondrial IAP binding proteins. Biochem Biophys Res Commun 2003; 304:499-504; PMID:12729584; http://dx.doi.org/10.1016/S0006-291X(03)00622-3.

5. Edison N, Zuri D, Maniv I, Bornstein B, Lev T, Gottfried Y, et al. The IAP-antagonist ARTS initiates caspase activation upstream of cytochrome C and SMAC/Diablo. Cell Death Differ 2012; 19:356-68; PMID:21860827; http://dx.doi.org/10.1038/cdd.2011.112.

6. O’Riordan MX, Bauler LD, Scott FL, Duckett CS. Inhibitor of apoptosis proteins in eukaryotic evolution and development: a model of thiamic conservation. Dev Cell 2008; 15:497-508; PMID:18854135; http://dx.doi.org/10.1016/j.devcel.2008.09.012.

7. Schle A, Garcia-Fernández M, Steller H. Regulation of apoptosis by XIAP ubiquitin-ligase activity. Genes Dev 2008; 22:2256-66; PMID:18705838; http://dx.doi.org/10.1101/gad.1663108.

8. Varfolomeev E, Vucic D. (Un)expected roles of c-IAPs in apoptotic and NFκB signaling pathways. Cell Cycle 2008; 7:1551-21; PMID:18469528; http://dx.doi.org/10.4161/cc.7.11.5959.

9. Reardon C, Mak TW. cIAP proteins: keystones in NOD receptor signal transduction. Immunity 2009; 30:755-6; PMID:19538823; http://dx.doi.org/10.1016/j.immuni.2009.06.005.

10. Zender L, Spector MS, Xue W, Fleming P, Corden-Caruso C, Silke J, et al. Identification and validation of oncopgenes in liver cancer using an integrative oncogenic approach. Cell 2006; 125:1253-67; PMID:16814713; http://dx.doi.org/10.1016/j.cell.2006.05.030.

11. Dogan T, Harms GS, Hekman M, Karterman C, Oberoi TK, Aleinri ES, et al. X-linked and cellular IAPs modulate the stability of c-RAF kinase and cell mortality. Nat Cell Biol 2008; 10:1447-55; PMID:19016199; http://dx.doi.org/10.1038/ncll1804.

12. Varfolomeev E, Blankenship JW, Wayson SM, Fedorova AV, Kayagaki N, Garg P, et al. IAP antagonists induce autoubiquitination of c-IAPs, NFκB inhibitor and TNFα-dependent apoptosis. Cell 2007; 131:669-81; PMID:18022362; http://dx.doi.org/10.1016/j.cell.2007.10.030.

13. Vince JE, Wong WW, Khan F, Felharam R, Chau D, Ahmed AU, et al. IAP antagonists target cIAP1 to induce TNFα-dependent apoptosis. Cell 2007; 131:682-93; PMID:18022363; http://dx.doi.org/10.1016/j.cell.2007.10.037.

14. Oberoi TK, Dogan T, Hocking JC, Schol RP, Mooz J, Anderson CL, et al. IAPs regulate the plasticity of cell migration by directly targeting Rac1 for degradation. EMBO J 2012; 31:14-28; PMID:22172719; http://dx.doi.org/10.1038/emoj.2011.423.

15. Friedl P, Wolf K. Tumour-cell invasion and migration: diversity and escape mechanisms. Nat Rev Cancer 2003; 3:562-74; PMID:12724747; http://dx.doi.org/10.1038/nrc1079.

16. Sahai E, Marshall CJ. RHO-GTPases and cancer. Nat Rev Cancer 2002; 2:133-42; PMID:12635176; http://dx.doi.org/10.1038/nrc725.

17. Vivicik O, Loris P, Boyer L, Chardin P, Lemichez E, Gaccon G. Activated Rac1, but not the tumorigenic variant Rac1B is ubiquitinated on Lys147 through a JNK-regulated process. FEBS J 2008; 275:386-96; PMID:18093184; http://dx.doi.org/10.1111/j.1742-4658.2007.06209.x.

18. Vega FM, Ridley AJ. Rho GTPases in cancer cell biology. FEBS Lett 2008; 582:2093-101; PMID:18460342; http://dx.doi.org/10.1016/j.febslet.2008.04.039.

19. de la Vega M, Burrows JF, Johnston JA. Ubiquitination: Added complexity in Ras and Rho family GTPase function. Small GTPases 2011; 2:192-201; PMID:22145991; http://dx.doi.org/10.4161/sgr.2.4.16707.

20. Castillo-Lluva S, Tatham MH, Jones RC, Jaffrey EG, Edmondson RD, Hay RT, et al. SUMOylation of the GTase Rac1 is required for optimal cell migration. Nat Cell Biol 2010; 12:1078-95; PMID:20955639; http://dx.doi.org/10.1038/ncb2112.

21. Nerhe M, Hordijk PL. The role of ubiquitination and degradation in RhoGTPase signalling. J Cell Sci 2010; 123:4011-8; PMID:21084561; http://dx.doi.org/10.1242/jcs.078360.

22. Hesshko A, Ciechanover A. The ubiquitin system. Annu Rev Biochem 1998; 67:425-79; PMID:9759494; http://dx.doi.org/10.1146/annurev.biochem.67.1.425.

23. Haghund K, Dikie I. Ubiquitination and cell signaling. EMBO J 2005; 24:3533-9; PMID:16148945; http://dx.doi.org/10.1038/emboj.2006.0808.

