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REVIEW ARTICLE

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Neuro-oncological ventral antigen 1 (NOVA1): Implications in neurological diseases and cancers

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Abstract

Neuro-oncological ventral antigen 1 (NOVA1) is a RNA-binding protein that interacts with RNA containing repeats of the YCAY sequence. This protein is a brain-specific splicing factor regulating neuronal alternative splicing. It has been increasingly recognized as an important contributor to neurological disorders and carcinogenesis. In this review, we summarize the biological functions and pathological roles of NOVA1. The clinical implications of NOVA1 will also be discussed.

1 | INTRODUCTION

Neuro-oncological ventral antigen (NOVA) was initially identified as an antigen in a rare neurological disorder known as paraneoplastic opsoclonus-myoclonus ataxia.[1,2] Involving in multiple steps of mRNA processing, RNA-binding proteins (RBP) are essential in gene expression. These include splicing, translation, transport, stability and degradation of RNA.[3] NOVA proteins are RBPs, which bind to the YCAY sequence of the target pre-mRNAs.[4–6] More than 200 NOVA RNA targets have been identified in the mouse brain.[7] The NOVA family comprises two members, namely NOVA1 and NOVA2.[8] Both of them have been demonstrated to regulate approximately 700 alternatively spliced exons through binding to the target pre-mRNAs. While NOVA1 was predominantly expressed in the ventral spinal cord and the midbrain, NOVA2 was localized in the neocortex and hippocampus.[9,10]

NOVA1 is a brain-specific splicing factor regulating alternative splicing in neurons.[1,6,11,12] Homonymous mutation of *NOVA1* in mice severely impaired the development of the motor system, suggesting its critical role in viability and functional maintenance of motor neurons.[5] Apart from neuronal functions, NOVA1 has other biological roles, such as brown adipogenesis.[13] Increasing evidence has demonstrated that NOVA1 is involved in numerous pathological processes and diseases, including neurological disorders and cancer.

In this review, we summarize the recent findings on the expression and function of NOVA1 in human diseases. Its role in neurological disorders and cancer will be emphasized. We will also discuss the potential clinical applications of NOVA1.

2 | NEUROLOGICAL DISORDERS

2.1 | Microcephaly, seizures and severe developmental delay

The involvement of NOVA1 in neurological defects was initially suggested by the detection of a chromosomal mutation in a 6-month-old girl with microcephaly, poor eye contact, dyspraxia and strabismus.[14]

Xin Yu and Zheng Li contributed equally to this work.

In the 14q12 region, there was a 4.09 Mb deletion spanning the loci encoding FOXG1 and NOVA1. Similarly, in a child with severe neurodevelopmental retardation, a de novo small supernumerary marker chromosome involving 14pter → q12 was detected, in which one of the encoded genes was *NOVA1*.[15] These suggest that NOVA1 might be important in neurological development. However, the causal relationship between the *NOVA1* gene disruption and the clinical manifestations remains uncertain. Further mechanistic studies using specific gene knockout in vivo may provide valuable insight into these neural defects.

2.3 | Cerebral ischaemia-reperfusion injury

In an animal model of focal cerebral ischaemia-reperfusion injury, the right middle cerebral artery of Sprague-Dawley rats was first occluded for 120 min followed by reperfusion for 1 day, 7 days or 14 days. Throughout the process, the animals exhibited dynamic changes of NOVA1 expression in the cerebral cortex, hypothalamus and hippocampus.[15] Expression of NOVA1 was detected throughout the entire brain. Notably, a higher density was observed in the hippocampus, cingulate cortex, hypothalamus and the medial habenular nucleus. Subsequent to 1-day reperfusion, the immunoreactivity of NOVA1 in neurons was significantly up-regulated, predominant in the CA1 region of the hippocampus. The ipsilateral hippocampal CA1 region became particularly active between day 1 and 7 of reperfusion. In the contralateral side of the striate cortex, dentate gyrus and hypothalamus, NOVA1 expression exhibited strong compensatory responses. In particular, NOVA1 was translocated to the dendrites and the growth cones of the axons in the hypothalamus on the ischaemic side by day 7 of reperfusion. Collectively, NOVA1 expression was positively correlated with neural repair upon ischaemia-reperfusion injury, contributing to neuronal responsiveness. As such, modulation of NOVA1 may provide a novel therapeutic approach for cerebrovascular accident.

