Stabilization of Physical RAF/14-3-3 Interaction by Cotylenin A as Treatment Strategy for RAS Mutant Cancers

ARTICLE *in* ACS CHEMICAL BIOLOGY · JUNE 2013

Impact Factor: 5.33 · DOI: 10.1021/cb4003464 · Source: PubMed

11 AUTHORS, INCLUDING:

Manuela Molzan
Max Planck Institute of Molecular Physiology
6 PUBLICATIONS **94** CITATIONS
SEE PROFILE

Stefan Kasper
University Hospital Essen
29 PUBLICATIONS **471** CITATIONS
SEE PROFILE

Lars Röglin
Max Planck Institute of Molecular Physiology
21 PUBLICATIONS **467** CITATIONS
SEE PROFILE

Christian Ottmann
Technische Universiteit Eindhoven
83 PUBLICATIONS **1,382** CITATIONS
SEE PROFILE

ACS chemical biology

Letters

pubs.acs.org/acschemicalbiology

Stabilization of Physical RAF/14-3-3 Interaction by Cotylenin A as Treatment Strategy for RAS Mutant Cancers

Manuela Molzan,[†,#] Stefan Kasper,[‡,#] Lars Röglin,[†] Malgorzata Skwarczynska,[†] Takeshi Sassa,[§] Takatsugu Inoue,[∥] Frank Breitenbuecher,[‡] Junko Ohkanda,[∥,⊥] Nobuo Kato,*[,∥] Martin Schuler,*[,‡,▽] and Christian Ottmann*[,†,¶]

[†]Chemical Genomics Centre of the Max-Planck-Society, Otto-Hahn-Strasse 15, 44227 Dortmund, Germany

[‡]Department of Medical Oncology, West German Cancer Center, University Hospital Essen, Essen, Germany

[§]Department of Bioresource Engineering, Yamagata University, Tsuruoka,Yamagata, Japan

[∥]The Institute of Scientific and Industrial Research, Osaka University, Ibaraki, Osaka, Japan

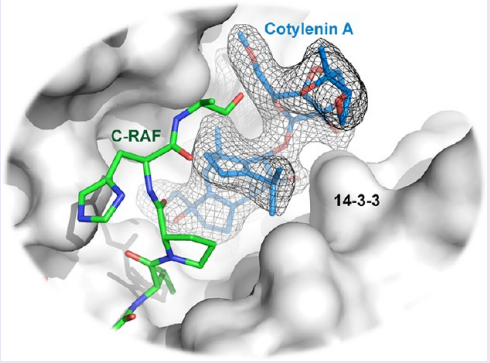
[⊥]Institute for Chemical Research, Kyoto University, Uji, Kyoto, Japan

[▽]German Cancer Consortium (DKTK), Heidelberg, Germany

[¶]Laboratory of Chemical Biology, Department of Biomedical Engineering, Eindhoven University of Technology, Den Dolech 2, 5612 AZ Eindhoven, The Netherlands.

Supporting Information

ABSTRACT: One-third of all human cancers harbor somatic *RAS* mutations. This leads to aberrant activation of downstream signaling pathways involving the RAF kinases. Current ATP-competitive RAF inhibitors are active in cancers with somatic RAF mutations, such as $BRAF^{V600}$ mutant melanomas. However, they paradoxically promote the growth of *RAS* mutant tumors, partly due to the complex interplay between different homo- and heterodimers of A-RAF, B-RAF, and C-RAF. Based on pathway analysis and structure-guided compound identification, we describe the natural product cotylenin-A (CN-A) as stabilizer of the physical interaction of C-RAF with 14-3-3 proteins. CN-A binds to inhibitory 14-3-3 interaction sites of C-RAF, pSer233, and pSer259, but not to the activating interaction site, pSer621. While CN-A alone is inactive in *RAS* mutant cancer models, combined treatment with CN-A and an anti-EGFR antibody synergistically suppresses tumor growth *in vitro* and *in vivo*. This defines a novel pharmacologic strategy for treatment of *RAS* mutant cancers.



One third of all human cancers harbor somatic RAS mutations. This leads to aberrant activation of downstream signaling pathways involving the RAF kinases. Recently, ATP-competitive RAF inhibitors have demonstrated preclinical and clinical activity in melanomas harboring B-RAF(V600) mutations, as exemplified by the approval of vemurafenib.[1] In the absence of mutant B-RAF vemurafenib seems inactive. In fact it may paradoxically activate MAPK signaling, which is explained by the complex interplay between the three RAF isoforms.[2] Moreover, promotion of RAS mutant cancers and leukemias has been described in patients treated with vemurafenib.[3] These findings seem to dismiss the development of ATP-competitive inhibitors of RAF kinases for treatment of RAS mutant cancers. However, the target itself is of high relevance and may demand more innovative pharmacologic strategies.

C-RAF activity is tightly regulated by phosphorylation and protein−protein interaction with the 14-3-3 proteins. Three 14-3-3 adapter protein binding sites have been identified in C-RAF: S233, S259, and S621.[4,5] Binding of 14-3-3 to C-RAF S233 and S259 plays an inhibitory role,[4] while 14-3-3 binding to Ser621 is reported to activate C-RAF.[5] 14-3-3 proteins are small (25−30 kDa) eukaryotic adapter proteins implicated in cancer pathophysiology.[6] Previously we have reported in structural detail how the natural products fusicoccin A (FC-A), and cotylenin A (CN-A) modulate the activated protein complex between the plant plasma membrane H^+-ATPase and 14-3-3 adapter proteins.[7,8] Since CN-A shows activity against cancer cells, we reasoned that this activity might at least partially be conferred by modulation of C-RAF/14-3-3 interactions.

