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Review

NEK2 Is an Effective Target for Cancer Therapy With Potential to Induce Regression of Multiple Human Malignancies

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Abstract. *Cancer is characterized by uncontrolled cell proliferation due to the aberrant activity of various proteins. Cell cycle-related proteins are thought to be important in several functions, such as proliferation, invasion and drug resistance in human malignancies. Never in mitosis gene A-related kinase 2 (NEK2) is a cell cycle-related protein. NEK2 is highly expressed in various tumor types and cancer cell lines. NEK2 expression is correlated with rapid relapse and poor outcome in multiple cancer types. Several researchers have demonstrated that NEK2 inhibition results in anticancer effects against many types of cancers, both in vitro and in vivo. Recent research strongly indicates the advantages of NEK2-targeted therapy for cancer. This review focuses on the current understanding of NEK2 in cancer and the rationale of a xenograft cancer model for cancer treatment. A possible therapeutic strategy, such as inhibitor and nucleic acid medicine targeting of NEK2, is also discussed.*

Cancer is characterized by uncontrolled cell proliferation due to the aberrant activity of various proteins (1). Recent studies have revealed that cell cycle-related proteins play important roles in multiple cancer types (2, 3). Many forms of cancers are uniquely dependent on these proteins and hence are selectively sensitive to their inhibition (1). In this regard, cell-cycle regulators are effective targets for cancer treatment.

Never in mitosis gene A-related kinase 2 (NEK2) is a cell cycle-related protein, along with aurora kinases and polo-like kinases (4, 5). Several studies have been published concerning the roles of NEK2 in chromosome instability, tumorigenesis, progression, and drug resistance in cancer (6-8).

This review focuses on the current understanding of NEK2 in cancer progression and the rationale of a xenograft cancer model for cancer treatment. A possible therapeutic strategy, such as inhibitor and nucleic acid medicine targeting of NEK2, is also discussed.

Structure of NEK2

NEK2 is structurally related to the mitotic regulator never in mitosis gene A (NIMA), which is cloned from *Aspergillus nidulans* (9). Eleven mammalian homologs of the NEK family, named NEK1 to NEK11, have been identified (5, 10). The NEK family comprises several serine/threonine kinases and is important for cell division and cell-cycle regulation, as well as NIMA (11). NEK2 is the closest mammalian isoform to NIMA and has structures with a serine-threonine kinase domain located at the amino-terminal and multiple regulatory motifs, such as a leucine zipper, coiled coil, centrosome and microtubule localization sites, protein phosphatase 1 (PP1) binding site, KEN-box, nucleolar localization sites, anaphase-promoting complex (APC) binding site, and destruction box (D-box) at the carboxyl-terminal site (12). NEK2 in mammals has three splice variants: NEK2A, NEK2B, and NEK2C (13, 14). NEK2A and NEK2B differ at their carboxy-termini (15, 16), and NEK2C lacks an eight-amino acid sequence from the carboxy-terminus of NEK2A (14). NEK2A is evenly distributed within the nuclei and cytoplasm, while NEK2B is mainly distributed in the cytoplasm, and NEK2C is mainly distributed in the nuclear region (14). As NEK2A, NEK2B and NEK2C exhibit overlapping or identical substrate usage, these variants are collectively referred to here as NEK2 (17-20).

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NEK2 Expression in Cancer

High expression of NEK2 was first identified in pediatric solid tumors called Ewing's sarcoma using microarray analysis (21). We reported that NEK2 is highly expressed in several cancer types, such as cholangiocarcinoma (22), breast (7, 23-31), colorectal (27, 32-35), and pancreatic (36, 37) cancer. Consistently, several researchers reported that high expression of NEK2 is identified in various cancer types, including testicular seminoma (27, 38, 39), cervical tumor (27), primary liver cancer (40), hepatocellular carcinoma (8, 41), prostate cancer (27, 42), lung cancer (27, 43-45), ovarian cancer (46), renal cell cancer (47, 48), myeloma (49), peripheral nerve sheath tumors (50), follicular lymphoma (51, 52) and diffuse large B-cell lymphoma (52).

NEK2 overexpression is significantly associated with histological differentiation, higher in TNM stage, lymph node metastasis and tumor invasion in colon (33), pancreatic (37), and lung (43) cancer. NEK2 is a promising predictor of poor prognosis in cancer because its expression is highly correlated with rapid relapse and poor outcome in multiple cancer types.

