Base excision repair protein dysregulation as a driver of genomic instability and cellular transformation

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DNA Repair Interest Group

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Multiple DNA repair pathways exist to handle various types of damage:

- **Base Excision Repair (BER)**
- **Homologous Recombination (HR)**
- **Mismatch Repair (MMR)**
- **Direct Reversal**
- **Nucleotide Excision Repair (NER)**
- Non-homologous End Joining Pathways (c-NHEJ) and (a-NHEJ)
DNA repair pathway crosstalk
Regulation of DNA damage management?
Multiple DNA repair pathways exist to handle various types of damage.

DNA Repair

- Nucleotide Excision Repair (NER)
- Non-homologous End Joining Pathways (c-NHEJ) and (alt-NHEJ)
- Base Excision Repair (BER)
- Oxidation, Alkylation, Deamination, Uracil
- Homologous Recombination (HR)
- Double strand breaks DSBs
- Bulky base damage
- Double strand breaks DSBs
**BER protein pathway crosstalk examples**

**NTHL1 glycosylase**
stimulates NTHL1 incision and turnover from DNA

**OGG1 glycosylase**
influences OGG1 binding and incision of substrate

**NEIL glycosylases**
stimulates NEIL activity
Endonuclease III-like glycosylase I (NTHL1)

- Highly conserved from bacteria to humans
- Functions in both the nucleus and mitochondria
- Repairs a suite of oxidative DNA damage
  - **Major substrate:** Pyrimidine derivatives (cytosine and thymine)
  - **Minor substrate:** ring opened guanine
- Catalytic activity resides in the HhH motif at lysine 220
  - Lysine to glutamine (K220Q) mutagenesis abolishes glycosylase and AP lyase activity (Ikeda, Mitra et al. JBC, 1999)
- Germline NTHL1 variant causes LOSS of NTHL1 protein
  - Patients are predisposed to colon cancer (Rivera et al. *NEJM*, 2015) (Weren et al. *Nat Gen* 2015)
  - loss of NTHL1 $\rightarrow$ accumulated DNA damage $\rightarrow$ mutations $\rightarrow$ cancer
NTHL1 amplification (instead of deletion) is found in multiple cancer types
Base Excision Repair (BER)
Main system for repairing oxidative base damage

- Creation of Apurinic/Apyrimidinic Site (AP site)
- Backbone 5’ cleavage
- Repair of Initial Damage Site
- Excision of Damaged Base N-Glycosylases
- Repair intermediates are DNA damage
- Backbone 3’ cleavage
  - N-glycosylase
  - AP Lyase Activity
- End Cleaning
- Further Processing

NTHL1
**Hypothesis**: NTHL1 overexpression contributes an oncogenic advantage
**NTHL1 protein levels are elevated in non-small cell lung cancer (NSCLC) cell lines**

**Steady-state NTHL1 levels are increased in NSCLC cell lines**
Base Excision Repair (BER)
Main system for repairing oxidative base damage

**Further Processing**

**Excision of Damaged Base**

*N*-Glycosylases

**Repair intermediates are DNA damage**

**Creation of Apurinic/Apyrimidinic Site (AP site)**

**Backbone 5’ cleavage**

**Backbone 3’ cleavage**

*N*-glycosylase AP Lyase Activity

**End Cleaning**

**Repair of Initial Damage Site**

**Further Processing**

**NTHL1**
Initial predicted consequence of NTHL1 overexpression:

Mutagenic BER repair intermediates overwhelm the downstream BER repair processes resulting in genomic instability.
An initial glimpse into the biology of BER protein overexpression: two versions of NTHL1 in non-cancerous human bronchial epithelial cells (HBEC-3KT)

<table>
<thead>
<tr>
<th>Protein Impact</th>
<th>Functional Impact</th>
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<tbody>
<tr>
<td>Wild Type</td>
<td>No catalytic activity</td>
</tr>
</tbody>
</table>

HBEC-3KT
Continuously replicating cell line via expression of hTERT and Cdk4 (no colonies in soft agar or tumors in nude mice)
NTHL1 is primarily localized to the nucleus in both NSCLC and overexpressing HBEC cells. NTHL1 is not mislocalized upon overexpression and is associated with chromatin.

