Pinched by RNA “fingers”: Long noncoding RNAs hitting signal transduction pathways

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\textbf{ABSTRACT}

We have recently reported a long noncoding RNA that interacts with nuclear factor \(\kappa B\) (NF\(\kappa B\)) and represses NF\(\kappa B\) activation by physically masking the phosphorylation site of inhibitor of NF\(\kappa B\) (I\(\kappa B\)). Our findings have revealed a new class of long noncoding RNAs (lncRNAs) that directly interact with proteins involved in signal transduction pathways and interfere with cell signaling. This implicates a potential strategy for the design of RNA-based targeted drugs.

The nuclear factor \(\kappa B\) (NF\(\kappa B\)) signaling pathway plays essential roles in multiple physiological and pathological processes such as development, immunity, inflammation, and cancer.\textsuperscript{1} Precise regulation of NF\(\kappa B\) activity is necessary for physiological homeostasis, whereas overactivation of NF\(\kappa B\) is found in many cancers, including breast cancer, and underlies the mechanisms of cancer formation and progression.\textsuperscript{2} Therefore, elucidating the regulatory mechanisms of NF\(\kappa B\) activation may help to understand cancer pathogenesis and identify therapeutic targets. It is widely accepted that the major trigger for canonical NF\(\kappa B\) activation is the IKK-I\(\kappa B\) axis.\textsuperscript{3} In resting cells, NF\(\kappa B\) transcription factors are sequestered in the cytoplasm by proteins called inhibitors of NF\(\kappa B\) (I\(\kappa Bs\)). Extracellular stimuli, including proinflammatory cytokines, lead to phosphorylation and activation of I\(\kappa B\) kinases (IKKs), which then phosphorylate I\(\kappa B\) proteins at serine 32 and serine 36. The phosphorylated I\(\kappa B\) undergoes conformational changes to expose the motif for ubiquitination-mediated degradation. This degradation leads to release of NF\(\kappa B\) transcription factors from I\(\kappa B\) and their subsequent nuclear translocation to activate transcription programs. Previous studies have identified many NF\(\kappa B\) regulators, most of which act to modulate IKK activation. However, it has been reported that elevation of IKK activity lasts much longer than NF\(\kappa B\) activation following inflammatory stimulation,\textsuperscript{4} and that basal IKK activities are sufficient to phosphorylate I\(\kappa B\).\textsuperscript{5} These findings suggest that some unknown mechanisms may protect I\(\kappa B\) from phosphorylation without affecting IKK activation.

Long noncoding RNAs (lncRNAs) are RNA transcripts that are longer than 200 nucleotides in length but do not encode proteins.\textsuperscript{6} LncRNAs have been shown to regulate gene expression by deploying epigenetic modification and modulating transcription, mRNA splicing, and translation, during which they may function as guides, decoys, or scaffolds for gene regulating proteins.\textsuperscript{7} Although these models predict most of the known lncRNA functions, the detailed mechanisms are poorly elucidated.

In our recent study we identified a cytoplasmic lncRNA called NF\(\kappa B\) interacting long noncoding RNA (NKILA) that interacts with NF\(\kappa B\) and represses I\(\kappa B\) phosphorylation by physically hindering the phosphorylation site of I\(\kappa B\).\textsuperscript{8} NKILA is transcriptionally activated by NF\(\kappa B\) signaling upon challenge by proinflammatory cytokines, and forms a negative feedback loop for NF\(\kappa B\) regulation. NKILA differs from other known NF\(\kappa B\) negative feedback loops in that it acts at the level of I\(\kappa B\) phosphorylation, which constitutes a physiological barrier to prevent NF\(\kappa B\) overactivation under conditions of persistently elevated IKK. As a “gate keeper” for aberrant NF\(\kappa B\) activation, NKILA is subjected to microRNA-mediated degradation and its expression is decreased in various types of cancer including breast, liver, lung, and colon cancers. Reduced NKILA expression in advanced cancers may result in NF\(\kappa B\) overactivation and the consequential cancer metastasis.\textsuperscript{8}

