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NEK9 depletion induces catastrophic mitosis by impairment of mitotic checkpoint control and spindle dynamics

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ABSTRACT

NEK9 is known to play a role in spindle assembly and in the control of centrosome separation, but the consequences of NEK9 targeting in cancer cells remain to be elucidated. In this study, we used siRNA to investigate the consequences of targeting NEK9 in glioblastoma and kidney cancer cells as a first step in assessing its potential as an anti-cancer therapeutic target. Live cell imaging revealed that NEK9 depletion of U1242 glioblastoma and Caki2 kidney carcinoma cells resulted in failure of cytokinesis. Interestingly, NEK9-depleted Caki2 cells overrode mitosis under incorrect chromosome alignment and were converted to a micronucleated phenotype, leading to cell death. Whereas, the RPE1 normal epithelium cell line was refractory to abnormal mitosis upon NEK9 knockdown. Nocodazole-induced mitotic arrest was compromised after NEK9 depletion, indicating that NEK9 has an important role in mitotic checkpoint system. Taken together, we propose that NEK9 inhibition represents a novel anti-cancer strategy by induction of mitotic catastrophe via impairment of spindle dynamics, cytokinesis and mitotic checkpoint control.

1. Introduction

The NIMA family of protein kinases is named after the Aspergillus nidulans protein kinase encoded by the nimA (never in mitosis A) gene [1]. NIMA is required for entry into mitosis and is involved in the control of chromatin condensation, spindle and nuclear envelope organization, and cytokinesis [2,3]. Eleven protein kinases, NEK1 to NEK11, with a catalytic domain related to NIMA have been identified in the human genome [4]. Nek9 is a 107-kDa polypeptide whose N-terminal catalytic domain is followed by a domain homologous to regulator of chromatin condensation (RCC1). NEK9 C-terminal coiled coil motif binds to DYNLL/LC8 and this interaction is regulated by NEK9 activity through the autophosphorylation [5]. Microinjection of anti-NEK9 antibodies in prophase results in spindle abnormalities and/or chromosomal misalignment [6]. NEK9 co-immunoprecipitates gamma-tubulin and the activated NEK9 localizes to the centrosomes and spindle poles during early mitosis, indicating that active NEK9 has important functions at the microtubular organizing center during cell division [7]. Nek6 and Nek7 can bind strongly to RCC1 domain of NEK9 and are phosphorylated and activated by NEK9 [8] and depletion of either NEK6 or NEK7 leads to defective mitotic progression [9]. It is clear that NEK6, NEK7 and NEK9 contribute to the establishment of the microtubule-based mitotic spindle [10].

Besides spindle tubule formation, Betran et al. reported that NEK9 activation by Polo-like kinase 1 (PLK1) contributed to the phosphorylation of the mitotic kinesin Eg5 is necessary for subsequent centrosome separation and timely mitosis [11]. In addition, NEK9 phosphorylates NEDD1 which recruits gamma-tubulin to centrosome to ensure the formation of two dense microtubule asters in cells entering mitosis [12]. These data indicate that NEK9 has important function not only in spindle formation but also in centrosome maturation and separation.

Spindle assembly checkpoint (SAC) is a fail-safe mechanism that monitors the fidelity of chromosome segregation in space and time. Until all chromosomes are properly aligned at the spindle equator, the mitotic checkpoint inhibits the anaphase-promoting complex/cyclosome (APC/C) that prevents cells from entering anaphase. If the kinetochore-microtubule attachment is not correct, SAC generates the wait-anaphase signal that propagates throughout the cell to inhibit the APC/C [13]. In various human cancers, mitotic checkpoint function is partially compromised, and altered expression or mutations in mitotic checkpoint genes have been shown to be related to chromosome instability (CIN) and aneuploidy. Nevertheless, no evidence for checkpoint malfunctions as a direct cause of CIN in tumor cells has been found [14–16]. However, complete inactivation of the mitotic checkpoint results in gross chromosomal missegregation and is not compatible with cell viability [17–20]. This has led to the suggestion that

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inhibition of the mitotic checkpoint could have therapeutic potential in cancer treatment. Moreover, tumor cells that have acquired a decreased checkpoint activity could be more sensitive to mitotic checkpoint inhibition when compared to healthy, checkpoint proficient cells [21].

Here, we explored the potential of NEK9 as an anti-cancer therapeutic target. We found that NEK9 plays an important role in the spindle checkpoint. NEK9 inhibition induced apoptosis through abnormal mitosis in tumor cells, whereas the hTERT-RPE1 (REP1) normal human retinal pigment epithelium cell line was refractory to abnormal mitosis.

2. Materials and methods

2.1. Human cell lines

Caki2 kidney carcinoma cells was obtained from the American Type Culture Collection and U1242 glioblastoma cells was obtained from Sugen Inc. were cultivated in RPMI1640 supplemented with 10% FCS and glutamine (Life Technologies). U1242 and Caki2 cells which were transfected with a human histone H2B/green fluorescence protein (GFP) fusion gene (H2B-GFP) were used to analyze mitotic processes. RPE1 cells which stably express H2B-GFP was a kind gift by Taylor [14]. Cell proliferation was measured by using CellTiter-Glo (Promega) according to the manufacturer's instructions.