24. Vucic D, Dixit VM, Wertz IE. Ubiquitination in apoptosis: a post-translational modification at the edge of life and death. Nat Rev Mol Cell Biol 2011; 12:439-52; PMID:21657991; http://dx.doi.org/10.1038/nrm3143.

25. Ye Y, Rape M. Building ubiquitin chains: E2 enzymes at work. Nat Rev Mol Cell Biol 2009; 10:755-64; PMID:19851334; http://dx.doi.org/10.1038/nrm2780.
26. Lemonnier M, Landraud L, Lemichez E. Rho GTPase-activating bacterial toxins: from bacterial virulence regulation to eukaryotic cell biology. FEMS Microbiol Rev 2007; 31:515-34; PMID:17680807; http://dx.doi.org/10.1111/j.1574-6976.2007.00078.x.

27. Torrino S, Visvikis O, Doye A, Boyer L, Stefani C, Munro P, et al. The E3 ubiquitin-ligase HACE1 catalyzes the ubiquitylation of active Rac1. Dev Cell 2011; 21:159-65; PMID:22006506; http://dx.doi.org/10.1016/j.devcel.2011.08.015.

28. Silke J, Kratina T, Chui D, Eckert PG, Day CL, Pakusch M, et al. Determination of cell survival by RING-mediated regulation of inhibitor of apoptosis (IAP) protein abundance. Proc Natl Acad Sci USA 2011; 108:1682-7; PMID:21626936; http://dx.doi.org/10.1073/pnas.0502828102.

29. Rajalingam K, Sharma M, Paland N, Hurwitz R, Thieck O, Oswald M, et al. IAP-IAP complexes required for apoptosis resistance of C. trachomatis-infected cells. PLoS Pathog 2006; 2:114; PMID:17069460; http://dx.doi.org/10.1371/journal.ppat.0020114.

30. Boulter E, Garcia-Maza R, Guilloy C, Dubash A, Rossi G, Beennwald PJ, et al. Regulation of Rho GTPase crosstalk, degradation and activity by RhoGDI1. Nat Cell Biol 2010; 12:477-83; PMID:19990198; http://dx.doi.org/10.1038/ncb2049.

31. Nerhe M, Anthony EC, Fernandez-Borja M, Dee R, Geerts D, Hensbergen PJ, et al. Focal-adhesion targeting links caveolin-1 to a Rac1-degradation pathway. J Cell Sci 2010; 123:1948-58; PMID:20460433; http://dx.doi.org/10.1042/JCS20091021.

32. Kim J, Park J, Choi S, Chi SG, Mowbray AL, Jo H, et al. X-linked inhibitor of apoptosis protein is an important regulator of vascular endothelial growth factor-dependent bovine aortic endothelial cell survival. Circ Res 2008; 102:896-904; PMID:18309102; http://dx.doi.org/10.1161/CIRCRESAHA.107.163667.

33. Sandrock K, Bielek H, Schrati K, Schmidt G, Klugbauer N. The nuclear import of the small GTPase Rac1 is mediated by the direct interaction with karyopherin alpha2. Traffic 2010; 11:198-209; PMID:19961560; http://dx.doi.org/10.1111/j.1600-0854.2009.00185.x.

34. Conze DB, Albert L, Ferrick DA, Goeddel DV, Yeh WC, Mak T, et al. Posttranscriptional downregulation of c-IP2 by the ubiquitin protein ligase c-IAP1 in vivo. Mol Cell Biol 2005; 25:3548-56; PMID:15798218; http://dx.doi.org/10.1128/MCB.25.8.3348-56.2005.

35. Geisbrecht ER, Montell DJ. A role for Drosophila IAP1-mediated caspase inhibition in Rac-dependent cell migration. Cell 2004; 118:111-25; PMID:15242648; http://dx.doi.org/10.1016/j.cell.2004.06.020.

36. Köster RW, Fraser SE. Direct imaging of in vivo neuronal migration in the developing cerebellum. Curr Biol 2001; 11:1858-63; PMID:11728308; http://dx.doi.org/10.1016/S0960-9822(01)00585-1.

37. Riegger S, Senghas N, Walch A, Köster RW. Cadherin-2 controls directional chain migration of cerebellar granule neurons. PLoS Biol 2009; 7:1000240; PMID:19901980; http://dx.doi.org/10.1371/journal.pbio.1000240.

38. Mack NA, Whalley HJ, Castillo-Lluna S, Malliri A. The diverse roles of Rac signaling in tumorigenesis. Cell Cycle 2011; 10:1571-81; PMID:21478669; http://dx.doi.org/10.4161/cc.10.10.15612.

39. Lopez J, John SW, Tenev T, Raatureau GJ, Gins M, Francoalca F, et al. CARD-mediated autoinhibition of c-IAP1's E3 ligase activity suppresses cell proliferation and migration. Mol Cell 2011; 42:569-83; PMID:21546926; http://dx.doi.org/10.1016/j.molcel.2011.04.008.

40. Liu J, Zhang D, Luo W, Yu Y, Yu J, Li J, et al. X-linked inhibitor of apoptosis protein (XIAP) mediates cancer cell motility via Rho GDP dissociation inhibitor (RhoGDI)-dependent regulation of the cytoskeleton. J Biol Chem 2011; 286:15650-40; PMID:21402697; http://dx.doi.org/10.1074/jbc.M110.176982.