To investigate whether CN-A can stabilize the inhibitory C-RAFpS233pS259/14-3-3ζ complex, anisotropy measurements were carried out employing a fluorescein-labeled, diphosphory-

Received: May 15, 2013
Accepted: June 28, 2013
Published: June 28, 2013

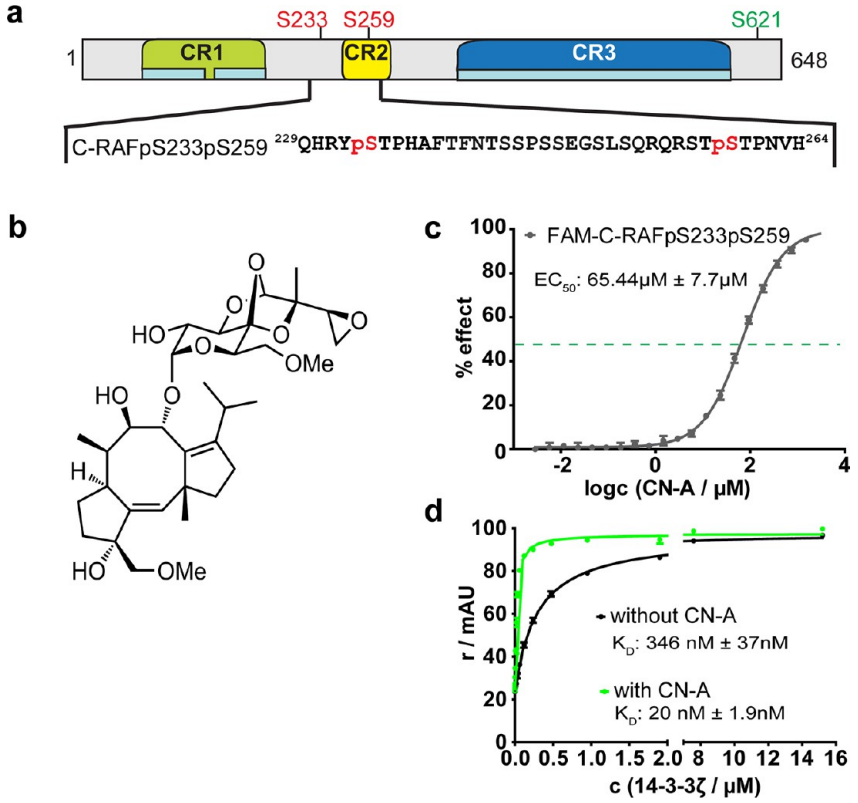


Figure 1. CN-A acting on the C-RAFpS233pS259/14-3-3ζ complex. (a) Schematic representation of the phosphorylated C-RAF peptide used for the biochemical measurements. (b) Structure of CN-A. (c) CN-A titration of the FAM-labeled C-RAF phosphopeptide (20 nM in 10 mM HEPES, 150 mM NaCl, 0.1% Tween-20, 0.1% BSA, pH 7.4) as depicted in panel a in the presence of 14-3-3ζ (0.25 μM) and subsequent anisotropy measurement. (d) Titration of C-RAFpS233pS259 peptide with 14-3-3ζ with and without CN-A. The affinity in the presence of 500 μM CN-A is increased 17-fold to 20 nM compared to the affinity in the absence of CN-A (346 nM).

lated C-RAFpS233pS259 peptide (Figure 1a). The EC_{50} value of CN-A acting on the 14-3-3ζ/C-RAFpS233pS259 complex was calculated to be in the range of 60 μM (Figure 1b). Subsequently, the influence of CN-A on the apparent affinity of this peptide to 14-3-3ζ was measured. In the presence of 500 μM CN-A the K_D of 14-3-3ζ and the diphosphorylated C-RAFpS233pS259 peptide was lowered 17-fold from 346 to 20 nM, which can be explained by a stabilizing effect of CN-A on this interaction (Figure 1c).

