ously bind to EGFR domain III, the binding of Matuzumab would interfere with formation of the active-like EGFR conformation (Figure 1A, middle panel). Thus, in the presence of Matuzumab, EGF could only contact domain III (or domain I), and its affinity for EGFR would be reduced—exactly what is observed. Schmiedel et al. also show that Cetuximab and Matuzumab do not compete for binding to EGFR, as predicted from comparison of crystal structures of their complexes with EGFR, and suggest that combination therapy with Cetuximab (or IMC-11F8) and Matuzumab may result in added clinical benefit.

It is clear that basic and clinical studies of the ErbB family of receptors have come a long way in the last few years. The results from each type of inquiry has informed the other, and together, they are leading to a deeper understanding of ErbB function and how to treat ErbB-involved diseases. It is also clear that much remains to be learned, and exciting times are ahead.

REFERENCES

Agus, D.B., Akita, R.W., Fox, W.D., Lewis, G.D., Higgins, B., Pisacane, P.I., Lofgren, J.A., Tindell, C., Evans, D.P., Maiese, K., et al. (2002). Cancer Cell *2*, 127–137.

Baselga, J., Albanell, J., Molina, M.A., and Arribas, J. (2001). Semin. Oncol. *28*, 4–11.

Burgess, A.W., Cho, H.S., Eigenbrot, C., Ferguson, K.M., Garrett, T.P., Leahy, D.J., Lemmon, M.A., Sliwkowski, M.X., Ward, C.W., and Yokoyama, S. (2003). Mol. Cell *12*, 541–552.

Cho, H.S., Mason, K., Ramyar, K.X., Stanley, A.M., Gabelli, S.B., Denney, D.W., Jr., and Leahy, D.J. (2003). Nature *421*, 756–760.

Clynes, R.A., Towers, T.L., Presta, L.G., and Ravetch, J.V. (2000). Nat. Med. *6*, 443–446.

Garrett, T.P., McKern, N.M., Lou, M., Elleman, T.C., Adams, T.E., Lovrecz, G.O., Kofler, M., Jorissen, R.N., Nice, E.C., Burgess, A.W., and Ward, C.W. (2003). Mol. Cell *11*, 495–505.

Holbro, T., and Hynes, N.E. (2004). Annu. Rev. Pharmacol. Toxicol. *44*, 195–217.

Hynes, N.E., and Lane, H.A. (2005). Nat. Rev. Cancer *5*, 341–354.

Li, S., Kussie, P., and Ferguson, K.M. (2008). Structure *16*, 216–227.

Li, S., Schmitz, K.R., Jeffrey, P.D., Wiltzius, J.J., Kussie, P., and Ferguson, K.M. (2005). Cancer Cell *7*, 301–311.

Schmiedel, J., Blaukat, A., Li, S., Knoechel, T., and Ferguson, K.M. (2008). Cancer Cell, this issue.

RanBP2: A Tumor Suppressor with a New Twist on TopoII, SUMO, and Centromeres

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In vertebrate cells, the small ubiquitin-like modifier SUMO plays a poorly defined role in targeting DNA topoisomerase II (TopoII) to centromeres (*CEN*s) during mitosis, presumably to facilitate the untangling of sister chromatids as cells transition into anaphase. A new study by Dawlaty in the April 4 issue of *Cell* identifies the nucleoporin RanBP2 as a novel tumor suppressor that acts as a SUMO ligase for TopoII. Analysis of this interaction reveals TopoII recruitment to *CEN*s is likely to play an important role in preventing chromosome segregation errors that lead to cancer.

RanBP2 is a remarkably large (350 kD!) protein that contains, as its only enzymatic function, an unusual SUMO E3 ligase domain (Pichler et al., 2002). In the final step of SUMO modification, the E2 conjugating enzyme Ubc9 transfers activated SUMO moieties to lysines on substrate proteins. This reaction typically requires, or is greatly stimulated by, SUMO E3 ligases. The best understood SUMO E3s are the PIAS family of proteins, which contain a RING finger motif and promote sumolyation by recruiting substrates to the E2 enzyme (Jackson, 2001). The RanBP2 E3 domain, in contrast, fits tidily within a ~300 amino acid segment that is structurally unrelated to PIAS proteins. Rather than binding substrates, this E3 acts more like a cofactor for Ubc9, possibly serving to directly stimulate E2 catalysis (Reverter and Lima, 2005).

