NEIL3-mediated mitotic base excision repair of oxidative lesions at telomeres prevents senescence in hepatocellular carcinoma

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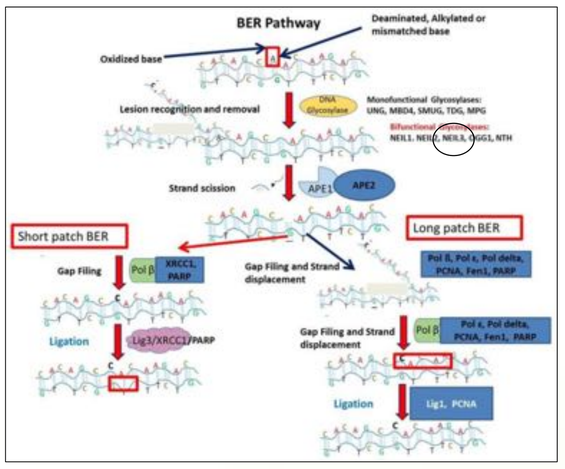
Introduction

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer and the third leading cause of cancer death worldwide. Chronic liver diseases contributes to accumulation of reactive oxygen species (ROS) and inflammation, leading to cirrhosis and eventually HCC. Although many approaches have been suggested to treat HCC (e.g., surgery, transarterial chemoembolization (TACE), immunotherapy and targeted therapies), effective drugs and non-surgical treatment for HCC patients remain very limited

ROS production increases during G2/M-phase and induction of oxidative damage in this phase arrests cells in pro-metaphase, suggesting that the level of endogenous DNA damage varies during the cell cycle and have more severe effects in certain phases.

It is well established that there is differential DNA repair at telomeres. Telomeres are sensitive to oxidative damage, resulting in cell senescence, chromosome fusion and apoptosis. While BER pathways are described to be active at telomeres, information about these processes or potential differential roles of glycosylases are generally lacking.



Oxidative damaged bases in DNA are repaired by Base Excision Repair (BER). NEIL3 is a multi-functional glycosylase removing hydantoins (spiroiminodihydantoin (Sp) and guanidinohydantoin (Gh)) and thymine glycol (Tg) from the DNA. NEIL3 has been implicated in repair of inter-strand crosslinks, replication associated damage and telomere damage. NEIL3 has an unstructured C-terminal domain that can interact with TRF1, FEN-1, PCNA and APE-1.

Summary

NEIL3 is overexpressed in HCC which correlates with poor survival.

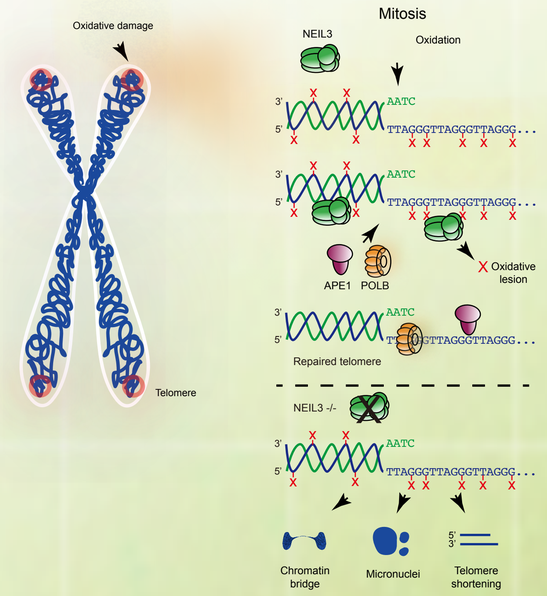
NEIL3 depleted HCC cell lines accumulate oxidative DNA lesions specifically at telomeres, resulting in Telomere dysfunctional foci (TIFs) and 53BP1 foci formation.

NEIL3 relocates to telomeres following oxidative DNA damage during mitosis and recruits apurinic endonuclease 1 (APE1), indicating activation of base excision repair.