Next, we explored the structural basis of CN-A's activity. We soaked crystals of the binary 14-3-3ζ/C-RAFpS233pS259 complex[9] with a solution containing CN-A, allowing the ternary structure to be solved at 2.20 Å (PDB ID: 4IHL). CN-A bound to the interface of both phosphorylation sites of the C-RAF peptide and 14-3-3 with the binding pockets of CN-A in the complex composed of both the 14-3-3 protein and C-RAF (Figure 2). Since in addition to the two inhibitory 14-3-3 binding sites (pS233, pS259) C-RAF harbors one activating site (pS621) as well, any strategy to modulate this protein−protein interaction must be site-specific. To precisely compare the respective interfaces of the activatory and the inhibitory sites, we co-crystallized the peptide C-RAFpS621 (residues 618−625) in complex with 14-3-3. The structure of C-RAFpS621/14-3-3σ (PDB ID: 4IEA) was solved to 1.7 Å by molecular replacement using 14-3-3σ (PDB ID: 1YWT) as a template (Figure 3a). Binding of the C-RAFpS621 peptide to 14-3-3 was similar to that of C-RAFpS233 and C-RAFpS259, the most important difference being the polar contacts established between the side chain of E622 of C-RAF and K49 and K122 of 14-3-3 (Figure 3a). This residue likely determines the specificity for the stabilization of the inhibitory C-RAF/14-3-3 interaction. Superimposition of the pS621 binding site with the CN-A-complexed binding sites pS233 and pS259 revealed a severe sterical and electrostatical conflict between the side chain of E622 and the ring system of CN-A (Figure 3b). The terminal carboxyl group of E622 not only clashes with the C3-hydroxyl of ring A in CN-A but in general causes a structural incompatibility with the simultaneous binding of the fusicoccane 5-8-5 ring system. This was confirmed by biophysical binding studies employing FAM-labeled phosphopeptides. Synthetic peptides of the three binding sites of C-RAF bound to 14-3-3 with comparable affinities in the low-micromolar range (Figure 3c). In contrast, titration with CN-A showed that it only stabilizes the binding of C-RAFpS259 and not C-RAFpS621 to 14-3-3ζ (Figure 3d). In support, binding of a C-RAFpS259,T260E peptide, in which threonine 260 has been changed to glutamic acid, to 14-3-3 was measured in the expected low-micromolar Kd range (Figure 3c) but could not be stabilized by CN-A (Figure 3d).

To study the potential of CN-A to specifically overcome RAS-dependent survival mechanisms, we devised A431 and Difi cancer cells. Both models are highly sensitive to the anti-EGFR antibody cetuximab *in vitro* and *in vivo*. Introduction of mutant KRAS, HRAS, or NRAS confers resistance of these cells against the clinically approved anti-EGFR antibodies cetuximab and panitumumab.[10] Neither effective doses of cetuximab nor CN-A at high micromolar concentrations reduced the viability of Difi-HRASG12 V cells. Interestingly, the combination of