2.2. RNA interference

Two independent siRNAs against NEK9 were used in all experiments. These are referred to as NEK9#115-siRNA (Ambion, Silencer Validated siRNA (#1115)) and NEK9#72-siRNA (Ambion, Silencer Select siRNA (#s40772)). Non-targeting RNA duplexes (Negative control siRNA (Ambion, #4390843)) were used as a negative control in all experiments. siRNAs were transfected using Lipofectamine RNAiMax (Invitrogen) according to the manufacturer's instructions (final concentration of siRNA was 3.75 nM).

2.3. Quantitative PCR

Total RNA was isolated using the RNeasy Mini Kit (Qiagen) and reverse transcribed into cDNA using AMV reverse transcriptase (Roche) with random hexamers. For real-time PCR, Assays-

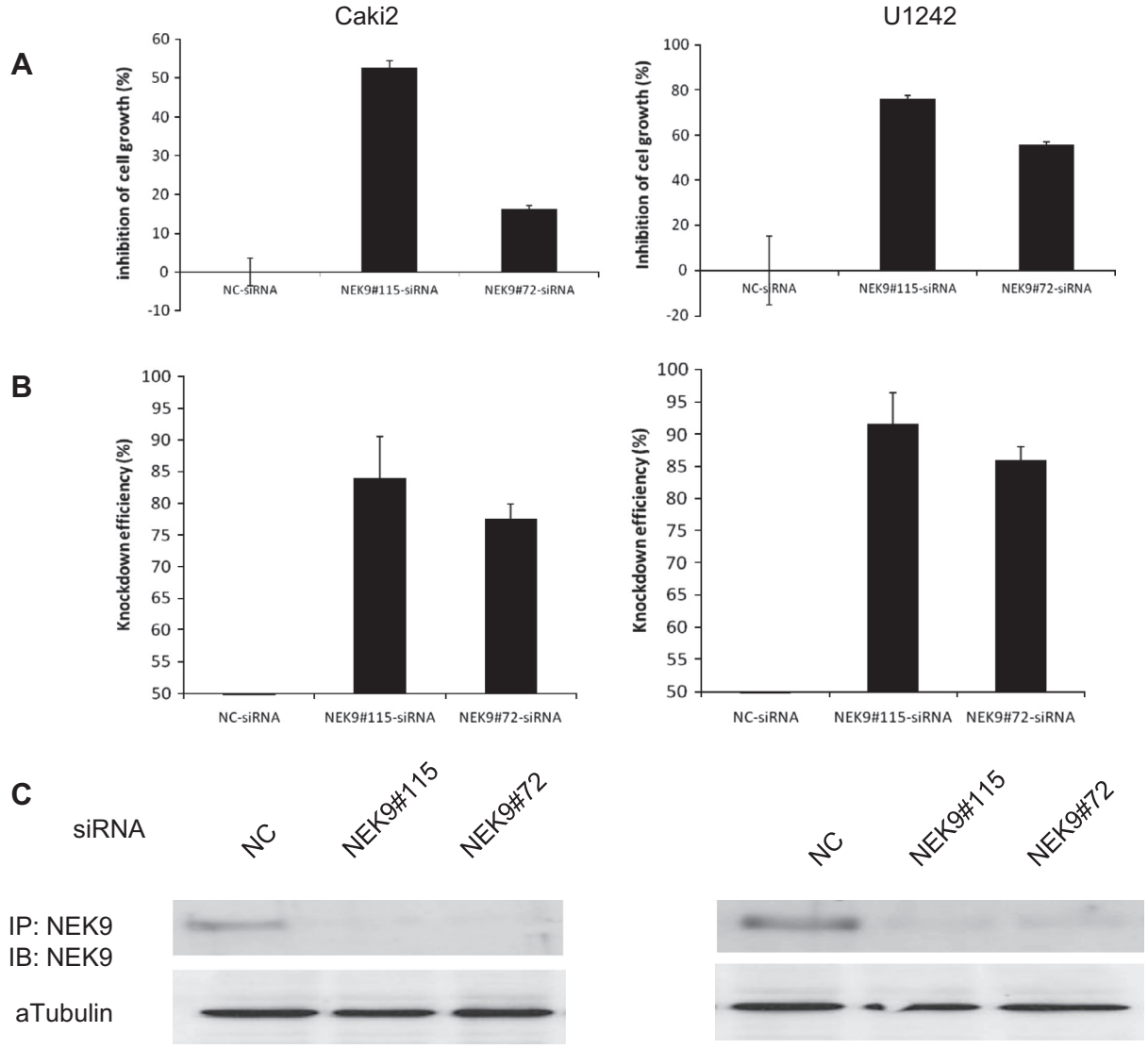

Fig. 1. Depletion of NEK9 induced growth inhibition in cancer cells. Inhibition of cell growth was measured 4 days post-transfection of siRNA. Growth inhibition of negative control siRNA (NC-siRNA) transfectants was normalized to zero (A). After siRNA transfection, cells were incubated for 4 days. Knockdown efficiency of NEK9 mRNA, which was normalized to GAPDH mRNA, was calculated by comparison to NC-siRNA transfectants (B). Knockdown efficiency was also confirmed by western blotting after immunoprecipitation (C).