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DNA glycosylase (NEIL3) overexpression associated with low tumor immune infiltration and poor overall patient survival in endometrial cancer

Cristofer Barry, Aashirwad Shahi & Dawit Kidane✉

Endometrial cancer (EC) is the most common gynecological malignancy. Although prognosis is favorable for patients with an early-stage disease, those with recurrent or more advanced disease have low response rates to chemotherapy and poor clinical outcomes. Previously, we have shown that DNA repair gene (NEIL3) is required for retaining replication fork integrity during replication stress. Here, we examined whether the overexpression of NEIL3 in endometrial cancer associated with altered genomic instability, tumor immunogenicity and anti-tumor immunity in endometrial tumor. In this study, we show that endometrial cancer patients with tumors that a have high NEIL3 expression associated with worse overall survival (OS) outcomes in patients. In addition, tumor with high NEIL3 expression is associated with high number of mutation and chromosomal instability. Furthermore, *NEIL3* expression in EC tumors positively correlated with mutation of DNA polymerase eta (POLE) and TP53 as well as high expression of replicative polymerases genes (POLE, POLD1 and POLA1). In contrast, tumor with high NEIL3 expression exhibit low tumor immunogenicity and poor anti-tumor immune cell infiltration. Our findings may have important clinical implications for utilizing NEIL3 as a potential prognostic biomarker to stratify EC patients and as a target to enhance immunotherapy response in endometrial cancer. However, our NEIL3 overexpression associated observation still requires further experimental-based scientific validation studies.

Keywords Endometrial cancer, NEIL3, Genomic instability, Tumor immunelandscape

Endometrial carcinoma (EC) is the most common gynecological malignancy in the United States, with an estimated 61,880 new cases and 12,160 deaths in 2019[1]. When diagnosed at an early stage without metastasis, the five-year survival rate was over 80%[2]. However, women diagnosed with advanced or recurrent disease have a poor prognosis, with a 5-year survival rate of only 17%[3] and poor responses to current therapies resulting in an overall survival of approximately 12 months[4]. Chemotherapy resistance remains a major challenge for at least 15–20% of patients[5,6]. Notably, treatment modalities in EC vary depending on the grade, the stage of the disease and the molecular subtypes of the EC. The four molecular subtypes of EC are DNA polymerase-ε hypermutation (mutation in the exonuclease domain), mismatch repair deficient tumor (microsatellite instability), TP53 mutated tumors, and tumors without none of the aforementioned classifications ('no specific molecular profile' or 'NSMP')[7]. These molecular classifications correlate with patient prognosis and can help to improve the identification of early-stage patients who may benefit from adjuvant therapy. To enhance a targeted and immune based therapy treatment responses and improve patient survival in EC patients, new prognostic genes and their association with patient survival should be continuously investigated,

NEIL3 is one of the DNA glycosylase repair genes involved in the base excision repair (BER) pathway, which is the predominant repair pathway of oxidative and alkylating DNA damage[8]. DNA glycosylases are the first BER factors that recognize DNA damaged bases, excise the lesions, and provide substrates for later enzymes in the pathway[9]. Base removal by a DNA glycosylase generates a apurinic/apyrimidinic site (AP-site) in DNA, which is then further processed by specific AP-endonuclease, DNA polymerase, and DNA ligase activities to restore the original DNA sequence[10]. Accordingly, cells lacking DNA glycosylase functions generally show increased

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levels of base damage in their DNA, elevated mutation rates, and hypersensitivity to specific DNA damaging agents[11]. There are five different types of DNA glycosylases that are specific for oxidative DNA base damages have been identified in human cells, namely OGG1, NTH1, NEIL1, NEIL2, and NEIL3[12]. NEIL3 was identified together with NEIL1 and NEIL2 as a gene product with significant structural similarities to the *E. coli* Fpg and Nei DNA glycosylases[13]. NEIL3 prefers substrates that contain single-stranded regions such as looped structures and structures representing replication forks[14]. NEIL3 has a very weak lyase activity[14] and the AP sites remaining after NEIL3 glycosylase activity is likely cleaved by APE1. NEIL3 can also function as a glycosylase in vivo as demonstrated by its ability to substantially reduce the spontaneous mutation frequency[14].