Functional Role of NEK2 in Cancer

NEK2 expression is low in the G_1 phase of the cell cycle, increasing through S and G_2 to reach a peak in late G_2/M, and decreasing upon cell entry into mitosis (5, 15). Similarly, NEK2 activity is stronger in the S and G_2 phases compared to that in other phases. NEK2 is responsible for starting centrosome separation at the G_2/M phase of the cell cycle (53). NEK2 overexpression results in chromosome instability and aneuploidy in cancer cells (6, 54, 55). In addition, NEK2 overexpression activates several oncogenic pathways and ATP-binding cassette transporters, thereby leading to cell proliferation, invasion, and drug resistance (55).

Proliferation. We demonstrated that increased NEK2 promoted cell growth and *NEK2*-targeting siRNA inhibited proliferation in cholangiocarcinoma (22). NEK2 siRNA suppressed tumor growth in a xenograft nude mouse model (22). Another group also demonstrated that increased expression of NEK2 promoted cell proliferation, while its suppression inhibited proliferation in various cancer types (24, 28, 31, 42, 54). Zhou *et al*. reported that both the PP1/AKT and the WNT signaling pathways may be involved in NEK2-induced cell proliferation (54). Several studies revealed that NEK2 expression is strongly related to Ki-67, a proliferation marker, in various malignancies (31, 44, 54). These data indicate the critical roles of NEK2 in cancer proliferation, both *in vitro* and *in vivo*.

Invasion and motility. Invasiveness and motility are associated with various genes in many cancer types (56-58). Hayward *et al*. first reported that NEK2 overexpression preceded metastasis in cancer cells (24). We previously reported that NEK2 expression affected invasion and motility in breast cancer and pancreatic cancer cells (23, 36). Xia *et al*. reported that NEK2 played an important role in tumor metastasis by regulating the expression and localization of β-catenin, because NEK2 overexpression induced nuclear accumulation of β-catenin in multiple myeloma and lung cancer cells (55). NEK2 induced metastasis in cooperation with RAS and SRC signaling and promoted chromosomal instability in cancer (59). From these data, NEK2 is believed to be involved in invasion and motility and to promote metastasis in cancer.

Apoptosis. The inactivation of apoptosis is central to cancer progression. Apoptosis is involved in the resistance to therapy of many kinds of cancer.

NEK2 depletion leads to aneuploidy and cell-cycle arrest; thereafter, apoptosis was found to be induced as a result of mitotic errors in various cancer cell lines (19, 27, 28, 54). Naro *et al*. reported that NEK2 inhibition in cancer cells led to high expression of cleaved PARP and activated caspase-3, caspase-8, and caspase-9 *in vitro* and in a xenograft mouse model of myeloma (27, 54). These data indicate an important role of NEK2 against the apoptosis pathway. However, Lee and Gollahon reported that NEK2 suppression did not induce strong mitotic arrest in the G_2/M phase, but instead induced apoptosis. This indicates that the role of NEK2 may be different from other cell cycle-related kinases in regulation of cell cycle (60). The mechanisms concerning apoptosis by NEK2 suppression remain unclear in cancer cells.

Drug sensitivity and resistance. Drug sensitivity and resistance are important issues in cancer treatment. Developing a novel strategy for enhancement of sensitivity to chemotherapeutic agents is one of the most serious challenges in improving the prognosis of patients with cancer. NEK2 was shown to regulate chemotherapeutic resistance through several genes, such as ATP binding cassette subfamily G (*ABCG*), aldehyde dehydrogenase 1 family, member A1 (*ALDH1A1*), and Retinoid X receptor alpha (*RXRA*) in malignancies (8, 61). NEK2 overexpression is associated with drug-resistant ovarian cancer and multiple myeloma (46, 62). Some researchers reported NEK2 to be involved in resistance to 5-fluorouracil, tamoxifen, trastuzumab, paclitaxel and doxorubicin (7, 60, 63). Therefore, NEK2 appears to contribute to resistance to several drugs in multiple cancer types.

Drug efflux plays an essential role in increasing drug resistance in a variety of malignancies. NEK2 depletion reduced drug efflux pump activity and inhibited drug resistance (61). NEK2 inhibition might be a therapeutic option for treating chemoresistant cancer cells.