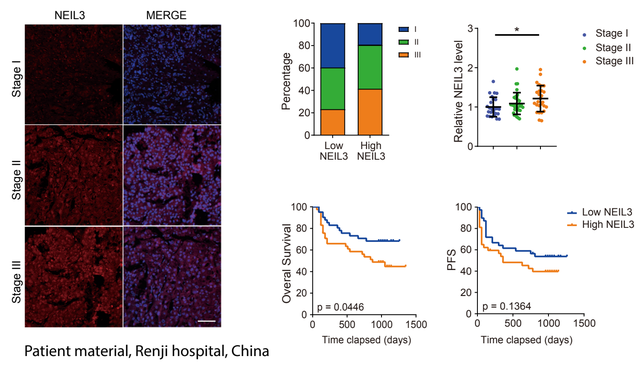
NEIL3, but not NEIL1 or NEIL2, is required to initiate base excision repair at oxidized telomeres that is dependent on APE1 and Polβ.

Repetitive exposure of oxidizing damage in NEIL3 depleted cells induced chromatin bridges and damaged telomeres.

These data suggest NEIL3 could be a target for therapeutic intervention of HCC, and perhaps a combination treatment with a NEIL3 inhibitor and oxidizing compounds (e.g., elesclomol) could prove to be a good strategy to induce ROS and prevent its repair in the tumour.

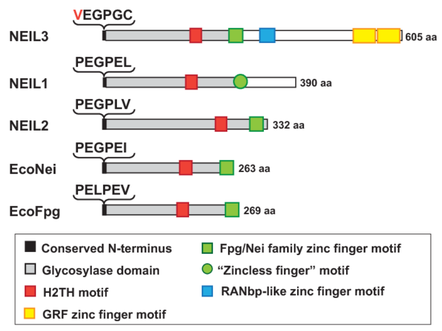


1 NEIL3 is overexpressed in HCC which correlates with poor survival.



Patient material, Renji hospital, China

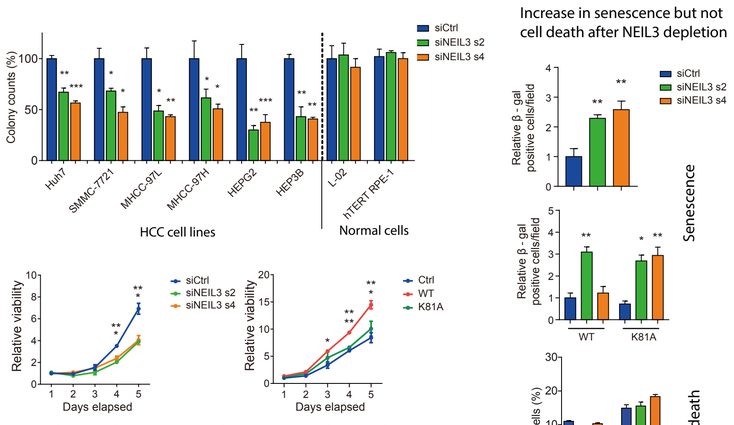
NEIL3 has an unique C-terminus with several protein binding motifs



Liu et al, Mutat Res. 2013

The increase of NEIL3 expression in G2/M phase and the observation that oxidative stress has profound effects in mitosis, suggests that NEIL3 has a crucial role in DNA repair in this phase.

2 HCC cell lines require NEIL3 for cell proliferation in vitro

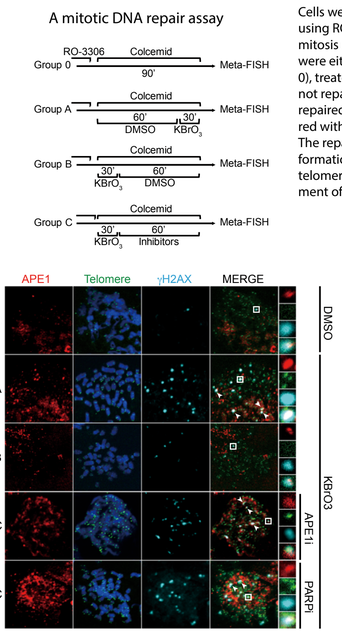


Transfection of HEP3B cells with NEIL3 siRNA which inhibit growth. Overexpression of WT, but not a catalytic dead mutant (K81A), increase growth.

