breast cancer model, which was reversed by NVP-BEZ235, a dual PI3K-mTOR inhibitor, treatment (Eichhorn et al., 2008) (Figure 1B, middle). Given the frequency of mutations in this pathway, PI3K appears to be an excellent target for therapy. The oncology field eagerly awaits further information on the clinical usefulness of PI3K inhibitors.

REFERENCES

Berns, K., Horlings, H.M., Hennessy, B.T., Madiredjo, M., Hijmans, E.M., Beelen, K., Linn, S.C., Gonzalez-Angulo, A.M., Stemke-Hale, K., Hauptmann, M., et al. (2007). Cancer Cell *12*, 395–402.

Eichhorn, P.J., Gili, M., Scaltriti, M., Serra, V., Guzman, M., Nijkamp, W., Beijersbergen, R.L., Valero, V., Seoane, J., Bernards, R., and Baselga, J. (2008). Cancer Res. *68*, 9221–9230.

Garcia-Echeverria, C., and Sellers, W.R. (2008). Oncogene *27*, 5511–5526.

Geyer, C.E., Forster, J., Lindquist, D., Chan, S., Romieu, C.G., Pienkowski, T., Jagiello-Gruszfeld, A., Crown, J., Chan, A., Kaufman, B., et al. (2006). N. Engl. J. Med. *355*, 2733–2743.

Holbro, T., Beerli, R.R., Maurer, F., Koziczak, M., Barbas, C.F., III, and Hynes, N.E. (2003). Proc. Natl. Acad. Sci. USA *100*, 8933–8938.

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

Johnston, S., Trudeau, M., Kaufman, B., Boussen, H., Blackwell, K., LoRusso, P., Lombardi, D.P., Ben Ahmed, S., Citrin, D.L., DeSilvio, M.L., et al. (2008). J. Clin. Oncol. *26*, 1066–1072.

Junttila, T.T., Akita, R.W., Parsons, K., Fields, C., Lewis Phillips, G.D., Friedman, L.S., Sampath, D., and Sliwkowski, M.X. (2009). Cancer Cell *15*, this issue, 429–440.

Leahy, D.J. (2008). Cancer Cell *13*, 291–293.

Nagata, Y., Lan, K.H., Zhou, X., Tan, M., Esteva, F.J., Sahin, A.A., Klos, K.S., Li, P., Monia, B.P., Nguyen, N.T., et al. (2004). Cancer Cell *6*, 117–127.

Stemke-Hale, K., Gonzalez-Angulo, A.M., Lluch, A., Neve, R.M., Kuo, W.L., Davies, M., Carey, M., Hu, Z., Guan, Y., Sahin, A., et al. (2008). Cancer Res. *68*, 6084–6091.

Inflamed Snail Speeds Metastasis

Chih-Cheng Yang[1] and Dieter A. Wolf[1,*]
[1]Signal Transduction Program, Burnham Institute for Medical Research, 10901 North Torrey Pines Road, La Jolla, CA 92037, USA
*Correspondence: dwolf@burnham.org
DOI 10.1016/j.ccr.2009.04.003

Macrophage infiltration and inflammatory cytokines are powerful drivers of tumorigenesis and metastasis. Wu et al., in this issue of *Cancer Cell*, show that TNFα-dependent NFκB activation induces COP9 signalosome-mediated inhibition of GSK3β and the $SCF^{\beta\text{-}TRCP}$ ubiquitin ligase, thus leading to stabilization of the transcription factor Snail and promoting cell migration and metastasis.

Transcription factors are prime targets for regulation by ubiquitin-dependent proteolysis. One of the best studied examples is β-catenin, a coactivator of the TCF-LEF family of DNA-binding proteins. In the absence of Wnt signaling, β-catenin is constitutively phosphorylated by glycogen synthase kinase 3β (GSK3β) on two serine residues. This phosphorylation also requires the scaffold protein axin and the adaptor protein adenomatous polyposis coli (APC), which appear to facilitate the recruitment of β-catenin to GSK3β. β-catenin phosphorylation triggers its recognition by the β-TRCP substrate receptor of a SKP1-CUL1-F-box protein (SCF) ubiquitin ligase, thus resulting in ubiquitylation and proteasomal degradation. Canonical Wnt signaling reverses β-catenin destruction by preventing its GSK3β-dependent phosphorylation.

