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NEWS AND VIEWS

"Smurf"-ing tumors on the chromatin through RNF20

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In the modern mythology of Dutch comic world, Smurfs are little blue creatures who say "smurf" for anything they do. In the parlance of molecular biology, there live two Smurfs so named because they are Smad ubiquitination regulator factor 1 and 2. Just like those blue devils on the television, the molecular Smurfs are also versatile; capable of controlling bone development, actin cytoskeleton dynamics, cellular senescence, and planar cell polarity in addition to their prototypic function in transforming growth factor β (TGF-β) signaling. Now, the realm of Smurf influence has expanded even further to epigenetic modifications of histones governing the chromatin landscape. A recent study from Ying E. Zhang's group in the National Cancer Institute of NIH published in *Nature Medicine* shows that Smurf2 regulates ubiquitin modification of histone H2B and trimethylation of histone H3 through targeting RNF20, the major H2B ubiquitin ligase. The importance of this regulation is underscored by the fact that genomic ablation of Smurf2 causes a loosening of chromatin compaction and over the long run leads to genomic instability and a disposition to a wide spectrum of tumors in aged mice. This novel cancer-causing mechanism has human relevance as well, since the study reports an inverse relationship between loss of Smurf2 function and up-regulation of RNF20 in many types of human cancers.

Smurf1 and 2 were initially characterized as key regulators in TGF-β and bone morphogenetic protein (BMP) signaling pathways, in which they function as ubiquitin E3 ligases by targeting Smad proteins and the TGF-β type I receptor for protesomal degradation. Due to the similarity in their protein sequences, Smurf1 and Smurf2 were thought to have overlapping functions. Indeed, mice with single knockout of Smurf1 (Smurf1$^{-/-}$) or Smurf2 (Smurf2$^{-/-}$) are viable, fertile and display no overt developmental phenotypes; nevertheless, double knockout of Smurf1 and Smurf2 simultaneously causes embryonic lethality, most likely due to defects in planar cell polarity (Narimatsu et al., 2009), indicating that Smurf proteins have an essential common function during embryonic development. Despite the lack of an embryonic defect, both Smurf1$^{-/-}$ and Smurf2$^{-/-}$ single knockout mice developed distinct phenotypes as they aged, suggesting that Smurf1 or Smurf2 each has unique targets (Yamashita et al., 2005). The Zhang group showed previously that aged Smurf1$^{-/-}$ mice accumulated a greater amount of bone mass than control littermates due to the increased activity of osteoblasts. The target of this osteogenic role of Smurf1 has been determined to be MEKK2. In the current study from the same group, Blank and colleagues report that aged Smurf2$^{-/-}$ mice developed a wide spectrum of tumors. Several earlier studies have suggested that Smurf2 may have a role in tumorigenesis; these include demonstration of elevated Smurf2 expression in breast cancers correlating with tumor progression by modulating TGF-β signaling (Fukuchi et al., 2002; Jin et al., 2009), and induction of senescence by Smurf2 leading to growth inhibition in certain cancer cell lines (Zhang et al., 2008). However, the current study from the Zhang group unequivocally demonstrated the tumor suppressor function of Smurf2 and determined the molecular mechanism. The story began when Blank and colleagues used the genetic approach to delete the *Smurf2* alleles in mice in an attempt to investigate the physiological function of Smurf2. They found that loss of Smurf2 causes a higher tumor incidence as the knockout mice aged. Surprisingly, a wide range of tumor types were revealed in different organs in Smurf2$^{-/-}$ mice, suggesting that Smurf2 plays a central role in controlling tumor formation instead of targeting a specific pathway in a particular organ. To elucidate the mechanism of Smurf2's role in tumorigenicity, Blank and colleagues characterized mouse embryonic fibroblast (MEF) cells isolated from Smurf2$^{-/-}$ embryos and showed that Smurf2$^{-/-}$ cells gain growth advantage and undergo oncogenic transformation in late passages. Interestingly, restoration of Smurf2 does not inhibit cell proliferation in late-passage Smurf2$^{-/-}$ cells, suggesting that loss of Smurf2 causes a chronic effect rather than an acute response in cell growth

regulation. In addition to increased cell proliferation, Blank and colleagues also found that Smurf2$^{-/-}$ MEF cells and freshly isolated dermal fibroblasts from Smurf2$^{-/-}$ mice are more sensitive to DNA damage response and exhibit genomic instability. Based on these observations, Blank and colleagues analyzed histone modifications and chromatin structure and revealed that the level of ubiquitination of H2B (ubH2B) is up-regulated and the chromatin structure is less compact in Smurf2$^{-/-}$ cells.