2.4 | Familial dysautonomia

Massive screening of non-coding RNAs revealed that the expression of 26 microRNAs (miRNA) was altered in familial dysautonomia, among which four of them target NOVA1. The expression of NOVA1 was correlated with a low level of IKAP, which is also known as ELP1.[16] Overexpression of miR-203a-3p inhibited NOVA1 expression but promoted the expression of IKAP. The interaction between NOVA1 and IKAP might be a novel regulator in the pathogenesis of familial dysautonomia.

2.5 | Biogenesis of neural microRNAs

A large-scale functional screen identified NOVA1 as a novel regulator of neuronal miRNA function.[17] By regulating neuronal miRNA and miRNA-induced silencing complex function *via* Argonaute proteins, NOVA1 governs synapse development and its plasticity. The Ago proteins are main catalysts for miRISC assembly on target mRNA. NOVA1 stimulates miRNA function by different mechanisms that converge to Ago proteins, the core component of the miRISC.

3 | TUMOURS

3.1 | Astrocytoma and oligodendroglioma

The expression of NOVA1 differs significantly between low-grade oligodendroglioma and grade II astrocytoma.[18] While the role of elevated NOVA1 in glioma remains largely unknown, such a difference provides an alternative way to differentiate these two clinical entities.

By interacting with non-coding RNAs, NOVA1 interferes with various neuronal functions. Overexpression of miR-181b-5p suppressed cellular growth, invasion and migration, promoting apoptosis in astrocytoma cells through directly targeting NOVA1. It has been shown that miR-181b-5p expression was lowered in astrocytoma, inversely correlated with the clinical stages of astrocytoma patients.[19] High level of NOVA1 was also associated with poor survival outcome. Down-regulation of NOVA1 by miR-181b-5p decreased cell proliferation, invasion and migration, suggesting that this NOVA1 was an oncoprotein.

3.2 | Gastric cancer

The stromal expression of NOVA1 in malignant gastric cancer is considerably lower in benign gastritis.[20] In gastric cancer, it is down-regulated in T lymphocytes and stromal spindle cells. Conversely, it is highly expressed in these cells in gastritis. Clinically, reduced level of NOVA1 in remnant tissues or T lymphocytes is associated with a poor prognosis in gastric cancer patients. In gastric cancer, NOVA1 was regulated by miR-146b-5p. Collectively, decreased NOVA1 expression might indicate occult residual disease in remnant tissues after gastric cancer removal, serving as a potential prognostic biomarker for gastric cancer.

Another study demonstrated that the expression of NOVA1 was down-regulated in gastric cancer cells, T cells and stromal spindle cells.[21] Clinically, reduced NOVA1 expression was correlated with increased mortality, advanced tumour stages and inhibited infiltration of forkhead box $P3^+$ regulatory T cells and increased infiltration of $CD163^+$

TABLE 1 NOVA1 expressions in human cancers

Cancer type	Expression	Role in invasion/metastasis	References
Astrocytoma	Increased	Oncogenic	18
Oligodendroglioma	Increased	Oncogenic	18
Gastric cancer	Increased	Oncogenic	20,22
Gastric cancer	Decreased	Tumour suppressor	21
Hepatocellular cancer	Increased	Oncogenic	23,24
Lymphomas	Increased	Oncogenic	25

M2 and CD68$^+$ macrophages. These data suggested that reduction of NOVA1 in the microenvironment of gastric cancer was associated with poor prognosis. This finding may be in part related to immune dysfunction through changes in the immune cell composition of T cells and macrophages.

However, contradictory evidence regarding the role of NOVA1 in gastric cancer also exists in the literature. Overexpression of miR-339 was found to dramatically inhibit gastric cell proliferation, invasion, migration and tumorigenicity through targeting NOVA1.[22] NOVA1 expression was significantly down-regulated in miR-339 overexpressed cells. Restored expression of NOVA1 partially reversed the inhibitory effects of miR-339-overexpressed gastric cancer cells. These findings support NOVA1 might have oncogenic function in gastric carcinogenesis.

3.3 | Hepatocellular carcinoma

Focal expression of NOVA1 was observed in the cytoplasm of both tumour and peri-tumoural cells in hepatocellular carcinoma (HCC).[23] Overexpression of intratumoural NOVA1 was associated with lower survival rate and increased recurrence in HCC patients, serving as an independent prognostic factor. Enforced expression of NOVA1 increased cell proliferation, invasion and migration in HCC cell lines.