In many colon cancers, this reversal is mimicked by mutations in APC, thus leading to increased β-catenin-TCF activity and growth promoting gene expression. Within the inflammatory tumor microenvironment, this effect can be potentiated by two additional stimuli that are provided by tumor-associated macrophages (TAMs). The first comes in the form of Wnt ligands, and the second is the proinflammatory cytokine TNFα. Even in the absence of APC mutations, TAM-derived TNFα can promote the nuclear accumulation of β-catenin and gastrointestinal tumorigenesis (Oguma et al., 2008). The cytokine does so not by activating its prominent target NF-κB, but by driving Akt1-mediated, inhibitory phosphorylation of GSK3β, which prevents β-catenin from being targeted to $SCF^{\beta\text{-}TRCP}$.

The new study by Wu et al. (2009) enacts a remarkably similar plot with partly the same cast. Once again, a transcription factor, Snail, escapes ubiquitylation by $SCF^{\beta\text{-}TRCP}$ and proteasomal degradation. Once again, this involves TAMs and their secreted TNFα. And once again, this potentiates the tumor phenotype, this time promoting metastasis. However, the newly discovered pathway has also distinctly novel aspects and brings fresh players to the stage.

Snail is a transcription factor that represses the expression of *E-cadherin* and thereby confers onto epithelial cells a fibroblast-like behavior that includes increased motility. This process, known as epithelial-mesenchymal transition (EMT), occurs at the invasive front of tumors, the same site where tumor infiltration by TAMs takes place. Wu et al. now link these events by demonstrating that TAM-derived TNFα leads to the stabilization of Snail, which is otherwise a highly unstable protein targeted for degradation by GSK3β-dependent phosphorylation and $SCF^{\beta\text{-}TRCP}$-mediated ubiquitylation (Zhou et al., 2004). As with β-catenin, TNFα-mediated Snail stabilization occurs through inhibition of GSK3β-dependent

