

AUTHOR'S VIEW

***NBR2*: A former junk gene emerges as a key player in tumor suppression**

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ABSTRACT

NBR2 (*neighbor of BRCA1 gene 2*) is a non-protein coding gene that resides adjacent to tumor suppressor gene *BRCA1*, but its role in cancer biology has remained unknown. Our recent study showed that *NBR2* encodes a long non-coding RNA and suppresses tumor development through regulation of adenosine monophosphate-activated protein kinase (AMPK) activation.

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The *BRCA1* tumor suppressor gene encodes a nuclear protein that plays critical roles in the maintenance of genome integrity, and germline mutations of this gene account for most familial cases of breast and ovarian cancer. *BRCA1* mutations are relatively rare in sporadic human cancers; instead, its expression is often downregulated, which has prompted detailed analysis of its gene promoter and transcriptional control. Previous genomic studies¹⁻³ revealed that the mouse *Brca1* gene lies head-to-head with the *Nbr1* (*neighbor of BRCA1 gene 1*) gene, with only several hundred base pairs (bp) between the transcription start sites of these 2 genes (Fig. 1A). Interestingly, during the evolution from mouse to primate, genomic duplication and subsequent genomic alterations resulted in the *BRCA1-NBR2-BRCA1P1* (*BRCA1* pseudogene 1)-*NBR1* genomic structure on human chromosome 17q21, in which the *BRCA1* gene lies head-to-head with the *NBR2* gene, while the *BRCA1P1* gene resides right next to the *NBR1* gene³ (Fig. 1A).

Although the *NBR1* and *NBR2* genes share high sequence homology at their 5' ends, the remaining sequences share no significant homology. *NBR1* encodes a protein of 966 amino acids that functions as an autophagy receptor.⁴ In contrast, current evidence suggests that the *NBR2* gene encodes a long non-coding RNA (lncRNA), a class of non-coding RNAs that are longer than 200 nucleotides.⁵ *NBR2* transcripts ranging from 1 to 3 kb were expressed in most of the tissues examined in a previous study.² Although the *NBR2* cDNA contains an open reading frame (ORF) of 112 amino acids, a strong Kozak signal has not been identified within this ORF. In addition, the stop codon is more than 55 bp upstream of the last splicing site for the putative *NBR2* ORF, suggesting that even if the *NBR2* transcript were subjected to protein synthesis it would have been degraded by nonsense-mediated mRNA decay.² There is only 218 bp between the transcription start sites of the human *BRCA1* and *NBR2* genes, and the 2 genes share a bi-directional promoter.² Given the close proximity of the human *BRCA1* and *NBR2* genes, it has been suggested that these 2 genes may

be coordinately expressed. Indeed, *BRCA1* and *NBR2* genes are often co-deleted in familial breast and ovarian cancers.^{6,7} These observations suggest that, like *BRCA1*, *NBR2* may play a role in tumor suppression. For many years, however, *NBR2* has been regarded as a “junk gene” as it does not appear to encode a protein, and its potential function in tumor biology has remained unexplored.

Our recent study revealed, for the first time, the function of *NBR2* in tumor suppression.⁸ The discovery stemmed from our analysis and characterization of energy stress-induced lncRNAs. RNA sequencing and computational analyses of non-coding RNA profiles of cells cultured in glucose-containing or glucose-free medium identified *NBR2* as one of several lncRNAs that were induced upon glucose starvation. Subsequent analyses revealed that *NBR2* functions to promote energy stress-induced activation of adenosine monophosphate-activated protein kinase (AMPK) (Fig. 1B), the major energy sensor in eukaryotic cells. In response to energy stress, AMPK is activated and functions to repress anabolic processes (such as protein/lipid/sterol synthesis) and promote catabolic processes (such as glycolysis, fatty acid oxidation, autophagy), resulting in restoration of energy balance.⁹ As anabolic processes are often required to support tumor growth, the AMPK pathway plays tumor suppressive roles in many cancers. Consistent with this, our data showed that *NBR2* knockdown promoted anchorage-independent growth and xenograft tumor development *in vivo*, whereas *NBR2* overexpression inhibited tumor cell proliferation. Accordingly, *NBR2* is downregulated and its low expression is correlated with poor prognosis in several human cancers. Further functional studies showed that *NBR2* exerted its tumor suppressive function at least in part through regulation of AMPK activity.⁸