Human NEIL3 is overexpressed in several tissues including thymus, testis, and tumor tissues[15,16] and involved in cellular proliferation for tumor progression[17]. The cell cycle-dependent expression pattern of NEIL3 indicate that the level of expression is high in early S phase and G2 phase[16]. Since NEIL3 is expressed in S-phase when replication takes place, it is also involved in replication associated oxidative damaged repair, recruitment of chromatin remodeling proteins, and topological DNA structure during DNA replication dynamics[18]. NEIL3 repairs telomere oxidative damage and protects telomere integrity during the S phase to enable accurate chromosome segregation in actively dividing cells[19]. NEIL3 has been suggested to play a role in replication-associated repair or transcription-coupled repair[20,21]. Stalling of the replication machinery during S phase instigate subsequent replication fork collapse and thereby induce genomic instability such as copy number variation[22], micronuclei formation[23], and loss of heterozygosity[24], leading to an increase in tumorigenesis[25].

The up-regulation of NEIL3 expression in diverse cancer types is consistent with the results from previously published data[15,17,26] but the effect of such up-regulation on clinical outcomes and anti-tumor immune response has not yet been determined. In this study, through TCGA data, we show that EC patients with tumors that a have high NEIL3 expression have significantly lower overall survival (OS) and progression free survival (PFS) rates compared to patients whose tumor expresses lower levels of NEIL3. Furthermore, NEIL3 overexpressing endometrial tumors harbor greater genomic instability. In contrast, tumor with overexpressed NEIL3 harbor low tumor immunogenicity as indicated with poor anti-tumor immune cell infiltration and innate immune signaling gene expression. Finally, our study showed a positive correlation between *NEIL3* expression in EC tumors and replicative polymerases gene expression (POLE, POLD1 and POLA1), mismatch repair (MMR) genes expression, POLE mutations, P53 mutations and high microsatellite instability. While the biological relevance of the correlations is unclear, it raises the possibility that this profile would help explain the worse prognosis observed in NEIL3 overexpressing endometrial cancer patients. Furthermore, these findings have clinical implications for endometrial cancer patients for whom NEIL3 should be explored as a potential prognostic marker for therapy selection and as an immune based a therapeutic target for endometrial cancer.

Results

NEIL3 overexpression leads to poor OS in endometrial cancer

Data were accessed from cBioPortal (www.cBioPortal.org). TCGA PanCancer RNA-seq data and corresponding clinic information for EC types were retrieved for validation. As shown in Fig. 1A the mean expression value of *NEIL3* in EC cancer tissues was 11 folds greater than the normal control group. Overall, we have found that 33.5% of primary tumors overexpressed NEIL3. Importantly, NEIL3 overexpressed significantly in tumor of serous endometrial carcinoma subtypes (Fig. 1B; $^{**}P<0.01$). Furthermore, NEIL3 overexpressed tumor exhibit high grade tumor (G3/4) versus tumor with low NEIL3 expression (Fig. 1C; $^{****}P<0.0001$). To investigate whether the expression profile of the NEIL3 in tumors could be related to patient survival, Kaplan–Meir survival curves were generated based upon the designation of individuals as either low expressing NEIL3 (z score <0.5) or high expressing NEIL3 (z-score >0.5) to assess the impact of survival for endometrial cancer. We found that NEIL3 overexpression significantly negatively impacted the OS of patients with EC (Fig. 1D; $^{**}P<0.01$). In addition, the progression free survival of patients with NEIL3 overexpressed tumor is significantly lower than in patients who harbor low NEIL3 expression (Fig. 1E; $^{*}P<0.05$).