The cell biology of RanBP2 has also provided surprises. In addition to binding Ubc9, the RanBP2 E3 domain interacts specifically with SUMO-modified forms of RanGAP1. During interphase, this complex localizes to the cytoplasmic face of the nuclear pore. But once mitosis is underway, the entire RanBP2-SUMO~RanGAP1-Ubc9 complex partners with the nuclear export receptor Crm1 and moonlights as a component of the kinetochore (K; Arnaoutov et al., 2005). This is arguably even more important than RanBP2's day job, as RanBP2 depletion produces severe mitotic defects, including perturbations to K-microtubule (MT) attachment, mis-

localization of K and spindle assembly checkpoint (SAC) proteins, and formation of multipolar spindles (Joseph et al., 2004). How RanBP2 promotes K assembly/activity and whether SUMO ligase activity is involved in these functions is unknown.

Against this backdrop, the current study (Dawlaty et al., 2008) sets out to examine RanBP2 function in an intact organism. To circumvent lethality associated with a complete loss of RanBP2, they used a clever RNA splicing strategy to create a hypomorphic allele (*ranBP2*^{H}) that lowers RanBP2 protein levels to ~31%. *ranBP2*^{H} was combined with knockout or wild-type alleles to create an allelic series of RanBP2-deficient animals. As expected, *ranBP2*$^{-/-}$ mice died during embryogenesis, but *ranBP2*$^{-/H}$ and *ranBP2*$^{H/H}$ individuals developed normally and survived into adulthood. Over a 2 year period, these mutants succumbed to a range of cancers, primarily lung carcinomas, and were also susceptible to chemically-induced tumorigenesis. Thus, RanBP2 plays an unanticipated role in tumor suppression.

Insight into how RanBP2 might act as a tumor suppressor came with the finding that RanBP2-deficient cancer cells displayed severe chromosome instability, pointing toward aneuploidy as a factor controlling tumor initiation or progression. Consistent with this, splenocytes and embryonic fibroblasts (MEFs) derived from *ranBP*$^{-/H}$ and *ranBP*$^{H/H}$ mice exhibited chromosome segregation errors, typically gain/loss of single chromosomes, although more dramatic ploidy alterations were also observed. Given the role of RanBP2 at the K, one might suspect these missegregation events would correspond with defective chromosome-spindle attachment. Surprisingly, this did not appear to be the case, and Dawlaty et al. went to some lengths to show that spindle structure, K-MT interactions, and localization of K and SAC proteins appeared normal in *ranBP*$^{-/H}$ and *ranBP*$^{H/H}$ cells. Instead, the most prevalent mitotic defect they observed was anaphase chromosome bridging, a hallmark of interference with TopoII function.

TopoII (TopoIIα in vertebrates) is essential for chromosome segregation because it performs the DNA surgery necessary to decatenate replicated chromosomes. In vertebrates, there is a specifically regulated component of decatenation in which TopoII preferentially accumulates at *CEN* regions as cells approach the metaphase to anaphase transition (Figure 1). Previous studies have implicated sumolyation in TopoII *CEN* localization. In *Xenopus*, PIASy, a PIAS E3 family member that binds mitotic chromosomes, is required for TopoII SUMO conjugation (Azuma et al., 2005). PIASy depletion prevents SUMO conjugates from accumulating at *CEN*s and interferes with sister chromatid disjunction. In a seemingly analogous fashion, RNAi knockdown of PIASy in human cells perturbs TopoII *CEN* targeting and delays anaphase entry (Diaz-Martinez et al., 2006). Chromatid connections persist in PIASy knockdown cells even when cohesin complexes dissociate from DNA, arguing the separation block is due to reduced TopoII activity.