dx.doi.org/10.1021/cb4003464 | *ACS Chem. Biol.* 2013, 8, 1869−1875

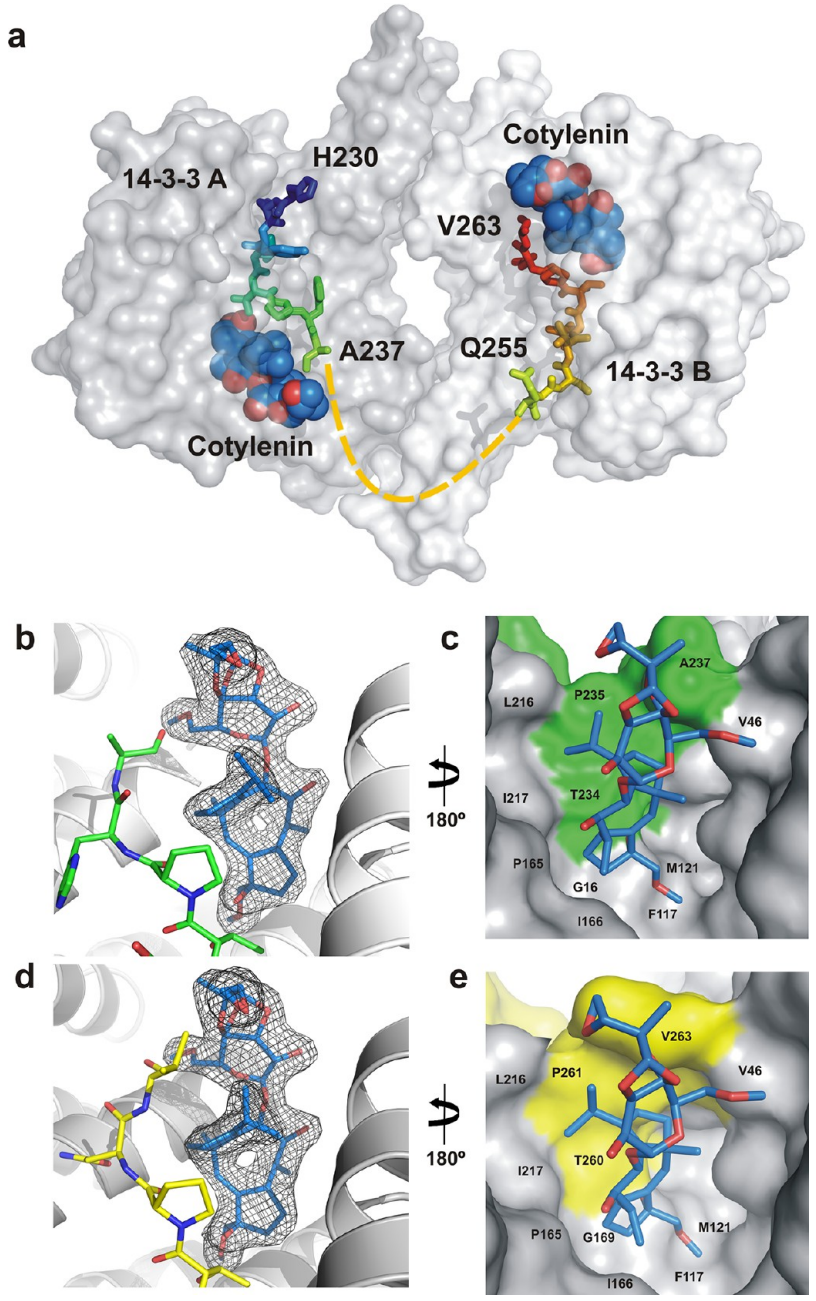


Figure 2. Structure of the ternary C-RAFpS233pS259/14-3-3ζ/CN-A complex. (a) Surface representation of the 14-3-3ζ dimer (light and dark gray) in complex with C-RAFpS233pS259 (rainbow-colored sticks) and CN-A (blue and red spheres). (b) Final $2F_o - F_c$ electron density (contoured at 1σ) for CN-A (blue sticks) bound to the interface of pS233 (green sticks) or (d) the pS259 site (yellow sticks) in C-RAF and 14-3-3ζ (gray ribbon). (c) Surface representation of the composite binding pocket of CN-A at the interface of 14-3-3 (gray) and C-RAFpS233 (green) or (e) C-RAFpS259 (yellow). Residues directly contacting CN-A are labeled.

cetuximab and CN-A was highly cytotoxic (Figure 4a). As expected the combination of cetuximab with a MEK inhibitor, U0126, was also efficacious. However, MEK inhibition was cytotoxic in itself (Figure 4a), which is in line with its dose-limiting clinical toxicities that partially overlap with those of cetuximab.[11] In line with observations with ATP-competitive B-RAF inhibitors,[2] CN-A induced activation of ERK, which was substantially attenuated by simultaneous treatment with cetuximab *in vitro* (Figure 4b). Transplanting A431 cancer cells into NOD/SCID mice leads to rapid tumor development, which can be prevented by cetuximab treatment. In this model, cetuximab acts as an inhibitor of EGFR signaling and activator of antibody-dependent cellular cytotoxicity, and both effector mechanisms are blocked by oncogenic RAS.[10] CN-A combined with an ineffective control antibody had no impact on development and growth of A431-HRASG12 V tumors. In contrast, CN-A significantly enhanced the therapeutic efficacy of cetuximab on A431-HRASG12 V tumor *in vivo* (Figure 4c). In summary, combined treatment with CN-A and cetuximab is effective in RAS mutant cancer models *in vitro* and *in vivo*. The absence of any apparent cytotoxicity of CN-A alone provides an opportunity to direct the combined cytotoxicity of CN-A and cetuximab toward cancer cells with high expression of EGFR.