NEIL3 overexpression significantly increases genomic alteration in EC patients

To assess deregulation of NEIL3 in human EC associated with genomic alteration, we analyzed whether *NEIL3* overexpression was associated with mutation count in tumors in patients with that showed poor OS. The number of mutations counts was significantly increased in *NEIL3* overexpressing tumors of EC in comparison to low NEIL3 expressing tumors (Fig. 2A; $^{***}P<0.001$). Importantly, NEIL3 overexpression may enhance chromosomal rearrangement and chromosomal instability as EC patients overexpressing *NEIL3* accumulated a higher aneuploidy score than EC patients with low NEIL3 expression (Fig. 2B; $^{***}P<0.001$). Furthermore, we examined whether NEIL3 overexpression was associated with microsatellite unstable tumors, and we found that microsatellite instability scores are significantly higher in NEIL3 overexpressed tumor (Fig. 2C; $^{**}P<0.01$). Lastly, EC tumors with high NEIL3 expression also harbor a significantly higher tumor mutation burden when compared to EC tumors with a low NEIL3 expression (Fig. 2D, $^{***}P<0.001$).

NEIL3 overexpression is associated with POLE hypermutation and TP53 types of EC

To determine whether NEIL3 overexpression is associated with the molecular subtypes of EC, we compared the co-occurrence of low and high NEIL3 expression with the molecular subtypes of EC. We found that NEIL3 overexpression strongly occurred in tumors with a POLE mutation and high chromosomal instability (Fig. 3A, $P<0.05$ and $P<0.001$). We examined other molecular signature of EC tumor suppressors genes and replicative polymerase mutations that may associated with overexpression of NEIL3. 38% of EC tumors harbor a TP53 mutation, 17% a POLE mutation, and 12% a POLA1 mutation that suggest that there is a strong association of cancer driving genes (Fig. 3B). Furthermore, 52% of EC tumor harbor the co-occurrence of a mutation TP53