Based on these observations, Dawlaty et al. tested whether RanBP2 E3 activity was involved in TopoII sumolyation. To summarize their data, they showed *ranBP2*$^{H/H}$ and *ranBP2*$^{H/-}$ MEFs failed to target TopoII to *CEN*s. Significantly, the RanBP2 E3 domain was sufficient to rescue RanBP2-deficient phenotypes, including TopoII *CEN* localization and chromosome-bridging defects. The E3 domain also stimulated TopoII sumolyation in vitro, and *ranBP2*$^{-/H}$ cells (but not *PIASy*$^{-/-}$ cells) lacked detectable TopoII SUMO conjugates. Finally, an overproduced N-terminal SUMO-TopoII fusion localized to *CEN*s and prevented chromosome bridging in RanBP$^{-/H}$ mutants, providing evidence that TopoII is likely to be the SUMO substrate responsible for these phenotypes. There is a potential caveat, as this construct was not compared to equivalently overproduced unfused TopoII. Nonetheless, the data suggest that, at least in MEFs, RanBP2 rather than PIASy is the E3 ligase used for TopoII sumolyation. Different cells may employ different E3s, and a next step should be to clarify the roles of PIASy and RanBP2 in human cells. The more significant conclusion, however, is that RanBP2's role in maintaining chromosome stability and potentially also tumor suppression appears to be directed through TopoII sumolyation. It will be important to determine whether these aspects of RanBP2 and TopoII function are associated with spontaneous or chemotherapy-induced tumors in humans.

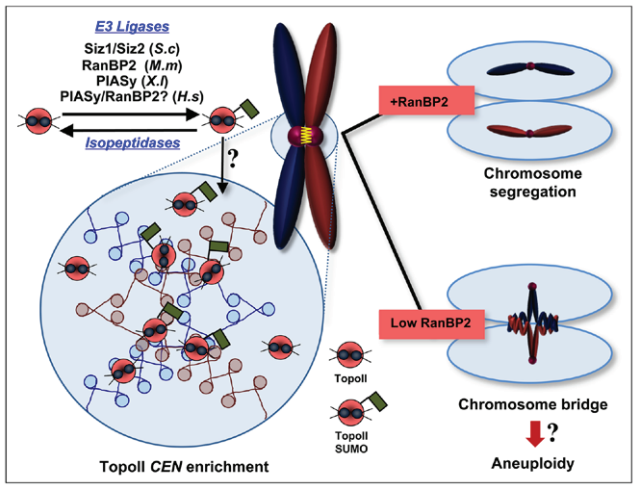

Figure 1. TopoII Sumolyation and Function within *CEN* Chromatin
TopoII sumolyation has been described in a number of organisms, occurring during mitosis and in response to some forms of DNA damage. In yeast, the SUMO acceptor lysines are located in the noncatalytic C terminus of the protein. Current information regarding SUMO E3 ligases catalyzing TopoII sumolyation is shown; the role of E1 and E2 enzymes is not depicted. SUMO isopeptidases (Ulp2/Smt4 in yeast, presumably a member of the SENP family in vertebrates) deconjugate SUMO from Topo II. Although the underlying mechanism is unclear (?), sumolyation facilitates TopoII enrichment to *CEN* regions of vertebrate chromosomes, presumably to help resolve a final population of catenates so chromatids disjoin properly—the act of segregation may even impart a direction to the TopoII reaction that favors this final decatenation step. As proposed by Dewlaty et al., reduced TopoII sumolyation in RanBP2-deficient cells leads to a failure to recruit TopoII to *CEN*s, producing anaphase chromosome bridging and the formation of aneuploid cells. Nondisjuction, as observed in yeast *top2* mutants, would be expected to be one mechanism contributing to aneuploidy, but the actual sequence(s) of events producing aneuploidy in RanBP2-deficient cells remains to be described (?).

Two additional issues merit further comment. First, how does SUMO ligation promote TopoII enrichment at *CEN*s? A straightforward model is that SUMO-conjugated forms of TopoII bind receptors within *CEN* chromatin. However, as is the case with most SUMO substrates, only a small fraction of TopoII is actually sumolyated at any one time. As suggested by Dawlaty et al., SUMO modification may only be required for an initial step in recruitment, allowing TopoII to remain associated with *CEN*s following SUMO deconjugation (Figure 1). Alternatively, sumolyation may promote a different aspect of TopoII dynamics, such as enzyme turnover on chromatin, which then indirectly facilitates *CEN* targeting through a different pathway. Further analysis of the genetic requirements for TopoII *CEN* localization, as well as determining if there are factors that bind sumolyated TopoII, should help clarify the recruitment mechanism.