In our study, we demonstrated that the functional domains of lncRNAs are regional hairpins formed by fold-backs of RNA segments, which may directly bind to active motifs of signaling proteins. Based on this observation, we hypothesize the existence of a unique lncRNA subclass called “signal transducer lncRNAs” that may have co-evolved with signaling proteins to regulate their activation by interacting with their functional domains. Lnc-DC, another lncRNA that was shown to interact with signal transducer and activator of transcription 3 (STAT3) and repress its dephosphorylation,\textsuperscript{9} may also belong to this group. In contrast to lncRNAs that serve as protein scaffolds, signal transducer lncRNAs exert their effects without mobilizing other regulatory proteins. The possible challenge to proving this hypothesis is to demonstrate evolutionary conserved
lncRNA families that co-evolved with their protein partners in the relevant signaling pathways, since lncRNAs are less conserved in evolution than proteins. Furthermore, the “loose” conservation in nucleic acid sequence may result in lncRNAs with functional diversity. Thus lncRNAs may serve as “fine-tuners” of signal transduction pathways throughout the history of evolution. Should this class of signal transducer lncRNAs exist, further investigation into their common structural and functional features would be needed to elucidate their role in a “protein dominated” biochemical world.

In addition, our study has demonstrated the flexibility and versatility of lncRNAs, as these molecules may employ various domains to carry out assorted functions in modulating signal transduction. We have identified 3 independent functional domains in NKILA (Fig. 1). Hairpin A mimics a canonical κB half-site that is recognized by NFκB p65 (encoded by v-rel avian reticuloendotheliosis viral oncogene homolog A [RELA], best known as NFκB p65 or p65) in a sequence-specific manner. Hairpin B interacts with the N-terminal domain of p65 to assist in the formation of a stable NKILA:p65:IkB complex. In this RNA:protein complex, hairpin C masks the phosphorylation site of IkB from active IKK and thus inhibits its phosphorylation and degradation. All 3 hairpins are indispensable for the function of NKILA, and mutation in any one of them abrogates NKILA’s ability to repress IKK-induced IkB phosphorylation (Fig. 1). Based on these findings, we propose that abridged versions of NKILA that preserve only the functional hairpins through appropriate linkages might also work to inhibit overactivation of NFκB. Indeed, our data showed that deleting nucleotides 1–300 from the 5′-end of NKILA, while preserving hairpins A and B, did not interfere with the ability of NKILA to interact with the p65:IkB complex. On the other hand, deleting the 3′-nucleotides up to nucleotide 1300 of NKILA did not dampen its ability to inhibit IKK-induced IkB phosphorylation. Therefore, the functional domains, or rather the motifs, of signal transducer lncRNAs are like “fingers” that “pinch” the signaling proteins. Analyzing these functional domains of signal transducer lncRNAs may be useful for the design of RNA-based targeted therapies to target aberrant signaling in related diseases.

In summary, our findings suggest the existence of a new lncRNA class that interacts with the functional motifs of signaling proteins and thus directly modulates cell signal

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**Figure 1.** Functional profiles of NKILA variants. The functional model of wild type or mutantNFκB interacting long noncoding RNA (NKILA) is shown. NKILA mutant A (mutA): mutation introduced in hairpin A disrupted NKILA-NFκB recognition. NKILA mutant B (mutB): mutation introduced in hairpin B did not interfere with recognition of NFκB p65 (product of gene v-rel avian reticuloendotheliosis viral oncogene homolog A, or RELA), but abrogated the ability of NKILA to bind to IkB-engaged NFκB p65, and therefore prevented NKILA from inhibiting IkB phosphorylation. NKILA mutant C (mutC): deletion of hairpin C did not affect interaction of NKILA with the NFκB:IkB complex, but abolished its ability to physically mask the phosphorylation site of IkB.
transduction as proteins themselves do. The working model of such "signal transducer IncRNAs" may represent a potential strategy for the design of RNA-based targeted drugs.

Disclosure of potential conflicts of interest
No potential conflicts of interest were disclosed.

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