dx.doi.org/10.1021/cb4003464 | *ACS Chem. Biol.* 2013, 8, 1869–1875

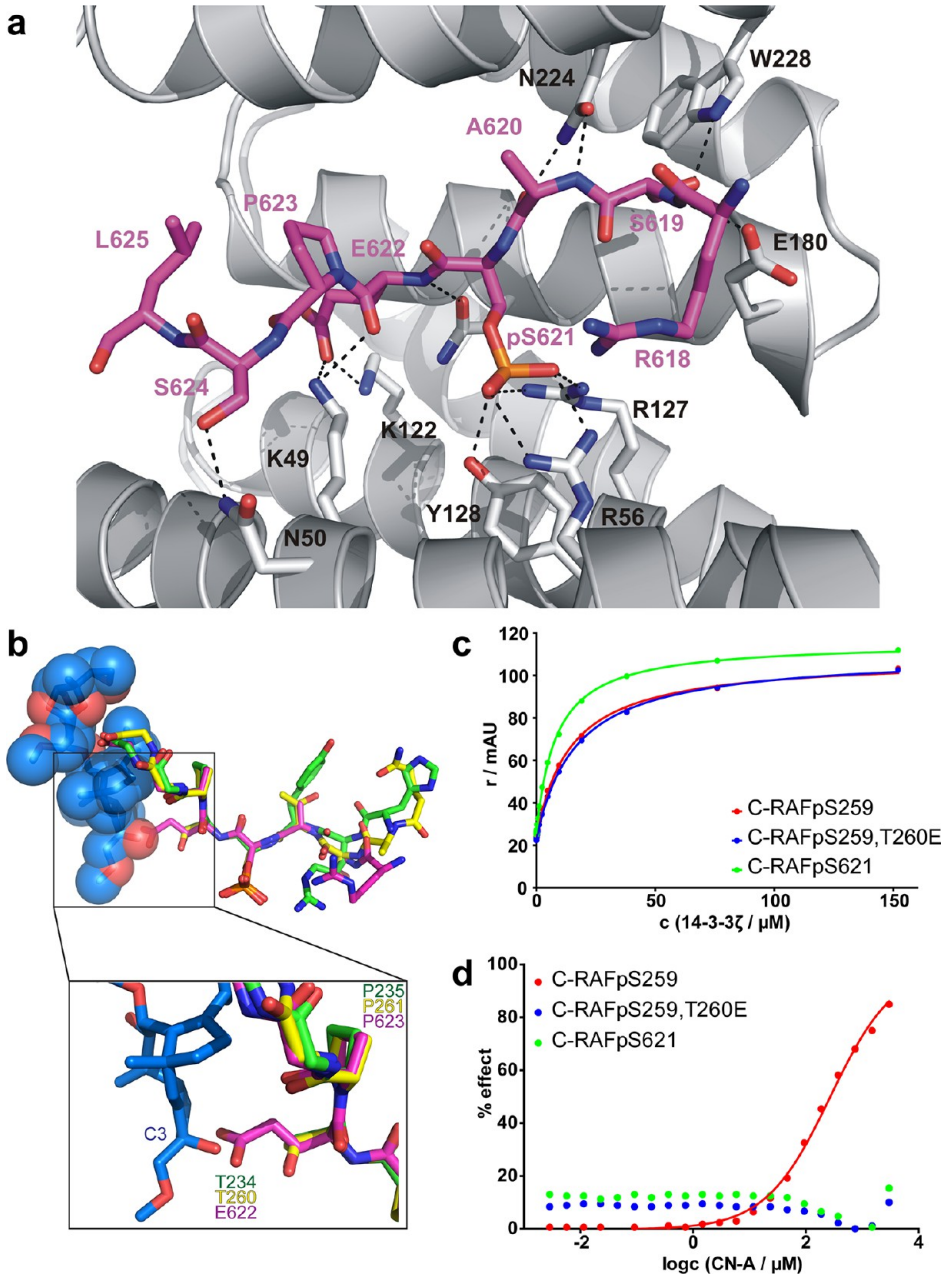


Figure 3. Specificity of CN-A for the N-terminal, inhibitory 14-3-3 interaction sites. (a) Electrostatic interactions (dotted lines) between C-RAF residues surrounding pS621 (magenta sticks) and 14-3-3ζ (gray sticks). (b) Superimposition of C-RAFpS233 (green), C-RAFpS259 (yellow), C-RAFpS61 (magenta), and CN-A (blue) bound to 14-3-3. (c) Binding of FAM-labeled C-RAFpS259, C-RAFpS621, or C-RAFpS259T260E peptides (20 nM) to 14-3-3ζ as measured by anisotropy. (d) Titration of CN-A to the FAM-labeled peptides used in panel c in the presence of 14-3-3ζ (8 μM) and subsequent anisotropy measurement. Only for the interaction of FAM-labeled C-RAFpS259 and 14-3-3ζ a stabilizing effect of CN-A can be observed.

The modulation of specific regulatory protein−protein interaction is an alternative pharmacologic strategy to active site inhibitors.[12] As demonstrated by the immunosuppressant rapamycin,[13] a small molecule that confers its biological activity by stabilizing a protein−protein interaction can display a formidable therapeutic effect. Compared to active site- or PPI-inhibitiors, stabilizers of PPIs exhibit certain features that might be therapeutically advantageous.[14] For example, the non-competitive nature of PPI-stabilizing small molecules explains the observation that such compounds do not always have to bind to their targets with high affinity to produce a significant physiological response, as in the case of brefeldin A.[15] The 14-3-3 binding sites in all three RAF kinases, A-RAF, B-RAF, and C-RAF, are largely conserved with the respective +1 amino acids that determine the ability of stabilization by CN-A being identical in all three isoform. This situation justifies the assumption that CN-A can stabilize 14-3-3 binding to the N-termini of all three RAF kinases and thereby attenuate the activity of A-, B-, and C-RAF. We certainly do not exclude the possibility that CN-A also acts on other 14-3-3 PPIs. In fact, the observation that CN-A shows *in vivo* activity at concentrations (1 μM) well below the EC_{50} of the *in vitro* measurement of the 14-3-3/C-RAFpS233pS259 interaction (60 μM) favors the view of a multitarget nature of CN-A. Therefore, the current