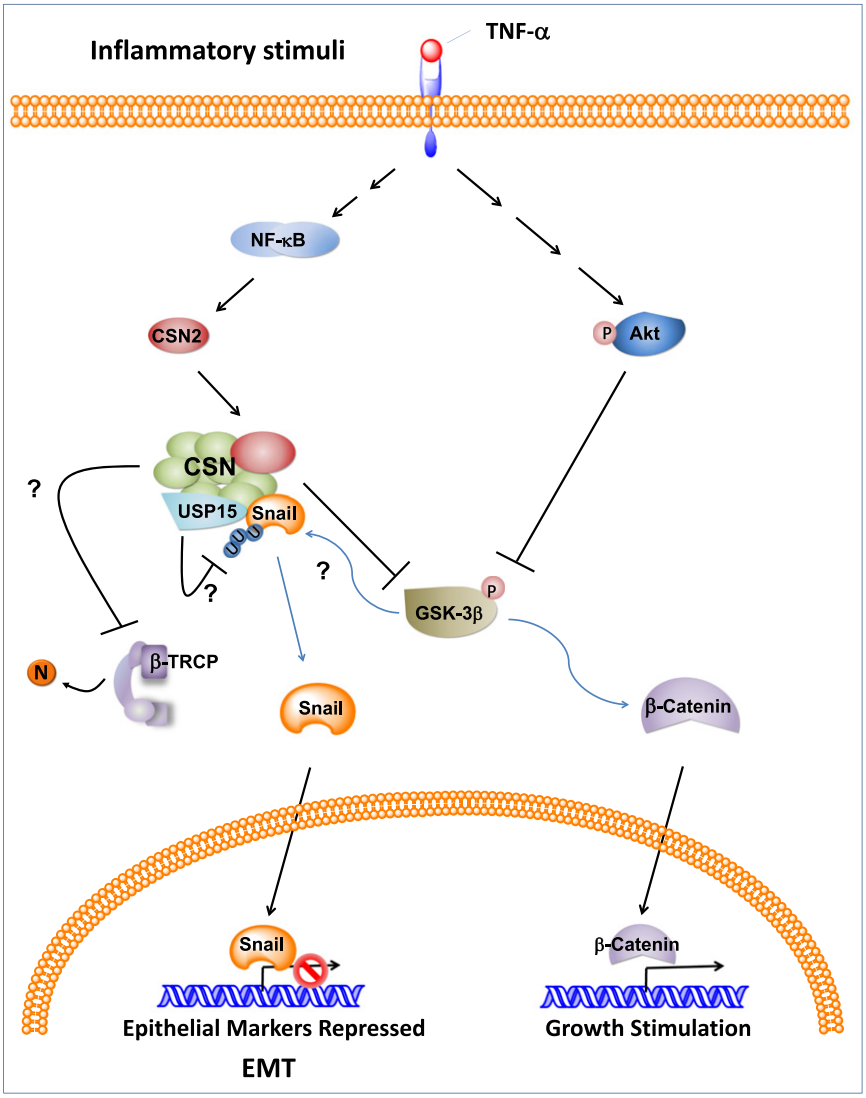

Figure 1. Model for Inflammation-Induced Tumorigenesis and Metastasis

TNFα secreted by tumor-associated macrophages sets in motion two distinct pathways that culminate in the stabilization and nuclear accumulation of Snail and β-catenin, thus effectively shortcircuiting the Wnt pathway in the absence of cognate ligand. Through activation of Akt1, TNFα causes the inhibitory phosphorylation of GSK3β, which in turn prevents the phosphorylation of β-catenin and recognition by $SCF^{β\text{-}TRCP}$ (Oguma et al., 2008). TNFα also stimulates NF-κB signaling, transcription of CSN2, de novo formation of CSN complex, and Snail stabilization by inhibition of GSK3β-mediated phosphorylation (Wu et al., 2009). The latter process is speculated to involve sequestration of Snail from GSK3β by binding to the CSN. Additional mechanisms might include deubiquitylation of Snail by CSN-associated USP15 or inhibition of $SCF^{β\text{-}TRCP}$ by CUL1 deneddylation.

phosphorylation. In diametrical opposition to β-catenin, however, Snail stabilization is not due to inhibitory phosphorylation of GSK3β by Akt1, but instead involves NF-κB signaling and transcriptional activity.

At this juncture, the critical question arose as to what are the transcriptional targets of NF-κB that trigger Snail stabilization. It is in answering this question that the Wu et al. study breaks the most new ground. They found that among ~50 genes rapidly induced by TNFα, only one can bring about Snail stabilization, *CSN2*. Overexpression of CSN2 is sufficient to stabilize Snail, whereas knockdown of CSN2 prevents TNFα-induced Snail accumulation.

CSN2 is a component of the eight subunit COP9 signalosome (CSN) complex, which is a major regulator of the family of cullin-RING ubiquitin ligases (CRLs) to which $SCF^{β\text{-}TRCP}$ belongs (Wolf et al., 2003). CSN is widely viewed as an activator of CRLs because it protects its associated substrate receptors from autocatalytic degradation (Wee et al., 2005). CSN exerts this stabilizing effect by removing the ubiquitin-like modifier NEDD8 from cullins in a reaction that is catalyzed by CSN subunit 5 (Cope et al., 2002). Deneddylated CRLs are enzymatically inactive and, therefore, prevented from self-digestion. At the same time, removal of NEDD8 allows cullins to bind CAND1, another regulator of CRLs that promotes the exchange of substrate receptors.

Since only one of the eight subunits of the CSN was induced by TNFα, it remained initially unclear whether Snail stabilization involved the entire CSN complex. In favor of this possibility is the demonstration that overexpression of CSN5 also caused Snail stabilization. In addition, it is well-established that overexpression of CSN2 is sufficient to drive the de novo assembly of CSN complexes (Schweitzer et al., 2007). Yet, if CSN is an activator of CRLs, why would an increased level of CSN complex prevent $SCF^{β\text{-}TRCP}$-dependent Snail ubiquitylation and degradation?

Although this question is not fully answered, Wu et al. provide some tantalizing clues that suggest a function of CSN that is altogether different from its control of CRLs by deneddylation. Importantly, they find that Snail stabilized by TNFα or CSN2 accumulates in the unphosphorylated state, whereas the protein is phosphorylated when it accumulates due to inhibition of the proteasome by MG132. This finding strongly suggests that the inhibition of Snail degradation by TNFα occurs upstream of $SCF^{β\text{-}TRCP}$, most likely at the level of GSK3β. Indeed, both TNFα and CSN2, but not MG132, prevent the recruitment of Snail to GSK3β. Thus, the main role of CSN may be to keep Snail apart from GSK3β. This could be achieved, for example, by transiently sequestering Snail into a complex with the CSN, thus precluding it from GSK3β or even promoting its dephosphorylation (Figure 1).

Precedence for such a scenario was previously provided by the study of another substrate of the GSK3β-$SCF^{β\text{-}TRCP}$ axis, IκBα (Schweitzer et al., 2007). There, CSN facilitates the

restabilization of IκBα after NF-κB activation, apparently through recruiting the protein to the CSN-associated deubiquitylating enzyme USP15. Whether this mechanism is also at play in CSN-mediated Snail stabilization remains to be investigated (Figure 1). Likewise, future work will be required to clarify the role, if any, of CSN-mediated cullin deneddylation in Snail stabilization. For example, one wonders whether the Snail stabilizing activity of CSN5 depends on the integrity of its JAMM metalloprotease domain. The finding that CAND1 is required for CSN2-induced Snail accumulation is intriguing in this regard, but difficult to reconcile with other reports demonstrating that, in vivo, CAND1 behaves as an activator of CRLs (Zheng et al., 2002), although this has not been established for $SCF^{\beta\text{-}TRCP}$. Based on the data available to date, it appears that preventing Snail phosphorylation is the primary role of the CSN in Snail stabilization.