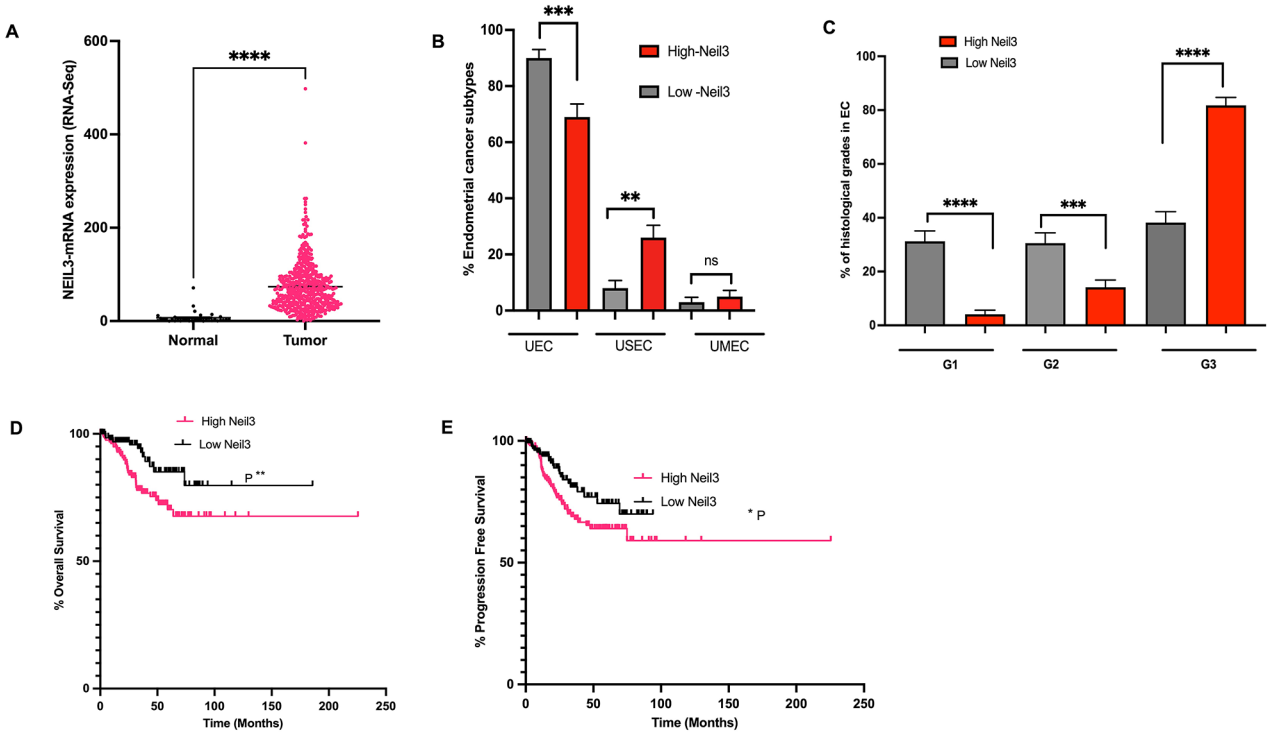


Fig. 1. Overexpression of NEIL3 in uterine endometrial cancer subtypes and its impact on patient survival. (**A**) NEIL3 expression of EC and adjacent normal tissue; (**B**) The landscape of NEIL3 expression on histologically categorized endometrial tumors (UEC: uterine endometrial cancer, USEC: Uterine serous endometrial cancer, UMEC: Uterine mixed endometrial cancer); (**C**) The histological tumor grades of EC patients with high and low NEIL3 expression; (**D**) Kaplan-Meier survival analysis of EC patients with low and high NEIL3 expression tumors; (**E**) The progression free survival of EC patients with low and high NEIL3 expression tumors. Mann-Whitney-U test was used to compare the significance of the change between histological grades. For Kaplan–Meier curves, P-values, were generated by log-rank tests. $p < 0.05$ was considered as statistically significant.

genes and high NEIL3 overexpression (Fig. 3C). Our data shows the co-occurrence of high NEIL3 expression with replicative polymerase (POLE, POLD1, POLD3, and POLA1) and TP53 mutations (Fig. 3C).

NEIL3 overexpression associated with low immune score and negatively correlated with immune cell infiltration

Increasing evidence suggests that cancer progression is strongly influenced by host immune response, which is represented by immune cell infiltrates in the tumor microenvironment. We compared the immune and stromal scores between low and high NEIL3 expressing tumor samples and found that NEIL3 overexpressing tumors were significantly associated with lower immune scores, stromal scores, and estimate scores (Fig. 4A, B, and C). Intriguingly, we performed a comprehensive investigation on the correlations between the expression levels of NEIL3 and immune cells infiltration on tumor using the TIMER database. High NEIL3 expression was associated with a reduced innate and adaptive immune cell abundance in tumor which included CD8T cells (correlation=-0.223; $P = 3.65e\text{-}02$), CD4 + T cells (correlation=-0.119; $P = 2.1e\text{-}01$), B cells (correlation= -0.04; $P = 6.5e\text{-}01$), macrophages (correlation = -0.22; $P = 3.7e\text{-}02$) and dendritic cells (correlation= -0.02; $P = 8.5e\text{-}02$) (Fig. 4D). In contrast, the infiltration of the myeloid derived suppressor cells (MDSC) positively correlated with NEIL3 expression in tumor (Fig. 4D, Correlation = 0.6, $P = 1.01e\text{-}30$). These results showed that overexpression of NEIL3 is significantly associated with the reduced infiltration of the dominate anti-tumor immune cells in the tumor microenvironment. In contrast, MDSC contribute a significant role in tumor development via a variety of immunosuppressive mechanisms in NEIL3 overexpressing tumor and modulate the tumor microenvironment. Additionally, we observed a significant negative correlation between NEIL3 expression with antigen processing and presentation genes in endometrial cancer suggesting that compromised antigen processing may contribute to the low immune score in NEIL3 overexpressing tumors (supplementary Table 1).