Second, previous studies have shown that TopoII inhibition (or PIASy knockdown) during mitosis can activate a preanaphase checkpoint that exhibits considerable overlap with the SAC (Diaz-Martinez et al., 2006). The lesion inducing this response is unclear, but one idea is that a catenate-counting mechanism delays anaphase until chromatid entanglements fall below a threshold level. In the current study, it is notable that failure to completely decatenate *CEN*s did not appear to activate such a checkpoint response. Similarly, yeast *top2* mutants that exhibit a lethal decatenation defect proceed into anaphase with normal cell-cycle kinetics. In yeast, TopoII SUMO modification is not required for efficient chromatid disjunction (Bachant et al., 2002) and is largely, but not completely, dispensable for chromosome segregation (Takahashi et al., 2006). One phenotype observed in yeast SUMO-resistant *top2* mutants, however, is a failure to maintain *CEN* compaction under tension as chromatids biorient on the spindle (Bachant et al., 2002). Could TopoII be mediating additional functions within *CEN* chromatin, and might these activities, rather than decatenation, be influencing the SAC? The newfound connections between TopoII, SUMO, *CEN*s, and cancer suggest the answers may prove interesting.

REFERENCES

Arnaoutov, A., Azuma, Y., Ribbeck, K., Joseph, J., Boyarchuk, Y., Karpova, T., McNally, J., and Dasso, M. (2005). Nat. Cell Biol. *7*, 626–632.

Azuma, Y., Arnaoutov, A., Anan, T., and Dasso, M. (2005). EMBO J. *24*, 2172–2182.

Bachant, J., Alcasabas, A., Blat, Y., Kleckner, N., and Elledge, S.J. (2002). Mol. Cell *9*, 1169–1182.

Dawlaty, M.M., Malureanu, L., Jeganathan, K.B., Kao, E., Sustmann, C., Tahk, S., Shuai, K., Grosschedl, R., and van Deursen, J.M. (2008). Cell, this issue.

Diaz-Martinez, L.A., Gimenez-Abian, J.F., Azuma, Y., Guacci, V., Gimenez-Martin, G., Lanier, L.M., and Clarke, D.J. (2006). PLoS ONE *1*, e53.

Jackson, P.K. (2001). Genes Dev. *15*, 3053–3058.

Joseph, J., Liu, S.T., Jablonski, S.A., Yen, T.J., and Dasso, M. (2004). Curr. Biol. *14*, 611–617.

Pichler, A., Gast, A., Seeler, J.S., Dejean, A., and Melchior, F. (2002). Cell *108*, 109–120.

Reverter, D., and Lima, C.D. (2005). Nature *435*, 687–692.

Takahashi, Y., Yong-Gonzalez, V., Kikuchi, Y., and Strunnikov, A. (2006). Genetics *172*, 783–794.

VHL Inactivation: A New Road to Senescence

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Loss of the *VHL* tumor suppressor gene promotes cancer in several ways, including activation of the HIF transcription factors. HIF overexpression is associated with increased malignancy in many tumor types. So why is the spectrum of tumors associated with *VHL* loss restricted only to a few specific organs? In a recent paper in the March issue of *Nature Cell Biology*, Kaelin and colleagues provide a possible explanation, suggesting that *VHL* loss can also trigger senescence, a potent tumor suppressor mechanism.

pVHL and HIF

von Hippel-Lindau (VHL) disease is a hereditary cancer syndrome characterized by a spectrum of benign and malignant tumors including retinal hemangiomas, cerebellar hemangioblastomas, pheochromocytomas, and renal cell carcinomas (Kaelin, 2002). Patients with VHL disease inherit a faulty allele of the ubiquitously expressed *VHL* tumor suppressor gene, and emergence of pathology in these patients follows the inactivation of the remaining wild-type allele.

The gene product of *VHL*, pVHL, acts as the substrate recognition component of an E3 ubiquitin ligase complex (Kaelin, 2002). While several proteins have been identified as pVHL-binding proteins that are subject to ubiquitin-mediated proteolysis, the best characterized putative substrates are the alpha subunits of the hypoxia-inducible factor (HIF1α, HIF2α, and HIF3α). The HIFs function as heterodimeric transcription factor