dx.doi.org/10.1021/cb4003464 | *ACS Chem. Biol.* 2013, 8, 1869−1875

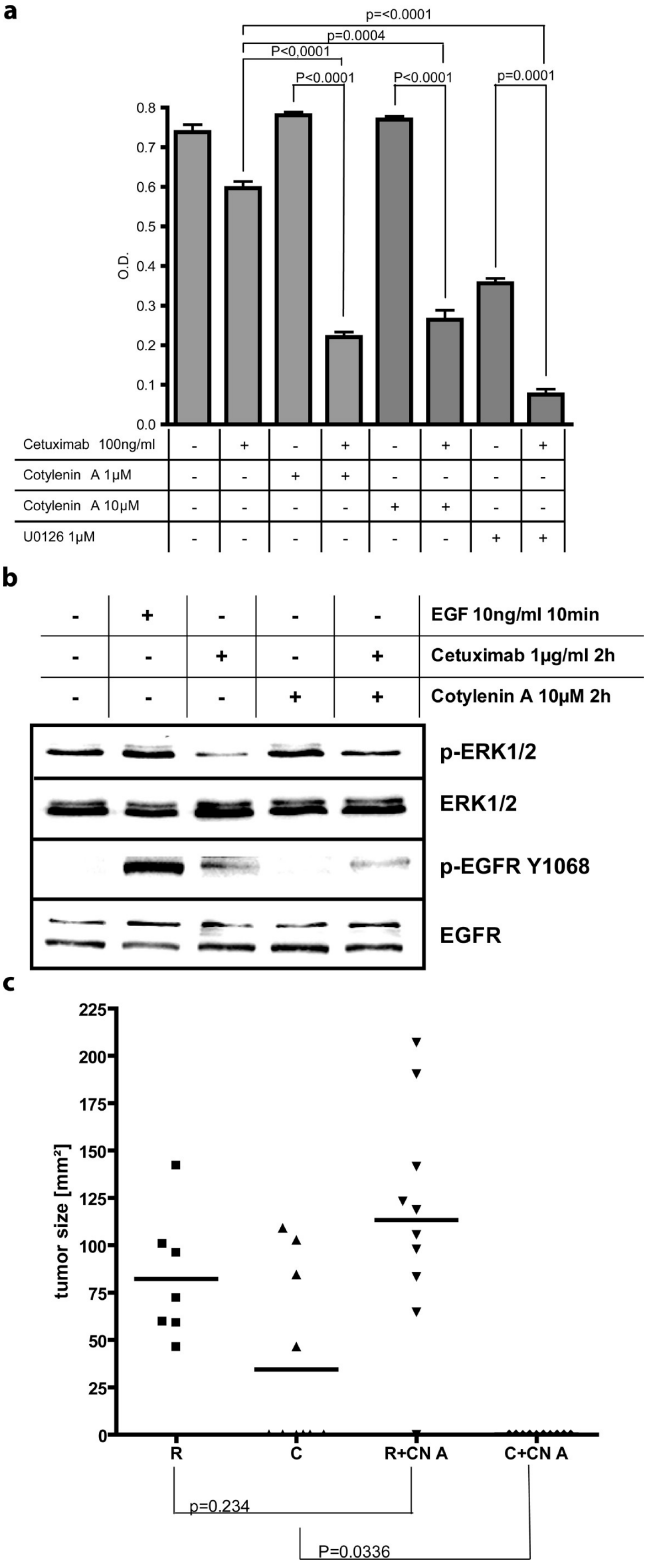


Figure 4. Anticancer activity of CN-A *in vitro* and *in vivo*. (a) Proliferation of Difi cells grown in the presence of CN-A, cetuximab, and the MEK inhibitor U0126 alone or in combination. Mean values (+ SD) of three independent experiments. (b) Whole cell lysates from A431-HRASG12 V cells were incubated with EGF, cetuximab, and CN-A, and combinations were analyzed by immunoblotting using phosphospecific antibodies for ERK and EGFR. Reprobing with anti-ERK and anti-EGFR served as loading control. (c) NOD/SCID mice received subcutaneous injections of A431-HRASG12 V cells on day 0. Starting on day 1 (arrow) mice were treated twice weekly by intraperitoneal injections of cetuximab or the control antibody rituximab (0.5 mg each) in combination with subcutaneous injections of CN-A (100 μg) or vehicle every other day. Tumor development was monitored by palpation, and tumors were measured using a caliper. Individual bidimensional tumor sizes and mean tumor size of 10 tumors per treatment group at day 10 are given. *P*-values were calculated by *t* test.

study may not represent the single structural explanation for the observed biological effects of combined treatment of RAS mutant cancer models with CN-A and cetuximab. It rather serves as an example of the mechanism of a new type of a small molecule mode of action. What PPIs of 14-3-3s with proteins other than RAF kinases might be influenced by CN-A or if additional, non-14-3-3 targets might play a role in CN-A biology has to be determined to exploit the full therapeutic potential of this natural product. In this report we provide evidence that it is possible to address the C-RAF/14-3-3 interaction with a small molecule leading to stabilization of the complex. Especially in the context of the possibility to identify further, less complex molecules stabilizing a target 14-3-3 PPI,[16,17] this establishes a novel therapeutic strategy for a major problem in oncology, which cannot be effectively overcome with the currently available clinical armamentarium.

METHODS

Crystallization and Biophysical Measurements. Detailed information for protein production, crystallization, and structure determination appears in Supporting Information, including data reduction and refinement statistics in Supplementary Table S2 and detailed description of anisotropy measurements.

Cell Lines and Reagents. The human EGFR-positive cancer cell lines A431 and Difi were obtained from DSMZ (Braunschweig, Germany) and Dr. Robert Coffey (Nashville, Tennessee), respectively. All cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS, PAA, Coelbe, Germany), L-glutamine, penicillin, and streptomycin (Invitrogen, Frankfurt, Germany). Stable expression of the HRASG12 V, cDNAs was achieved by retroviral transduction. Clinical grade cetuximab (Erbitux, Merck Serono, Darmstadt, Germany) and rituximab (Mabthera, Roche, Grenzach-Wyhlen, Germany) were purchased from the pharmacy of the University Hospital Essen. The following primary antibodies were used for immunoblotting and immunolabeling following standard protocols: phospho-ERK1/2 (T202/Y204), ERK1/2, EGFR, phospho-EGFR(Y1068) (all from Cell Signaling Technology, Danvers, MA).

Cellular Assays. Proliferation was quantified by means of the MTT assay according to the manufacturer's instruction (Roche, Mannheim, Germany). All results were obtained from at least three independent experiments. For statistical analysis the unpaired *t* test was used.