The integrity of the chromatin structure is an important form of cellular memory for maintaining tissue homeostasis (Kouzarides, 2007). Alternations of the chromatin structure are linked to the pathology of diseases, including cancer, diabetes, and neuro-degeneration. Epigenetic modifications, such as DNA methylation, histone modifications, and nucleosome remodeling, can influence the chromatin landscape (Misteli, 2007). Dysregulation of DNA methylation and histone modifications often causes silencing of tumor suppressor genes and re-expression of oncogenes leading to tumor development. Moreover, aberrant patterns of histone modifications may contribute to substantial changes of the chromatin structure and lead to increased risk for DNA damage and oncogenic transformation of the cells. Among different histone modifications, ubH2B has been shown to regulate gene expression because it promotes methylation of H3K4 and H3K79, which are considered as "active" modifications associated with transcription activation (Chandrasekharan, et al., 2010). In addition to transcription regulation, it has also been proposed that ubH2B plays a role in regulating the chromatin structure. The ubiquitin moiety on ubH2B was initially thought to function like a "wedge" to loosen the chromatin structure for the access of transcription factors (Jason et al., 2002). However, later studies showed that ubH2B can alter the chromatin dynamics by enhancing nucleosome stability (Shema et al., 2008; Chandrasekharan et al., 2009). Thus, the effect of ubH2B on the chromatin structure is still controversial. In Smurf2$^{-/-}$ cells, Blank and colleagues observed that the level of ubH2B increased alongside up-regulation of H3K4me3 and H3K79me3. This observation is consistent with the established model of ubH2B regulatory functions. Surprisingly, they found that the chromatin in Smurf2$^{-/-}$ cells is more sensitive to digestion by micrococcal nuclease (MNase) and restoration of Smurf2 can reduce the sensitivity to MNase, suggesting that loss of Smurf2 leads to a more loosely compacted chromatin structure. These findings were corroborated with a recent biochemical study using chemically reconstituted nucleosomes showing that ubH2B interferes with chromatin fiber compaction (Fierz et al., 2011). To further elucidate the molecular mechanism underlying the up-regulation of ubH2B by loss of Smurf2, Blank and colleagues characterized the RNF20/RNF40 (hBre1A/hBre1B) complex, which is the major E3 ligase responsible for H2B ubiquitination, and found that RNF20 protein is stabilized in cells that lost Smurf2. With a series of biochemical experiments, they concluded that Smurf2 can directly target RNF20 for proteasomal degradation. More importantly, they found that high RNF20 expression is linked to tumor development in several tumor types and there is an inverse relationship between Smurf2 and RNF20 protein expression in tumor cells. To further verify whether RNF20 is a direct mediator of the oncogenic role of Smurf2, they depleted RNF20 in Smurf2$^{-/-}$ cells and showed that knockdown of RNF20 decreased cell growth, reduced sensitivity to MNase digestion, and prevented cells from undergoing oncogenic transformation. Overexpression of RNF20 in wild-type MEF cells also promotes cell growth. Collectively, these findings indicate that Smurf2 mediates cell growth and maintenance of chromatin structure through RNF20. More interestingly, they found that Smurf2 and RNF20 co-localize with phosphorylation of H2AX (γ-H2AX) foci in cells in response to DNA damage and loss of Smurf2 enhances accumulation of γ-H2AX. They proposed that enhancement of DNA response leads to rapid DNA repair and may cause incomplete repair and genetic lesions. Indeed, they observed that late-passage Smurf2$^{-/-}$ cells display several chromosome abnormalities such as increased numbers of Robertsonian translocations. These data further provide a link between Smurf2/RNF20 and DNA repair pathway.

In summary, the current study from the Zhang group builds on a substantial body of evidence to demonstrate that Smurf2 possesses a tumor suppressor function in maintaining genomic stability by targeting RNF20. When Smurf2 is lost, the level of RNF20 becomes up-regulated, leading to increased ubH2B and consequentially H3K4me3 and H3K79me3. These epigenetic changes in the chromatin landscape are the root causes of dysregulated gene expression and genomic instability, which ultimately promote the tumor formation. One interesting question in regard to this model is what signaling can trigger Smurf2 to degrade RNF20. It has been shown that Smad degradation by Smurf1/2 occurs independent of receptor activation. Thus, Smurf1/2 control free amounts of Smad proteins in the cytoplasm to modulate BMP and TGF-β signaling. It will be important to know whether Smurf2 regulates RNF20 in the same manner.

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