Further study showed that overexpression of NOVA1 increased subcutaneous HCC growth in vivo in nude mice.[24] NOVA1 expression was inversely correlated with that of GABAARγ2 and GABA. GABAARγ2 was expressed in HCC and was an independent prognostic factor for overall and disease-free survival in HCC patients. It is therefore likely that NOVA1 acts as a prognostic marker in HCC patients through its interaction with GABAARγ2.

3.4 | Lymphomas

NOVA1 expression was higher in T- and NK-cell lymphomas than in normal paracortical T cells.[25] In addition, diffuse and strong expression of NOVA1 was observed in 56.5% of T- and NK-cell lymphoma cases. The expression level of NOVA1 varied in different subtypes. NOVA1 expression was higher in angioimmunoblastic T-cell lymphoma, T lymphoblastic leukaemia/lymphoma and ALK-negative anaplastic large cell lymphoma. However, its expression was lower in ALK$^+$ anaplastic large cell lymphoma. NOVA1 expression was lower in almost all subtypes of B-cell lymphomas. These findings suggested that up-regulation of NOVA1 might play a role in the development of NK- and T-cell lymphomas. It might also be a potential diagnostic and therapeutic target for lymphoma (Table 1).

4 | OTHER FUNCTIONS

4.1 | Human immunodeficiency

The transduction of PWWP-interacting region of NOVA1 significantly decreased human immunodeficient virus infectivity, while it showed no effect on murine leukaemia virus infection. This region has been shown to localize with LEDGF/p75 of human immunodeficient virus in HeLa cells.[26] However, the role of NOVA proteins in viral replication remains unclear.

4.2 | Pancreatic beta cell function

NOVA1 expression in pancreatic beta cells was more than 10-fold higher than in other tissues. NOVA1 controls the expression of key genes involved in transcription and insulin release.[27] Specific knockdown of NOVA1 led to altered splicing of nearly 5,000 transcripts involved in exocytosis, insulin receptor signalling, apoptosis, transcription and splicing. Inhibition of NOVA1 also decreased insulin secretion by interacting with its potential targets, such as PLCβ1 and Snap25. These observations indicate that NOVA1 as a splicing factor has a major role in the regulation of beta cell function.

5 | CONCLUSIONS

NOVA1 plays a significant role in carcinogenesis and neurological disorders. Its differential expression may permit the differentiation of distinct clinical entities, which would otherwise be difficult to achieve. Currently, the mechanism governing the expression of NOVA1 and its interaction with miRNAs remain largely unknown. Further studies are needed to clarify their roles in more tumours such as osteosarcoma, cutaneous squamous cell carcinoma, melanoma and other tumours.

CONFLICTS OF INTEREST

The authors declare no competing financial interests.

REFERENCES

1. Buckanovich RJ, Posner JB, Darnell RB. Nova, the paraneoplastic Ri antigen, is homologous to an RNA-binding protein and is specifically expressed in the developing motor system. *Neuron*. 1993;11:657-672.
2. Darnell RB, Posner JB. Paraneoplastic syndromes involving the nervous system. *N Engl J Med*. 2003;349:1543-1554.
3. Moore MJ. From birth to death: the complex lives of eukaryotic mRNAs. *Science*. 2005;309:1514-1518.
4. Buckanovich RJ, Darnell RB. The neuronal RNA binding protein Nova-1 recognizes specific RNA targets in vitro and in vivo. *Mol Cell Biol*. 1997;17:3194-3201.
5. Jensen KB, Dredge BK, Stefani G, et al. Nova-1 regulates neuron-specific alternative splicing and is essential for neuronal viability. *Neuron*. 2000;25:359-371.
6. Lewis HA, Musunuru K, Jensen KB, et al. Sequence-specific RNA binding by a Nova KH domain: implications for paraneoplastic disease and the fragile X syndrome. *Cell*. 2000;100:323-332.
7. Ule J, Jensen KB, Ruggiu M, et al. CLIP identifies Nova-regulated RNA networks in the brain. *Science*. 2003;302:1212-1215.
8. Saito Y, Miranda-Rottmann S, Ruggiu M, et al. NOVA2-mediated RNA regulation is required for axonal pathfinding during development. *Elife* 2016;5:e14371.
9. Yang YY, Yin GL, Darnell RB. The neuronal RNA-binding protein Nova-2 is implicated as the autoantigen targeted in POMA patients with dementia. *Proc Natl Acad Sci USA*. 1998;95:13254-13259.