Animal Models. All animal studies were conducted in compliance with institutional guidelines and German Animal Protection Law and were approved by the responsible regulatory authority (Landesamt für Natur, Umwelt and Verbraucherschutz Nordrhein-Westfalen, Az. G969/08). NOD/SCID mice (Charles River Laboratories, France) received single subcutaneous flank injections of 2×10^6 A431-HRASG12 V cells suspended in 200 μL of saline. Animals were monitored for tumor development twice weekly, and tumor growth was bidimensionally quantified using a caliper. Antibodies were dissolved in 200 μL of saline and were administered as biweekly intraperitoneal injections. A stock solution of CN-A for administration was prepared in DMSO at 100 mg mL^{-1}. Mice received subcutaneous injections every other day of 0.2 mL of PBS, including 100 μg CN-A. Kaplan–Meier plots for tumor-free survival were analyzed using the

dx.doi.org/10.1021/cb4003464 | *ACS Chem. Biol.* 2013, 8, 1869–1875

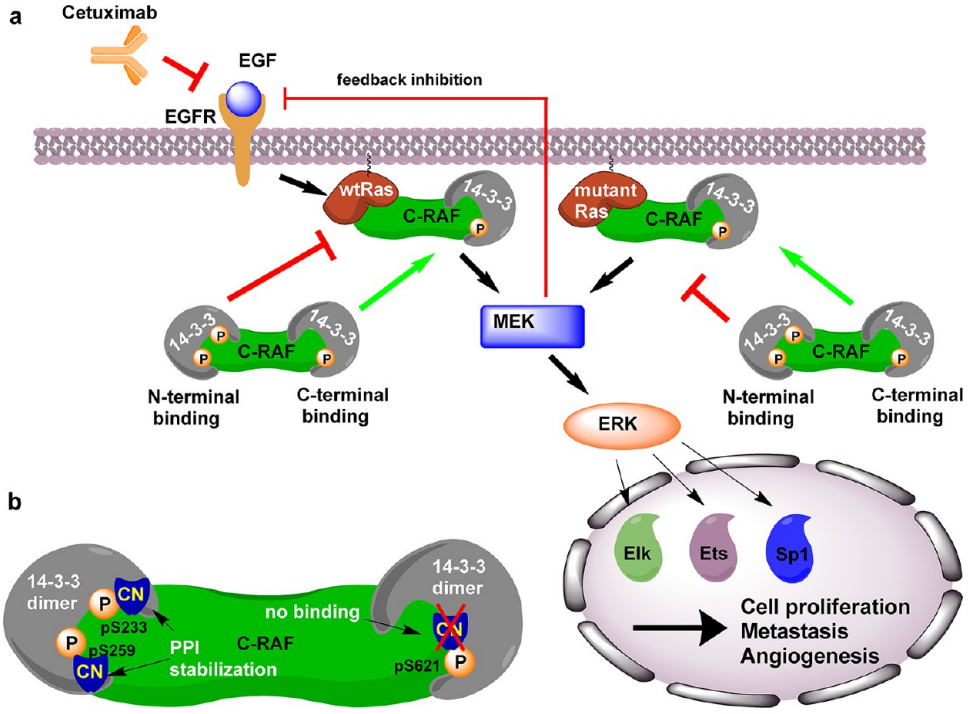


Figure 5. Role of 14-3-3 in C-RAF regulation. (a) 14-3-3 proteins are important regulators of C-RAF. Binding of 14-3-3 to the N-terminus of C-RAF prevents Ras-mediated plasma membrane recruitment and activation of C-RAF, whereas C-terminal binding of 14-3-3 promotes C-RAF activation. The RAF kinases are the central effectors of Ras signaling. Whereas wtRas is dependent on activation by receptor tyrosine kinases (RTKs) such as the EGF receptor (EGFR), oncogenic mutant Ras is constitutively activated. The pro-proliferative Ras-RAF-MEK-ERK pathway is often activated in cancer with both mutant Ras signaling as well as RTK-dependent wtRas signaling contributing to oncogenesis. Hence simultaneous inhibition of EGFR and C-RAF can lead to a synergistic effect on cancer cells. (b) Cotylenin A (CN) discriminates between the pS233/pS259 and the pS621 14-3-3 binding site in C-RAF and stabilizes only the inhibitory binding of 14-3-3 to the N-terminus of C-RAF.

log rank test. For statistical analysis of tumor size the unpaired *t* test was used.

■ ASSOCIATED CONTENT

Supporting Information

Additional experimental details and the crystallographic data table. This material is available free of charge *via* the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: kato-n@sanken.osaka-u.ac.jp; Martin.Schuler@uk-essen.de; c.ottmann@tue.nl; christian.ottmann@cgc.mpg.de.

Author Contributions

#These authors contributed equally to this work.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

C.O. thanks AstraZeneca, Bayer CropScience, Bayer Healthcare, Boehringer Ingelheim, and Merck KGaA for support. We thank the SLS beamline staff for data collection at the Swiss Light Source, beamline PXII-X10SA, Paul Scherrer Institute, Villigen, Switzerland. We thank J. Markowetz, S. Stergar, and S. Hoffarth for technical assistance and R. Rose and P. Thiel for helpful interpretation and discussion of crystallographic data. M.S. acknowledges grant support from the Deutsche Forschungsgemeinschaft (SCHU 1541/5-1), Deutsche Krebshilfe (107993), Wilhelm Sander-Stiftung (2005.136.3), and Bio.NRW (z0911bt026f). S.K. and C.O. received grant support from MERCUR (An-2011-0031).