We previously reported that the combination of *NEK2* siRNA and cisplatin showed additive antitumor effects on colorectal cancer cells (32). siRNA and antisense oligonucleotide against NEK2 worked synergistically with paclitaxel and doxorubicin by promoting apoptosis of breast cancer cells (60). NEK2 combined treatment would be useful to abrogate the resistance to chemotherapy and further improve clinical outcomes.

Therapeutic Potential of NEK2 in a Xenograft Cancer Model

The studies using various cancer cell lines suggest that the inhibition of NEK2 may be beneficial for cancer treatment. Even if agents are effective against cell lines, they often demonstrate no efficacy in xenograft model. In this regard, it is important to confirm the therapeutic potential of NEK2 for cancer treatment in several xenograft cancer models, such as subcutaneous tumors, peritoneal dissemination and liver metastasis. Several researchers reported the efficiency of NEK2 inhibition in various xenograft cancer models.

Subcutaneous tumors. We reported that *NEK2* silencing suppressed xenograft tumor growth of cholangiocarcinoma (22), and breast (23), colorectal (32), and pancreatic (36) cancer. In addition, NEK2 inhibition showed efficiency in other xenograft cancer models, such as myeloma, and prostate, liver, colorectal, and breast (42, 54, 64-66) cancer.

Peritoneal dissemination. Peritoneal dissemination is a major undesirable complication frequently associated with inoperable cases of cancer. Many patients with cancer die of peritoneal dissemination because of lack of effective and useful treatment. NEK2 overexpression is associated with serosal invasion, lymphatic invasion, and peritoneal dissemination (34).

We previously reported that *NEK2* siRNA improves the survival of nude mice with peritoneal dissemination of cholangiocarcinoma (22) and pancreatic cancer (36) xenografts. The total nodule number and weight of peritoneal dissemination in the *NEK2* siRNA-treated group were significantly lower than those in the control siRNA-treated group.

Liver metastasis. Multiple metastases are considered to be detrimental to the outcome of cancer because it is impossible to remove all lesions during an operation. Although NEK2 is highly expressed in liver metastases, the significance of NEK2 expression using xenografts has not been investigated.

We previously examined the potential of *NEK2* siRNA for metastasis of pancreatic cancer (36). *NEK2* siRNA reduced the number and the area of liver metastases from pancreatic cancer in a rat xenograft model (36). *NEK2* siRNA was able to prevent the progression of liver metastasis efficiently.

NEK2-targeted Cancer Therapy

Despite the development of chemotherapy for cancer, clinical outcomes have not been markedly improved in many types of cancer. Novel strategies are therefore required to treat cancer, especially in the case of local recurrence, peritoneal dissemination and liver metastasis. As NEK2 has a critical role in the progression of malignancies, as mentioned previously, it is attractive as a target for novel anticancer therapies (55, 67).

NEK2 inhibitors. Several small-molecule inhibitors of NEK2 were developed in high-throughput screening (68) (Table I). These inhibitors showed their therapeutic effectiveness against cancer cells both *in vitro* and *in vivo*.

Propynamide16 was designed as an irreversible, cysteine-targeted inhibitor of NEK2 through a structure-based approach. This compound inhibited cellular NEK2 without affecting the mitotic kinases, cyclin-dependent kinase 1 (CDK1), aurora B, or polo-like kinase 1 (PLK1). This compound was the first small molecule shown to inactivate NEK2 kinase activity in cells (69).

Highly expressed in cancer 1 (HEC1) is a critical mitotic regulator, which is phosphorylated by NEK2 in proper chromosome segregation (70). TAI-95 and TAI-1, small molecules targeting the HEC1/NEK2 pathway, inhibited tumor growth in xenograft mouse models of liver, colorectal , and breast cancer (60, 64-66). A 4-aryl-N-arylcarbonyl-2-aminothiazole was designed and synthesized as an HEC1/NEK2 inhibitor. This compound also demonstrated inhibition of tumor growth in a xenograft breast cancer model (71).