10. Graus F, Rowe G, Fueyo J, et al. The neuronal nuclear antigen recognized by the human anti-Ri autoantibody is expressed in central but not peripheral nervous system neurons. *Neurosci Lett*. 1993;150:212-214.
11. Barash Y, Calarco JA, Gao W, et al. Deciphering the splicing code. *Nature*. 2010;465:53-59.
12. Buckanovich RJ, Yang YY, Darnell RB. The onconeural antigen Nova-1 is a neuron-specific RNA-binding protein, the activity of which is inhibited by paraneoplastic antibodies. *J Neurosci*. 1996;16:1114-1122.
13. Lin JC, Chi YL, Peng HY, et al. RBM4-Nova1-SRSF6 splicing cascade modulates the development of brown adipocytes. *Biochim Biophys Acta*. 2016;1859:1368-1379.
14. Fryssira H, Tsoutsou E, Psoni S, et al. Partial monosomy14q involving FOXG1 and NOVA1 in an infant with microcephaly, seizures and severe developmental delay. *Mol Cytogenet*. 2016;9:55.
15. Li H, Sun C, Wang Y, et al. Dynamic expression pattern of neuro-oncological ventral antigen 1 (Nova1) in the rat brain after focal cerebral ischemia/reperfusion insults. *J Histochem Cytochem*. 2013;61:45-54.
16. Herve M, el Ibrahim C. MicroRNA screening identifies a link between NOVA1 expression and a low level of IKAP in familial dysautonomia. *Dis Model Mech*. 2016;9:899-909.
17. Storchel PH, Thummler J, Siegel G, et al. A large-scale functional screen identifies Nova1 and Ncoa3 as regulators of neuronal miRNA function. *EMBO J*. 2015;34:2237-2254.
18. Gimenez M, Marie SK, Oba-Shinjo S, et al. Quantitative proteomic analysis shows differentially expressed HSPB1 in glioblastoma as a discriminating short from long survival factor and NOVA1 as a differentiation factor between low-grade astrocytoma and oligodendroglioma. *BMC Cancer*. 2015;15:481.
19. Zhi F, Wang Q, Deng D, et al. MiR-181b-5p downregulates NOVA1 to suppress proliferation, migration and invasion and promote apoptosis in astrocytoma. *PLoS ONE*. 2014;9:e109124.
20. Yoon SO, Kim EK, Lee M, et al. NOVA1 inhibition by miR-146b-5p in the remnant tissue microenvironment defines occult residual disease after gastric cancer removal. *Oncotarget*. 2016;7:2475-2495.
21. Kim EK, Yoon SO, Jung WY, et al. Implications of NOVA1 suppression within the microenvironment of gastric cancer: association with immune cell dysregulation. *Gastric Cancer* 2016.
22. Shen B, Zhang Y, Yu S, et al. MicroRNA-339, an epigenetic modulating target is involved in human gastric carcinogenesis through targeting NOVA1. *FEBS Lett*. 2015;589:3205-3211.
23. Zhang YA, Zhu JM, Yin J, et al. High expression of neuro-oncological ventral antigen 1 correlates with poor prognosis in hepatocellular carcinoma. *PLoS ONE*. 2014;9:e90955.
24. Zhang YA, Liu HN, Zhu JM, et al. RNA binding protein Nova1 promotes tumor growth in vivo and its potential mechanism as an oncogene may due to its interaction with GABAA Receptor-gamma2. *J Biomed Sci*. 2016;23:71.
25. Kim EK, Yoon SO, Kim SH, et al. Upregulated Neuro-oncological Ventral Antigen 1 (NOVA1) Expression Is Specific to Mature and Immature T- and NK-Cell Lymphomas. *J Pathol Transl Med*. 2016;50:104-112.
26. Morchikh M, Naughtin M, Di Nunzio F, et al. TOX4 and NOVA1 proteins are partners of the LEDGF PWWP domain and affect HIV-1 replication. *PLoS ONE*. 2013;8:e81217.
27. Villate O, Turatsinze JV, Mascali LG, et al. Nova1 is a master regulator of alternative splicing in pancreatic beta cells. *Nucleic Acids Res*. 2014;42:11818-11830.

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