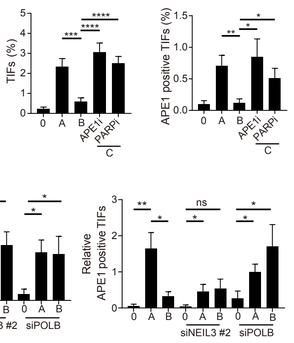
Increase in senescence but not cell death after NEIL3 depletion

6 NEIL3 recruits APE1 to damaged telomeres and promotes repair of oxidized lesions in mitosis

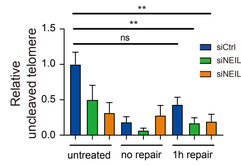
A mitotic DNA repair assay



Cells were synchronized in G2 using RO3306 and arrested in mitosis using Colcemid. Cells were either untreated (group 0), treated with KBrO3 and not repaired (group A), repaired (group B) and repaired with inhibitors (group C). The repair was measured by formation of damaged telomeres (TIFs) and recruitment of APE1 to TIFs.

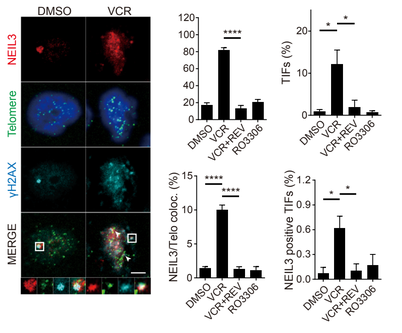


Telomere qPCR assay of mitotic cells



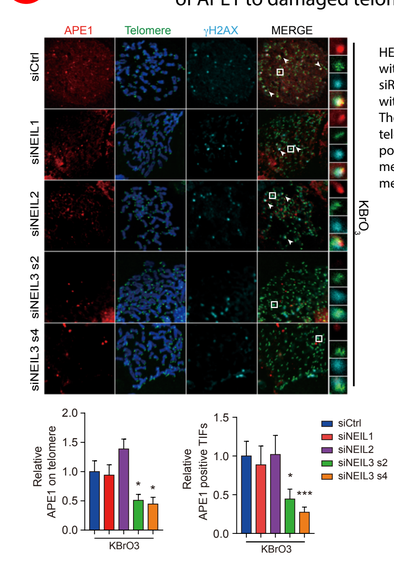
HEP3B cells were transfected with NEIL3 siRNA and synchronized n G2-phase with 2 mM thymidine for 24h and RO-3306 for 18h. Cells were released into mitosis and treated with KBrO3. After arresting cells in mitosis for 1h, oxidized lesions were measured at the telomere using the telomere qPCR assay with NEIL1 digestion.

4 NEIL3 is localized to damaged telomeres induced by prolonged mitotic arrest



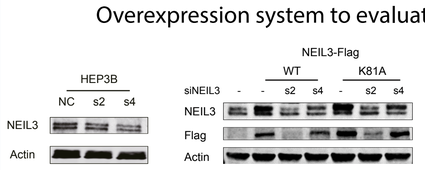
Vincristine induce (VCR) recruitment of NEIL3 to damaged telomeres (γH2AX-Telomere colocalization). Co-treatment with reversine (REV) that prevents mitotic arrest, also prevents NEIL3 recruitment

5 NEIL3, but not NEIL1 and 2, are required for recruitment of APE1 to damaged telomeres



HEP3B cells were transfected with NEIL1, NEIL2 or NEIL3 siRNA and after 72h treated with 5 mM KBrO3 for 30 min. The APE1 colocalization with telomere and TIFs (γH2AX positive telomeres) was measured by Meta-FISH on metaphase spreads.

