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AUTHOR'S VIEW

# Stabilization of nuclear oncoproteins by RNF4 and the ubiquitin system in cancer

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**ABSTRACT**
RNF4, a SUMO-targeted ubiquitin ligase, stabilizes a selected group of oncoproteins. It potentiates oncoprotein activity and serves as a positive feedback agonist of Wnt and Notch pathways. RNF4 is essential for cancer cell survival and its levels are elevated in human cancers, correlating with poor outcome in a subset of cancer patients.

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## Dysregulated proteostasis in cancer

Cancer is intimately linked to dysregulated proteolysis. Twenty-five years ago, Ciechanover *et al.* observed that nuclear oncoproteins are short-lived and degraded by the ubiquitin system.[1] Over the next $2^1/_2$ decades it became apparent that degradation of oncoproteins requires phosphorylation by mitogenic kinases. Mutations of these phosphorylation sites (e.g., $Myc^{T58A}$, $\beta$-$Catenin^{S33A}$) are identified frequently in cancer patients and are sufficient to impair degradation and promote tumorigenesis in mouse models. In addition, enzymes and scaffold proteins that mediate the degradation of these phospho-oncoproteins are often mutated or inactivated in human cancers (e.g., the tumor-suppressors F-box/WD repeat-containing protein 7 [FBXW7] and adenomatous polyposis coli [APC]). Collectively, deregulated stabilization and increased abundance of oncoproteins are at the heart of tumorigenesis.[2-4]

In many cases, destabilized phosphorylation is preceded by a priming phosphorylation event that initially potentiates the activity of oncogenes by poorly understood mechanisms. These phosphorylations are essential for the subsequent recruitment of kinases that catalyze secondary destabilizing phosphorylations. For example, phosphorylation of c-Myc S62 by mitogen-activated protein kinases (MAPKs) primes for glycogen synthase kinase-3$\beta$ (GSK3$\beta$) phosphorylation of T58 of c-Myc and subsequent ubiquitylation-dependent degradation by the FbW7 ligase complex.[3] Likewise, phosphorylation of $\beta$-catenin S45 by casein-kinase-I (CKI) is required for phosphorylation by GSK3$\beta$ and ubiquitylation by the $SCF^{\beta TRCP}$ ubiquitin ligase complex.[4] However, the mechanism(s) by which these initial phosphorylations enhance the stability and activity of oncogenic transcription factors, as well as their contribution to cancer, is less clear.

In a recent study,[5] we identified that RNF4, a ubiquitin ligase, and the ubiquitin system is part of the machinery involved in stabilization, rather than degradation, of selected short-lived oncoproteins such as $\beta$-Catenin, c-Myc, c-Jun, and Notch intracellular domain protein (N-ICD). RNF4 potentiates the transcriptional activity of these oncoproteins, enhances the tumorigenic properties of cancer cells, and is essential for cancer cell survival. Importantly, high RNF4 levels correlate with poor outcome in a subset of cancers.[5]

RNF4 belongs to a small group of RING (really interesting new gene) ubiquitin ligases termed SUMO-Targeted Ubiquitin ligases (STUbL). Conserved from yeast to man, these ligases bind to SUMO chains of SUMOylated proteins via multiple SIM domains at the N-termini of the protein and catalyze their ubiquitylation via the RING domain, thus physically connecting the ubiquitin and SUMO pathways. In many cases, ubiquitylation by RNF4 leads to proteasomal degradation; for example, SUMO-dependent ubiquitylation by RNF4 leads to degradation of the promyelocytic leukemia (PML) protein and its related oncogenic fusion protein PML-RAR (a fusion between PML and the retinoic acid receptor $\alpha$). Moreover, expression of RNF4 results in differentiation of PML-RAR leukemic cells,[6] suggesting that RNF4 functions as a tumor suppressor in promyelocytic leukemia. In contrast, in the case of epithelial cancers RNF4-dependent ubiquitylation potentiates the tumorigenic properties of cancer cells.