Regardless of the exact mechanism, the current study deepens the link between inflammation and metastasis and introduces the CSN as a further player in these processes. The new findings also provide initial mechanistic insight into the emerging connection between CSN activation and tumorigenesis (Adler et al., 2008).

REFERENCES

Adler, A.S., Littlepage, L.E., Lin, M., Kawahara, T.L.A., Wong, D.J., Werb, Z., and Chang, H.Y. (2008). Cancer Res. *68*, 506–515.

Cope, G., Suh, G.S.B., Aravind, L., Schwarz, S.E., Zipursky, S.L., Koonin, E.V., and Deshaies, R.J. (2002). Science *298*, 608–611.

Oguma, K., Oshima, H., Aoki, M., Uchio, R., Naka, K., Nakamura, S., Hirao, A., Saya, H., Taketo, M.M., and Oshima, M. (2008). EMBO J. *27*, 1671–1681.

Schweitzer, K., Bozko, P.M., Dubiel, W., and Naumann, M. (2007). EMBO J. *26*, 1532–1541.

Wee, S., Geyer, R.K., Toda, T., and Wolf, D.A. (2005). Nat. Cell Biol. *7*, 387–391.

Wolf, D.A., Zhou, C., and Wee, S. (2003). Nat. Cell Biol. *5*, 1029–1033.

Wu, Y., Deng, J., Rychahou, P.G., Qiu, S., Evers, B.M., and Zhou, B.P. (2009). Cancer Cell *15*, this issue, 416–428.

Zheng, J., Yang, X., Harrell, J.M., Ryzhikov, S., Shim, E.H., Lykke-Andersen, K., Wei, N., Sun, H., Kobayashi, R., and Zhang, H. (2002). Mol. Cell *10*, 1519–1526.

Zhou, B.P., Deng, J., Xia, W., Xu, J., Li, Y.M., Gunduz, M., and Hung, M.C. (2004). Nat. Cell Biol. *6*, 931–940.

YB-1 Translational Control of Epithelial-Mesenchyme Transition

Ghassan Mouneimne[1] and Joan S. Brugge[1,*]
[1]Department of Cell Biology, Harvard Medical School, Boston, MA 02115, USA
*Correspondence: joan_brugge@hms.harvard.edu
DOI 10.1016/j.ccr.2009.04.006

Transitions between epithelial and mesenchmal phenotypes play critical roles in normal development and cancer progression. In this issue of *Cancer Cell*, Evdokimova et al. demonstrate that YB-1 regulates epithelial-mesenchyme transition (EMT) by inducing cap-independent translation of mRNAs encoding EMT-promoting factors and suppressing cap-dependent translation of mRNAs encoding growth-promoting factors.

Metastasis is a multistep process that mediates the spread of cancer cells from primary tumors to distant sites. This process relies on structural and phenotypic changes that enable tumor cells to dissociate from the tumor mass, invade the surrounding tissue, intravasate into vascular or lymphatic vessels, and extravasate and proliferate at a secondary site. An increasing number of reports provide evidence that epithelial cancer cells adopt embryonic transcription programs during the invasive phase of metastasis, which allow them to suspend some or all of their epithelial properties and acquire those of mesenchymal cells (Thiery and Sleeman, 2006; Yilmaz and Christofori, 2009). This epithelial-to-mesenchymal transition (EMT) is associated with changes in cell-cell adhesion, remodeling of cell-matrix adhesion, and enhanced migratory activity. Several transcription factors have been implicated in this transition, including Snail, Slug, Zeb1, and Twist. The canonical Ras pathway has also been shown to play a crucial role in EMT in certain contexts. Ras activity, however, is not sufficient to induce EMT; other factors collaborate with Ras to orchestrate this process (Thiery, 2003). The relevance of EMT as an integral and obligate phase of cancer cell invasion/metastasis in vivo is controversial; in fact, several cancer cell types are able to invade by adopting modes of migration that don't involve an EMT, such as collective or amoeboid (Friedl et al., 2004). Nevertheless, an increasing body of evidence demonstrates the occurrence of EMT in tumor cell subpopulations during the progression of certain types of cancers (Yilmaz and Christofori, 2009; Polyak and Weinberg, 2009), and a recent study