Taken together, our results not only reveal the biological function of *NBR2* in tumor suppression but also provide a new conceptual framework to understand the regulation of kinase signaling by lncRNAs. Several important questions remain to

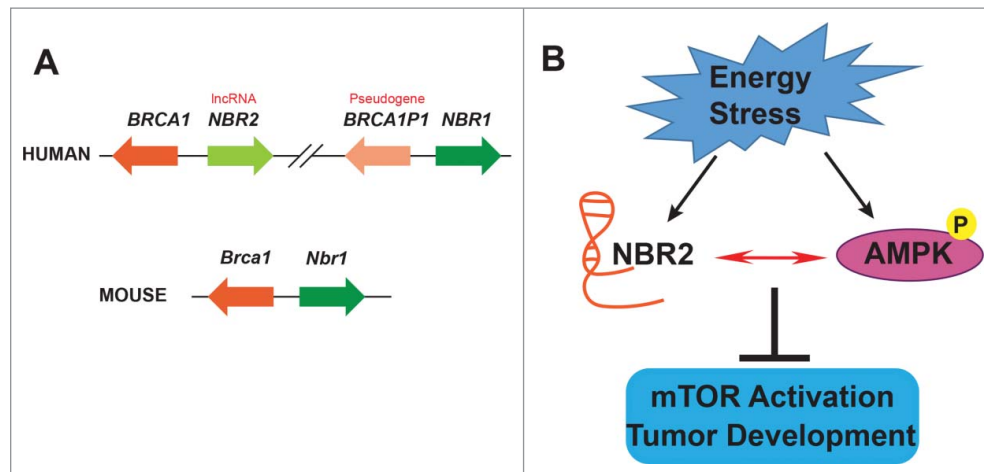


Figure 1. Genomic structure of the *NBR2* gene and its proposed role in the regulation of adenosine monophosphate-activated protein kinase (AMPK). (A) Human and mouse *BRCA1* loci, with arrows indicating the direction of transcription. Genes are not drawn to scale. Orthologous genes are shown in identical colors. (B) *NBR2* and AMPK reciprocally regulate each other in response to energy stress and function in mTORC1 (mammalian target of rapamycin complex 1, also known as mechanistic target of rapamycin complex 1) inactivation and tumor suppression.

be addressed in future studies. First, how *NBR2* regulates AMPK kinase activity remains incompletely understood. It will be particularly important to determine the crystal structure of the *NBR2*-AMPK complex, which undoubtedly will provide further mechanistic insight into how *NBR2* regulates AMPK activation. Whether the *NBR2*-AMPK regulation discovered in our study can be applied to other lncRNAs that may regulate kinase function also awaits further investigation. In addition, our data show that *NBR2* overexpression still partially inhibited tumor cell proliferation in *AMPK*-deficient cells,⁸ suggesting the existence of AMPK-dependent and -independent mechanisms that mediate *NBR2* function in tumor suppression. Since many lncRNAs function to regulate the transcription of neighboring genes, the hypothesis that *NBR2* regulates *BRCA1* transcription is of particular interest. Although our current data do not support such a hypothesis,⁸ another study showed that *NBR2* knockdown modestly downregulated *BRCA1* mRNA level.¹⁰ Since these studies were conducted in different cell lines with different knockdown efficiencies, it is possible that *NBR2* regulation of *BRCA1* expression is context dependent, and it will be important to further clarify this question in future studies.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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