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AUTHOR'S VIEW

Two-faced activity of RNF8: What "twists" it from a genome guardian to a cancer facilitator?

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ABSTRACT
The RING finger protein 8 (RNF8)-induced ubiquitination signaling cascade promotes DNA repair and maintains genomic stability. Our study reveals an unexpected action of RNF8 in promoting cancer metastasis, cancer stem cell formation, and chemoresistance through the regulation of TWIST lysine 63 (K63)-linked ubiquitination, suggesting that RNF8 may serve as a new cancer prognosis marker and therapeutic target.

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Triple-negative breast cancer (TNBC), a subtype of breast cancer classified by the absence of estrogen receptor, progesterone receptor, and the human epidermal growth factor 2, accounts for 15-20% of breast cancer cases and is notorious for its high metastasis rate and abysmal prognosis. Lack of protein expression of those 3 hormone receptors causes patients to be unresponsive to hormonal therapy such as herceptin or tamoxifen. The ability of metastatic tumors to resist initial treatment and spread is the major cause of cancer-related death. TNBC is a heterogeneous group of diseases that exhibit poor overall outcome to current dose-dense chemotherapy regimens, particularly in cases of advanced and metastatic TNBC.[1] As a master transcriptional regulator of epithelial-to-mesenchymal transition (EMT), TWIST (a basic helix-loop-helix transcription factor 1) has been shown to trigger EMT, cancer stem cell (CSC) self-renewal, and cancer metastasis.[2] In a genetically engineered mouse model, upregulated TWIST combined with active GTPase HRas (Ras) were demonstrated to initiate tumor formation of TNBC with EMT features. Gene profiling of primary and metastatic breast tumors in murine mouse models revealed that TWIST is a key driver of breast cancer metastasis. Suppression of TWIST expression in highly metastatic cancer cells specifically inhibits their ability to metastasize to the lung in both murine and human xenograft models, confirming TWIST as an appealing therapeutic target for metastatic tumors. However, as a class of molecules, transcription factors like TWIST have proven elusive as drug targets. The poor "druggability" of TWIST creates an emerging need to understand the detailed regulatory mechanisms of TWIST in order to develop TWIST-based therapy.

To this end, we conducted a systemic ubiquitin E3 ligase screening in 2 independent TNBC cell lines and identified RING finger protein 8 (RNF8) as a novel activator of TWIST and EMT, the main cause of chemotherapy failure. RNF8 is a RING-finger E3 ligase best known for its function in maintaining genomic stability by facilitating DNA double-strand break (DSB) repair and protecting telomere ends.[3-6] A recent study revealed that RNF8 is released from DSB sites through cyclin-dependent kinase (CDK)-mediated phosphorylation during cell division, thus arresting DNA repair.[7] However, the biological characteristics of DSB-dissociated RNF8 remain a mystery.

In our study, we discovered previously unrecognized pathologic roles of RNF8 in regulating CSC formation, cancer cell migration/invasion, and metastasis through mediation of TWIST ubiquitination.[8] First, we found that RNF8 associates with TWIST and promotes lysine (K)63-linked ubiquitination on the TWIST K38 residue. This post-translational modification event is critical for TWIST nuclear localization and protein stability. Second, we revealed a novel role of DSB-dissociated RNF8 in CSC regulation and chemoresistance. RNF8 activates EMT and CSC self-renewal by promoting TWIST K63-linked ubiquitination, leading to acquisition of resistance to chemotherapy. Intriguingly, our histologic analysis showed that RNF8 is overexpressed in breast tumors and its overexpression correlates with disease progression, EMT features, and patient survival. Moreover, we elucidated that both RNF8 deficiency of either one alone and TWIST ubiquitination deficiency impairs TWIST-mediated cancer cell migration and invasion and thus inhibits cancer metastasis. Our study provides proof-of-principle that targeting RNF8 for inhibition of TWIST K63-linked ubiquitination is an appealing strategy to prevent chemoresistance and tumor progression.