■ REFERENCES

(1) Sosman, J. A., Kim, K. B., Schuchter, L., Gonzalez, R., Pavlick, A. C., Weber, J. S., McArthur, G. A., Hutson, T. E., Moschos, S. J., Flaherty, K. T., Hersey, P., Kefford, R., Lawrence, D., Puzanov, I., Lewis, K. D., Amaravadi, R. K., Chmielowski, B., Lawrence, H. J., Shyr, Y., Ye, F., Li, J., Nolop, K. B., Lee, R. J., Joe, A. K., and Ribas, A. (2012) Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib. *N. Engl. J. Med. 366*, 707−714.

(2) Poulikakos, P. I., Zhang, C., Bollag, G., Shokat, K. M., and Rosen, N. (2010) RAF inhibitors transactivate RAF dimers and ERK signalling in cells with wild-type BRAF. *Nature 464*, 427−430.

(3) Callahan, M. K., Rampal, R., Harding, J. J., Klimek, V. M., Chung, Y. R., Merghoub, T., Wolchok, J. D., Solit, D. B., Rosen, N., Abdel-Wahab, O., Levine, R. L., and Chapman, P. B. (2012) Progression of RAS-mutant leukemia during RAF inhibitor treatment. *N. Engl. J. Med. 367*, 2316−2321.

(4) Dumaz, N., and Marais, R. (2003) Protein kinase A blocks Raf-1 activity by stimulating 14−3-3 binding and blocking Raf-1 interaction with Ras. *J. Biol. Chem. 278*, 29819−29823.

(5) Tzivion, G., Luo, Z., and Avruch, J. (1998) A dimeric 14−3-3 protein is an essential cofactor for Raf kinase activity. *Nature 394*, 536−539.

(6) Morrison, D. K. (2009) The 14−3-3 proteins: integrators of diverse signaling cues that impact cell fate and cancer development. *Trends Cell Biol. 19*, 16−23.

(7) Ottmann, C., Marco, S., Jaspert, N., Marcon, C., Schauer, N., Weyand, M., Vandermeeren, C., Duby, G., Boutry, M., Wittinghofer, A., Rigaud, J. L., and Oecking, C. (2007) Structure of a 14−3-3 coordinated hexamer of the plant plasma membrane H^+-ATPase by

combining X-ray crystallography and electron cryomicroscopy. *Mol. Cell 25*, 427−440.
(8) Ottmann, C., Weyand, M., Sassa, T., Inoue, T., Kato, N., Wittinghofer, A., and Oecking, C. (2009) A structural rationale for selective stabilization of anti-tumor interactions of 14−3-3 proteins by cotylenin A. *J. Mol. Biol. 386*, 913−919.
(9) Molzan, M., and Ottmann, C. (2012) *J. Mol. Biol. 423*, 486−495.
(10) Kasper, S., Breitenbuecher, F., Reis, H., Brandau, S., Worm, K., Köhler, J., Paul, A., Trarbach, T., Schmid, K. W., and Schuler, M. (2012) Oncogenic RAS simultaneously protects against anti-EGFR antibody-dependent cellular cytotoxicity and EGFR signaling blockade. *Oncogene 32*, 2873−2881.
(11) Leijen, S., Middleton, M. R., Tresca, P., Kraeber-Bodéré, F., Dieras, V., Scheulen, M. E., Gupta, A., Lopez-Valverde, V., Xu, Z. X., Rueger, R., Tessier, J. J., Shochat, E., Blotner, S., Naegelen, V. M., Schellens, J. H., and Eberhardt, W. E. (2012) Phase I dose-escalation study of the safety, pharmacokinetics, and pharmacodynamics of the MEK inhibitor RO4987655 (CH4987655) in patients with advanced solid tumors. *Clin. Cancer Res. 18*, 4794−4805.
(12) Wells, J. A., and McClendon, C. L. (2007) Reaching for high-hanging fruit in drug discovery at protein-protein interfaces. *Nature 450*, 1001−1009.
(13) Choi, J., Chen, J., Schreiber, S. L., and Clardy, J. (1996) Structure of the FKBP12-rapamycin complex interacting with the binding domain of human FRAP. *Science 273*, 239−242.
(14) Thiel, P., Kaiser, M., and Ottmann, C. (2012) Small-molecule stabilization of protein-protein interactions: an underestimated concept in drug discovery? *Angew. Chem., Int. Ed. 51*, 2012−2018.
(15) Peyroche, A., Antonny, B., Robineau, S., Acker, J., Cherfils, J., and Jackson, C. L. (1999) Brefeldin A acts to stabilize an abortive ARF-GDP-Sec7 domain protein complex: involvement of specific residues of the Sec7 domain. *Mol. Cell 3*, 275−285.
(16) Rose, R., Erdmann, S., Bovens, S., Wolf, A., Rose, M., Hennig, S., Waldmann, H., and Ottmann, C. (2010) Identification and structure of small-molecule stabilizers of 14−3-3 protein-protein interactions. *Angew. Chem., Int. Ed. 49*, 4129−4132.
(17) Milroy, L. G., Brunsveld, L., and Ottmann, C. (2012) Stabilization and inhibition of protein-protein interactions: the 14−3-3 case study. *ACS Chem. Biol. 8*, 27−35.