A small molecule, *N*-(4-[2,4-dimethyl-phenyl]-thiazol-2-yl)-benzamide (INH1), specifically disrupted HEC1/NEK2 interaction *via* direct HEC1 binding. This INH-bound HEC1 triggered NEK2 degradation and eventually induced cell death. INH1 effectively inhibited the proliferation of breast cancer cell lines (72, 73). Several INH derivatives were designed and synthesized, which significantly suppressed xenograft tumor growth without obvious toxicity (74).

The epidermal growth factor receptor /human epidermal growth factor receptor 2 (EGFR/HER2) inhibitors neratinib and pelitinib also inhibited human NEK2 activity *in vitro* (59). Aminopyridine (R)-21, a potent and selective inhibitor based on an aminopyridine scaffold, modulated NEK2 activity in cells (75). Two viridian-like compounds, CC004731 and CC004733, suppressed NEK2 activity and inhibited the proliferation of human cancer cell lines (76). HCI-2389 was designed as a NEK2 inhibitor by virtual screening, and successfully mitigated drug resistance in bortezomib-resistant multiple myeloma (62).

Nucleic acid medicine. Several studies demonstrated that NEK2 inhibition using nucleic acids such as siRNA,

Table I. *Inhibitory effect of never in mitosis gene A-related kinase 2 (NEK2) inhibitors in subcutaneous xenograft animal models.*

Cancer type	Cell line	Animal	Agent	Dose, mg/kg		Administration method and DDS	Ref
Breast	MDA-MB-231	CB.17, SCID mouse	TAI-1	20	*i.v.*	2/d×28 d	(66)
				150	*p.o.*	2/d×28 d	
Colorectal	Colo-205	CB.17, SCID mouse	TAI-1	7.5, 22.5, 50→75	*p.o.*	2/d×28 d	(66)
Liver	Huh-7	CB.17, SCID mouse	TAI-1	7.5, 22.5, 50→75	*p.o.*	2/d×28 d	(66)
Liver	Huh-7	CB.17, SCID mouse	TAI-95	1, 2.5, 10	*p.o.*	2/d×28 d	(64)
Breast	MDA-MB-231	CB.17, SCID mouse	TAI-95	10, 25	*p.o.*	2/d×28 d	(65)
Breast	BT474	CB.17, SCID mouse	TAI-95	10, 25, 50	*p.o.*	2/d×28 d	(65)
Breast	MCF7	Balb/c, nude mouse	TAI-95	10, 25, 50	*i.v.*	2/d×28 d	(65)
Breast	MDA-MB-231	Balb/c, nude mouse	Compound 32	20	*i.v.*	1/d×28 d	(71)
				150	*p.o.*	2/d×28 d	
Breast	MDA-MB-468	Balb/c, nude mouse	INH1	50, 100	*i.v.*	Every other day ×7 w (25 cycles)	(73)
Breast	MDA-MB-468	Balb/c, nude mouse	INH41	10, 50	*i.p.*	3/w×7 w	(74)
Breast	MDA-MB-468	Balb/c, nude mouse	INH154	5, 20	*i.p.*	3/w×7 w	(74)

i.s.: Local administration; *i.p.*: intraperitoneal; *i.v.*: intravenous; *p.o.*: oral; w: week; d: day; DDS: Drug Delivery System.

Table II. *Inhibitory effect of nucleic acid medicine targeting never in mitosis gene A-related kinase 2 (NEK2) in xenograft animal models.*