Our study is the first to demonstrate the oncogenic activity and clinical significance of RNF8 in cancer and represents the groundbreaking discovery of a mechanism that cancer cells exploit to evade chemotherapy, survive, and ultimately metastasize. Our discoveries not only indicate that clinical assessment of RNF8 may be warranted to guide optimal use of chemotherapy but also open up a new perspective on cancer-promoting

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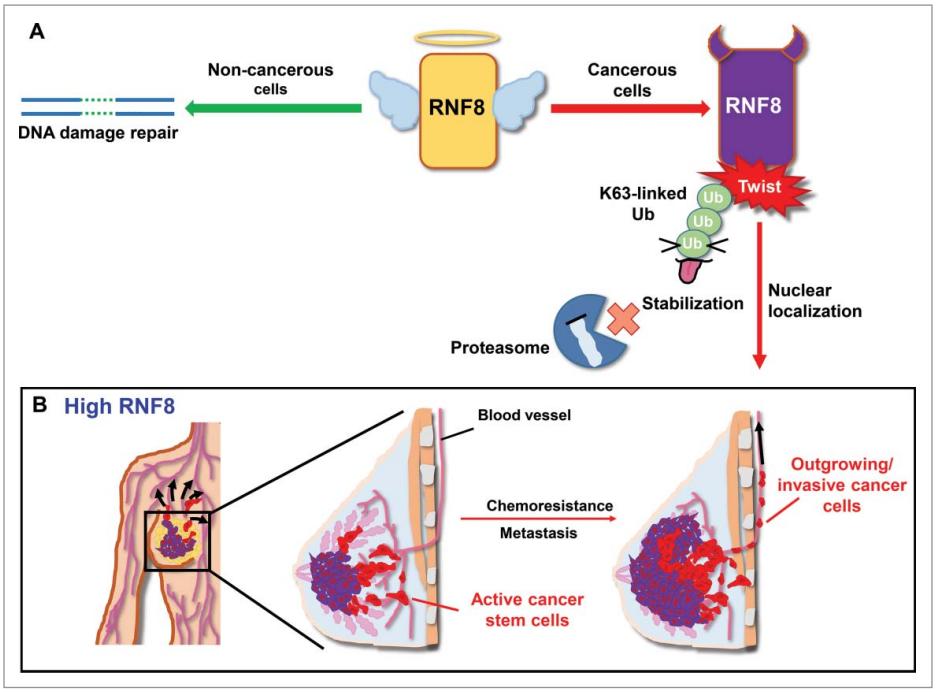

Figure 1. Molecular mechanisms through which RNF8 promotes tumor progression and chemoresistance. In non-cancerous cells (A), RNF8 positively regulates the DNA damage response. In contrast, overexpressed RNF8 in cancerous cells directly promotes the nuclear localization and protein stability of TWIST via K63-linked ubiquitination. In breast cancer patients (B), high expression of RNF8 correlates with tumor progression, conferring chemoresistance and promoting cancer metastasis. Ub, ubiquitination.

actions of DNA damage regulators. In fact, drugs developed to specifically target E3 ligases, such as S-phase kinase-associated protein 2 (SKP2), have been shown to effectively restrict cancer stem cell formation and cancer progression.[9] Our work demonstrated that the RNF8-TWIST signaling axis promotes cancer metastasis and that the RNF8 expression level positively correlates with tumor progression and poor outcome, suggesting that small compounds that interrupt the interaction between RNF8 and TWIST might improve TNBC chemotherapy. A recent study conducted by Shao and colleagues supports our findings by showing that RNF8 is upregulated in malignant breast cancer cells and that overexpressed RNF8 can promote EMT in cell-based experiments and a nude mouse xenograft model.[10]

Although DSB-associated RNF8 was known to be a guardian of the genome, we have revealed a novel role for DSB-dissociated RNF8 in cancer metastasis and chemoresistance (Fig. 1). However, the detailed mechanistic switch between those 2 faces of RNF8 remains unclear. Unlike the RNF8-mediated ubiquitination cascade in the DNA damage response, RING finger protein 168 (RNF168), a critical E3 ligase amplifying downstream signals triggered by RNF8 in the DNA damage response, is not required for RNF8-mediated TWIST activation, EMT, and cancer cell invasion. This suggests that the selectivity for collaboration with various E3 ligases may determine the function of RNF8 in cancerous versus non-cancerous cells. Based on previous studies demonstrating that RNF8 failed to be recruited to DSB sites in mitotic cells with upregulation of CDK1 activity and that CDK1 expression level and activity are increased in some breast cancer cell lines, it would be interesting to investigate whether the functions of DSB-dissociated RNF8 in TNBC cells are dependent on CDK1. Together, our findings suggest a new and broad opportunity to overcome chemoresistance via targeting the E3 ligases that activate K63-linked ubiquitination of EMT transcription factors. The implications of our findings should be of interest to cancer researchers, basic research scientists, physicians, and cancer patients.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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