## *Mechanisms of RNF4-dependent stabilization and oncogene activation*

STUbL ligases bind to their SUMOylated substrates via multiple SIM domains. However, in the case of oncoprotein stabilization, RNF4 binding requires phosphorylation and not SUMOylation of its substrates. Importantly, these are the priming phosphorylations mediated by mitogenic kinases. These p-oncoproteins are recognized by RNF4 via a short

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arginine motif (ARM) located at the N-terminal part of RNF4 downstream of the SIM motifs.[5,7] Mutation of these phosphorylation sites or the ARM region prevented binding, ubiquitylation, stabilization, and transcriptional enhancement of oncoproteins by RNF4. Moreover, RNF4 stabilized and enhanced the transcriptional activity of otherwise stable oncoprotein mutants such as $\beta$-catenin$^{S33A}$ and c-Myc$^{S62A}$. Thus, inhibition of RNF4 may be a potent strategy to target cancers in which degradation of these oncoproteins is compromised.

We found that protein stabilization requires the catalysis of atypical polyubiquitin chains by RNF4. These chains are characterized by the generation of internal ubiquitin linkages via K11 and K33, most likely by RNF4 in concert with a specific ubiquitin conjugating protein (E2 ligase). Indeed, RNF4 was shown to interact with multiple E2s, and sequential ubiquitylation by the ubiquitin conjugating enzymes RAD6 and UbcH5b is required for the catalysis of SUMO-Ub hybrid chains.[8] However, the identity of the E2 enzyme involved in RNF4-dependent protein stabilization is yet to be determined. RNF4 stabilizing activity also requires its association with nucleosomes. A point mutation, K179D, that blocks this association prevents ubiquitylation, stabilization, and transcriptional enhancement,[5,6] suggesting that RNF4 acts on, or in the vicinity of, chromatin. Nevertheless, how atypical ubiquitylation and association with nucleosomes results in oncoprotein potentiation is not clear. These mixed chains may be poorly recognized by the proteasome, hence attenuating protein turnover. Alternatively, they may serve to tether these factors to the nuclear lamina or be involved in transcription-related, high-order nuclear organization. In this regard, phosphorylation of Ser62 of c-Myc was recently shown to enhance c-Myc association with Lamin A/C.[9] Additional studies are required to elucidate the mechanisms involved beyond the formation of atypical chains.

### *RNF4 and non-oncogene addiction genes (NOA) in cancer*

RNF4 serves as a positive agonist of oncogenic pathways such as Wnt and Notch. In both pathways, a critical target is c-Myc.[3] RNF4 is a direct c-Myc target and its expression is elevated upon pathway activation. In turn, RNF4 stabilizes and potentiates c-Myc, $\beta$-catenin, and N-ICD. Therefore, RNF4 may be a potential target in cancers where Myc proteins and/or these pathways are abnormally activated, such as colon cancer and acute T-cell leukemia. Supporting this notion, conditional RNF4 expression potentiates the tumorous phenotype of colon and breast cancer cells and is essential for the survival of Myc-dependent aggressive breast cancer cells (MDA-MB-231). Moreover, RNF4 protein levels were found to be elevated in ~30% of biopsies of human colon carcinoma but not adenoma, correlating with adenoma to carcinoma transition. Likewise, high *RNF4* mRNA levels correlated with poor outcome in a cohort of breast cancer patients with a relatively less severe type of breast cancer (ER$^{+}$, luminal type A).[5]

In summary, RNF4 fits into a class of genes termed "non-oncogene addiction" (NOA) genes encoding enzymes and proteins that are essential for the activity of oncogenes or negatively regulate tumor suppressor functions.[10] Generally, NOA genes are not oncogenic on their own but are crucial for tumor progression and maintaining the cancerous state. While NOA genes are essential in cancer cells they make a smaller contribution to the viability of non-transformed cells, making them excellent potential targets for cancer therapies. A current challenge is to characterize RNF4-dependent tumors as a basis for future design of RNF4-based precise interventions in these cancers.

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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