Cancer type	Cell lines	Animal	Xenograft	siRNA for *Nek2*	Conc. Vol.	Administration method and DDS			Ref
Cholangiocarcinoma	HuCCT1	Balb/c nude mouse	Subcutaneous	siRNA	20 μM, 100 μl	*i.s.*	Biocollagen	1/w×3 d	(22)
Breast	MDA-MB-231	Balb/c nude mouse	Subcutaneous	siRNA	20 μM, 100 μl	*i.s.*	Biocollagen	1/w×3 d	(23)
Breast	MCF7	Balb/c nude mouse	Subcutaneous	siRNA	20 μM, 100 μl	*i.s.*	Biocollagen	1/w×3 d	(23)
Colorectal	DLD1	Balb/c nude mouse	Subcutaneous	siRNA	20 μM, 100 μl	*i.s.*	Biocollagen	2w×3 d	(32)
Colorectal	DLD1	Balb/c nude mouse	Subcutaneous	siRNA (CDDP)	50 μM, 100 μl (4 mg/kg)	*i.s.* *i.p.*	Biocollagen –	2/w×2 w (2/w×2 w)	(32)
Pancreas	KLM1	Balb/c nude mouse	Subcutaneous	siRNA	50 μM, 100 μl	*i.s.*	Biocollagen	1/w×3 d	(36)
Cholangiocarcinoma	HuCCT1	Balb/c nude mouse	Peritoneal	siRNA	20 μM, 100 μl	*i.p.*	Liposome	1/w×3 d	(22)
Pancreas	KLM1	Balb/c nude mouse	Peritoneal	siRNA	50 μM, 100 μl	*i.p.*	Liposome	1/w×3 d	(36)
Pancreas	KLM1	F344/njcl rat	Liver metastasis	siRNA	50 μM, 100 μl	*i.v.*	Liposome	1/d×5 d	(36)
Myeloma	APR1	*Nod-Rag*/null mouse	Subcutaneous	*Nek2* silenced cell using shRNA		*i.s.*	–	–	(54)
Prostate	LNCAP	Balb/c nude mouse	Subcutaneous	*Nek2* silenced cell using siRNA		*i.s.*	–	–	(42)

i.s.: Local administration; *i.p.*: intraperitoneal; *i.v.*: itravenous; *p.o.*: oral; w: week; d: day; CDDP: cisplatin; DDS: drug-delivery system.

antisense and miRNA may be beneficial for cancer therapy (34, 60, 77) (Table II). Nucleic acid medicine is positioned to be a promising therapy. For instance, *NEK2*-targeting siRNA reduced the viability and proliferation of several cancers, including cholangiocarcinoma (22), breast (23), colorectal (32), and pancreatic (36). *miR-128* suppressed NEK2 expression in cancer cells and thereafter inhibited cell proliferation and induced cell-cycle arrest (34).

Although siRNA is a beneficial tool for inhibiting the expression of a specific gene through a drug delivery system, an *in vivo* delivery system is especially important for clinical application. Mowa *et al*. reported that viral vectors such as adenoviral vectors and retroviral vectors were good systems for drug delivery of siRNA (78). However, viral vectors carry a risk of severe side-effects in clinical use.

We used biocollagen and liposome as delivery carriers for *NEK2* siRNA in a xenograft mouse model, such as subcutaneous tumor, peritoneal dissemination and liver metastasis (22, 23, 32, 36). Both delivery carriers were efficient in the transfection rate and for the effect of *NEK2*

siRNA in cancer cells (36). Therefore, biocollagen and liposomes have advantages for clinical application as delivery carriers for nucleic acid medicine, including siRNA.

In addition, we have focused on the venous port-catheter system as another drug-delivery system for siRNA for liver metastasis. The system has already been applied in the clinic, and is effective as a drug delivery system for siRNA, because anticancer drugs are administered directly into tumors (36).

Further investigations are required in terms of safety and side-effects of the use of *NEK2* siRNA. We consider it possible that *NEK2* siRNA will be a novel therapeutic strategy for the treatment of cancer.

Future Perspectives

Several researchers have demonstrated that the suppression of NEK2 results in inhibitory effects in many cancer types both in vitro and in vivo. Current research strongly indicates an advantage for NEK2-targeted cancer therapy. However, several crucial problems remain to be resolved. For instance, the signaling pathway of NEK2 in cancer may be partly shared by normal cells. We did not identify this complication related to NEK2 inhibition in several xenograft mouse models. However, the side-effects in NEK2-targeting therapy are difficult to predict completely. Further investigation of the role of NEK2 in cancer cells is thus crucial to prevent damage to normal cells. Taken together, these findings suggest that NEK2 is an effective target for cancer therapy and has the potential to promote the regression of multiple human malignancies.

Conflicts of Interest

The Authors have no conflicts of interest in regard to this study.

Authors' Contributions

In regard to our own work cited here, Toshio Kokuryo, Yukihiro Yokoyama and Masato Nagino conceived and planned experiments; Junpei Yamaguchi and Nobuyuki Tsunoda carried out experiments; Tomoki Ebata contributed to sample preparation and the interpretation of results. Toshio Kokuryo took the lead in writing the article. All Authors provided critical feedback and helped shape the research, analysis and article preparation.

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