IHC staining: Emory University Pathology Core
Limpose et al NAR in revision
DNA damage accumulates upon overexpression of NTHL1 and CATmut

Cells embedded in agarose

Lysis

electrophoresis

Comet tail

% DNA in comet tail

Vector NTHL1 CATmut

NT Empty WT (K220Q) CATmut

NTHL1 Flag Actin

Vector NTHL1 CATmut

Comet Assay: Erica Werner

Limpse et al NAR in revision
**DSBs accumulate upon overexpression of NTHL1 and CATmut**

Elevated NTHL1 levels increase the cellular load of DNA damage in a manner that does not depend on NTHL1 enzymatic activity (greater effect seen with enzymatically active NTHL1)

[Limpse et al. NAR in revision]
DSBs accumulate upon overexpression of NTHL1 and CATmut

Is this caused by direct induction of DSBs or inhibition of DSB repair (HR or NHEJ)?

**HR measurement**: Gene conversion in direct repeat GFP (DR-GFP) reporter construct integrated into the DR-U2OS cell line (Pierce et al Genes Dev. (1999)) – Interrogates HR after I-SceI-induced DSB in GFP – Cleaved GFP repaired by second, transcriptionally inactive GFP – Results in recovery of GFP fluorescence.
DSB repair (HR) is compromised by overexpression of NTHL1 and CATmut

Increased NTHL1 or CATmut protein inhibits HR repair of DSBs in a manner that does not depend on NTHL1 enzymatic activity (~50% decrease in HR)

DR-GFP assay: Kelly Trego
Limpose et al NAR in revision
NHEJ is moderately elevated by overexpression of NTHL1 and CATmut
Results suggest: Increased NTHL1 or CATmut protein inhibits HR-mediated repair of DSBs
Micronucleus assay: A gold standard for measuring genomic instability

Cytochalasin-B block Cytokinesis-block

whole chromosomes

chromosome fragments

Binucleated

Micronucleus
Genomic instability is induced by transient NTHL1 and CATmut overexpression

Genomic instability is present at 48 hours following overexpression.

NTHL1 and CATmut overexpression results in multiple micronuclei.

Occurs independent of NTHL1 enzymatic activity (greater effect seen with enzymatically active NTHL1).

Limpose et al NAR in revision
Genomic instability is proportional to the level of NTHL1 overexpression

![Graph showing genomic instability and NTHL1-GFP intensity]

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>% Binucleated cells with micronuclei/nuclear buds</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTHL1-GFP_purified_002.fcs</td>
<td>NT</td>
</tr>
<tr>
<td>NTHL1-GFP_purified_Med_003.fcs</td>
<td>GFP</td>
</tr>
<tr>
<td>NTHL1-GFP_purified_High_004.fcs</td>
<td>Low</td>
</tr>
<tr>
<td>NTHL1-GFP_purified_001.fcs</td>
<td>Int</td>
</tr>
<tr>
<td>NTHL1-GFP_purified_002.fcs</td>
<td>High</td>
</tr>
<tr>
<td>NTHL1-GFP_purified_003.fcs</td>
<td>Unsrted</td>
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Limpose et al NAR in revision
Does replication stress contribute to the observed cellular phenotypes?

Approach: immunoblot for known replication stress signaling proteins.

