RNA helicase DP103 and TAK1: a new connection in cancer

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DEAD-box protein-103 (DP103, also known as DDX20 or Gemin3 [protein component of gems number 3]) belongs to the family of DExd/H-box RNA helicases that contain the highly conserved motif Asp-Glu-Ala-Asp/His in the helicase domain. DP103 is a multifunctional protein that binds and unwinds RNA secondary structures, thus functioning in RNA metabolism from birth to death. DP103 was first identified as a component molecule of survival of motor neuron (SMN) together with SM ribonucleoproteins and other Gemin proteins.1 This 824-amino acid protein is known to be a transcriptional repressor for early growth response 2 (Egr2) in hindbrain development2 and forms a repressor complex with PE-1/METS (PU-Its related 1/mitogenic Ets transcriptional suppressor) to suppress E26 transformation-specific (Ets) target genes involved in the Ras-dependent proliferation and differentiation of macrophages.3 It was previously reported that DP103 transcriptionally represses the nuclear receptor steroidogenic factor 1 (SF1), the central transcription factor in reproductive organ development, by functioning as a co-factor of the E3 ligase protein inhibitor of activated STAT protein gamma (PIASy) in a small ubiquitin-like modifier (SUMO)-dependent manner.4 Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is the central transcription factor in innate immunity against infection. As a result of its broad regulatory capability to trigger immune responses upon various stimuli, its precise regulation has played a pivotal role in survival and evolution.5 Five NF-κBs and 8 inhibitors of NF-κB (IκBs) are known today, and at the center of its signaling cascade lie the inhibitor of κB kinases (IKK) complexes, consisting of IKK1/IKKα, IKK2/IKKβ, and the NF-κB essential modulator NEMO/IκK3/IκKγ, which control NF-κB activation through phosphorylation of IκBs. Activation of NF-κB in normal cells is both inducible and reversible to tightly control physiological cell functions through the binding of NF-κB to IκB proteins. However, NF-κB is known to be constitutively active in many cancers, including breast cancer. It is both natural and ideal for cancers to maintain a constitutively active NF-κB signal in order to escape apoptosis induced by self and non-self antigens and to become resistant to therapies and irradiation.6,7 Nevertheless, constitutive NF-κB activation through overexpression of its central kinase IKK2 and its upstream transforming growth factor β-activated kinase 1 (TAK1) has not been documented in human malignancies.

Breast carcinoma is a highly heterogeneous disease and the most common malignancy in females. Despite progress in diagnosis and treatment 30% of patients with early breast cancer experience relapse, and advanced metastatic diseases are a major cause of death. In our search for new markers of tumor metastasis, we found that expression of DP103 was significantly upregulated in the metastatic basal subtype of breast cancers in 3 independent cohorts.8 Furthermore, this elevated DP103 expression correlated with metastatic breast cancer gene signatures and was strongly associated with patient survival. Suppression of DP103 decreased the migratory and invasive ability of breast cancer cells, both in vitro and in vivo. Through qPCR array, we found that matrix metalloproteinase 9 (MMP9) levels positively correlated with increased DP103 levels. Conversely, suppression of DP103 expression decreases MMP9 expression, suggesting that MMP9 mediates the effect of DP103 on breast cancer invasiveness.

**KEYWORDS:** cancer, DP103, MMP9, metastasis, NF-κB

**ABBREVIATIONS:** DP103, DEAD-box protein 103; MAPK, mitogen-activated protein kinase; MMP9, matrix metalloproteinase 9; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PIASy, protein inhibitor of activated STAT protein gamma; SUMO, small ubiquitin-like modifier; TAK1, transforming growth factor β-activated kinase 1.
To validate the hypothesis that DP103’s mechanism of action involves regulation of MMP9, we first investigated the SUMOylation of NEMO during the DNA damage response. Indeed, we found that DP103 could affect PIASy SUMOylation of NEMO; however, this did not have a functional role in its regulation of MMP9 transcription. Instead, the action of DP103 on NF-κB activation involved its canonical pathway. While testing the specificity of DP103’s role in PIASy SUMOylation of NEMO, and hence NF-κB activation, we found that DP103 regulates NF-κB–dependent gene expression in response to multiple stimuli such as tumor necrosis factor α (TNFα), interleukin-1 (IL-1), and lipopolysaccharides (LPS), in addition to DNA damaging reagents including etoposide (VP-16), camptothecin (CPT), and doxorubicin, which initiate NF-κB activation from distinct signaling relays. DP103 knockdown experiments clearly showed downregulation of NF-κB activation by a broad range of general stimuli, including TNFα and LPS, pointing to involvement of the central controlling molecules IKKs and TAK1, a member of the mitogen-activated protein kinases (MAPK) family that is known to be an upstream kinase of IKK2 and MAPK. Using endogenous and purified proteins, we revealed that DP103 can directly bind to TAK1 and function as a cofactor, thus enhancing TAK1-mediated IKK2 phosphorylation, and hence NF-κB activation (Fig. 1; ref.9).

The concept of RNA helicase-enhancing kinase activity in human disease has recently been reported. For example, Cruciat et al.9 identified the DEAD-box RNA helicase DDX3 as a regulator of the Wnt/β-catenin signaling. They demonstrated that DDX3 binds casein kinase 1, epsilon (CK1ε) in a Wnt-dependent manner and directly stimulates its kinase activity, thus promoting phosphorylation of the scaffold protein dishevelled (Dsh). Li et al.10 also reported that, during infection, hepatitis C virus (HCV) interacts with DEAD box polypeptide 3, X-linked (DDX3X) to activate NF-κB–independent IKKα and induce a cAMP-response element-binding protein (CREB)-binding protein (CEBP)/p300-mediated transcriptional program involving sterol regulatory element-binding proteins (SREBPs).

In summary, we have provided the first evidence that the RNA helicase DEAD-box protein DP103 is an NF-κB target that could form part of a positive feedback loop contributing to DP103-mediated regulation of TAK1 kinase activity on the major NF-κB kinase IKK2, thus implicating DP103 in the maintenance of this oncogenic signaling arm in human cancer.

![Figure 1. Role for the RNA helicase DP103 in the activation of NF-κB in cancer. Schematic model based on our study showing a role for the RNA helicase DP103 through its ability to bind and stabilize TAK1 and thus activate NF-κB signaling in cancers. DP103, DEAD-box protein 103; IκB: inhibitor of NF-κB; IKK, inhibitor of κB kinases; NEMO, NF-κB essential modulator; p65, nuclear factor NF-κB p65 subunit; p50: nuclear factor NF-κB p50 subunit; TAK1, transforming growth factor β-activated kinase 1.](image)

Since we have shown that DP103 affects PIASy SUMOylation of NEMO, we are currently extending our studies to the role of DP103 in the activation of NF-κB in response to DNA damaging agents.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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