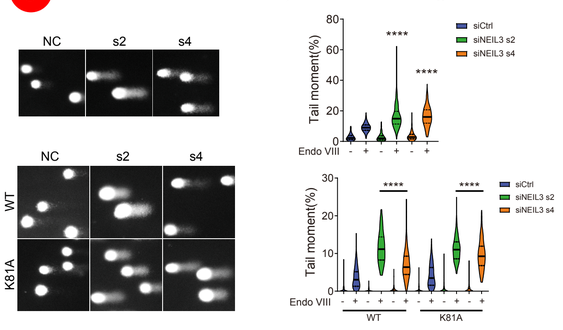
Overexpression system to evaluate specificity of NEIL3 siRNA



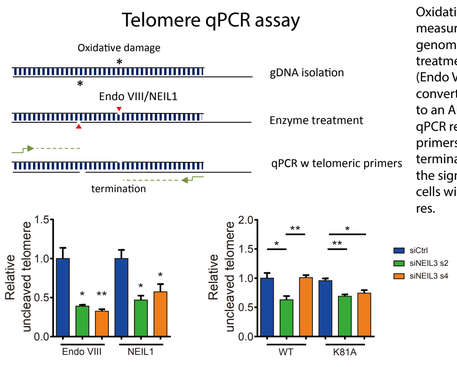
NEIL3-FLAG (WT and catalytic dead K81A) is resistant to NEIL3 siRNA s4 but not s2. In a rescue experiment, overexpression of NEIL3 WT is expected to revert the effect of siRNA s4.

NEIL3 silencing induces oxidative damage at telomeres

3 Modified comet assay with Endo VIII digestion

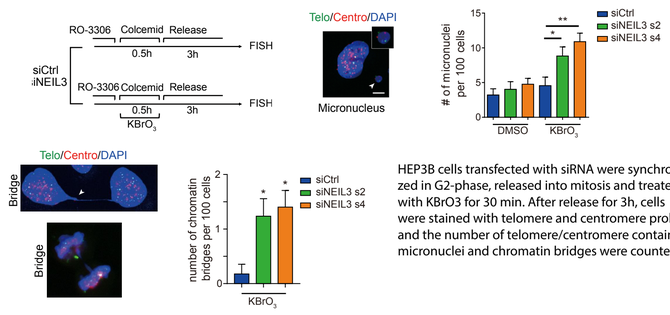


Telomere qPCR assay



Oxidative lesions can be measured by isolating genomic DNA (gDNA) and treatment with a glycosylase (Endo VIII or NEIL1) to convert the oxidative lesion to an AP-site. In a subsequent qPCR reaction with telomeric primers, the reaction will terminate at the AP-site and the signal will be lower in cells with damaged telomeres.

7 NEIL3 protects HEP3B cells from genomic instability induced by oxidative damage



HEP3B cells transfected with siRNA were synchronized in G2-phase, released into mitosis and treated with KBrO3 for 30 min. After release for 3h, cells were stained with telomere and centromere probes and the number of telomere/centromere containing micronuclei and chromatin bridges were counted.

Overexpression of NEIL3 protects cells from long-term effects of oxidative stress

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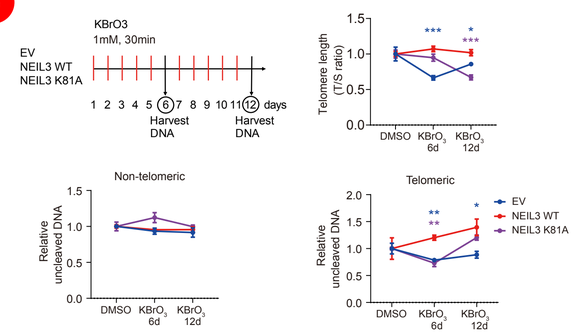

HEP3B cells, overexpressing WT or K81A NEIL3 or the empty vector control, were treated with 1 mM KBrO3 for 30 min periods, once a day for 6 or 12 consecutive days. The telomere length and the EndoVIII sensitive lesions were measured.

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