- ATR phosphorylation (pATR T19898)
- Chk1 phosphorylation – intra-S phase checkpoint (S317)

Replication fork collapse -> DSBs -> ATM activation

- phosphorylation of downstream effectors -> Chk2 (pChk2 T68)
- phosphorylation of RPA (pRPA S4/S8)
DSBs can occur during each replication cycle

Markers

Replication stress:
- pATR
- pChk1

DSB signaling:
- γH2Ax
- 53BP1
- pChk2

HR signaling:
- pRPA S4/S8

Fork collapse and double strand break (DSB) formation

Generation of one-sided DSB

HR Repair
Replication stress signaling assessment following NTHL1 and CATmut overexpression

**Replication stress:**
- pATR
- pChk1

**DSB signaling:**
- γH2Ax
- 53BP1
- pChk2

**HR signaling:**
- pRPA S4/S8

Limorese et al. NAR in revision
Conclusion:

NTHL1 and CATmut overexpression induce replication stress signaling
Early cancer hallmarks are conferred by NTHL1 and CATmut overexpression

HBEC cells overexpressing NTHL1 and CATmut form colonies in soft agar

Limpose et al NAR in revision
Early markers of cellular transformation are conferred by NTHL1 and CATmut overexpression

Loss of contact inhibition develops in cells that acquired the capability to grow in soft agar

Persistent genomic instability is a permanent characteristic of these transformed cells

Limpose et al NAR in revision
Acquired markers of cellular transformation

Loss of contact inhibition

Anoikis
Anchorage independent growth

DSBs
Inhibition of HR
Micronuclei

In addition to DNA damage in the form of BER intermediates caused by catalytic activity of NTHL1, what could the other effects be?

NTHL1 Glycosylase

NTHL1 AP Lyase

APE1 End Cleaning

interaction stimulates NTHL1 turnover
NTNL1 interacts with XPG (Co-IP)

D

<table>
<thead>
<tr>
<th></th>
<th>Vector</th>
<th>NTNL1</th>
<th>NTNL1</th>
</tr>
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<tbody>
<tr>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Bound

- NTNL1
- XPG
XPG is essential for HR repair of DSB resulting from collapsed replication forks (Trego et al. *Mol. Cell* 2016)

- HR function of XPG is not dependent on catalytic function
DNA repair pathway crosstalk regulates/impacts DNA damage management

Limpose, Corbett, and Doetsch. DNA Repair 2017
Current Model

![Diagram showing the relationship between NTHL1 expression and genome stability]

- **Loss, Decreased Expression**
  - Unrepaired DNA Damage
  - Genetic Instability
  - Tumorigenesis

- **Increased Expression**
  - DNA Damage
  - Genomic/Genetic Instability
  - Tumorigenesis
Model

Replication stress

DSB Generation

Initiation of HR Repair

pRPA coats resected DNA

NTHL1 Sequestration of XPG

Inefficient loading of Rad51

Alt-NHEJ Initiated?

Genomic Instability

Supporting Result(s)

<table>
<thead>
<tr>
<th>p-ATR T1989</th>
<th>p-Chk1 S317</th>
</tr>
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<tbody>
<tr>
<td>γH2Ax/53BP1 foci</td>
<td>Comet Assay</td>
</tr>
<tr>
<td>p-Chk2 T68</td>
<td>p-RPA2 S4/S8</td>
</tr>
<tr>
<td>Decrease in DR-GFP Comet assay</td>
<td>Micronucleus Formation</td>
</tr>
</tbody>
</table>
Conclusions

• Overexpression of a BER glycosylase causes DSB accumulation

• Overexpression of NTHL1 impairs DSB repair
  – independent of NTHL1 enzymatic activity

• Outcome of impaired DSB repair is genomic instability

• DSBs generated during replication likely exacerbate genomic instability

• Acquisition of multiple cellular transformation markers appear when NTHL1 is overexpressed
Clinical Implications

Prediction: tumor cells that overexpress NTHL1 could be sensitized to agents that induce DSBs
- Ionizing radiation (IR)
- Topoisomerase inhibitors

Novel combination strategies
- Sensitization to PARP inhibitors
- Exploiting the defect in HR

Biomarker potential
- Use to predict patient outcomes
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