Association of NEIL3 with immune checkpoint genes and innate immune signaling landscape in EC

We examined whether altered level of NEIL3 expression was associated with innate immune signaling genes expression and immune checkpoints mediator genes. We performed a spearman correlation analysis on endometrial tumors with low and high NEIL3 expression using the publicly available cancer genomic database

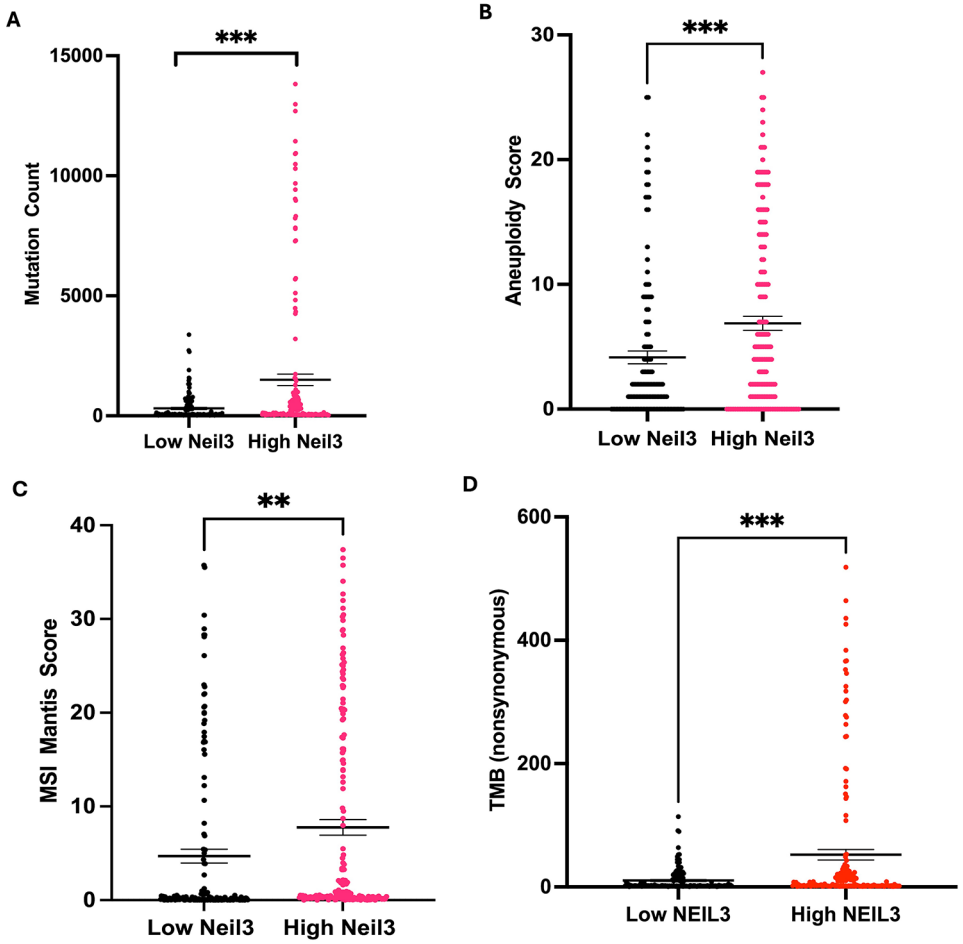
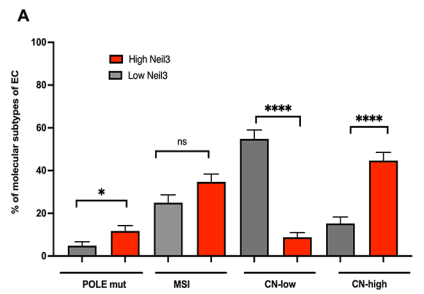
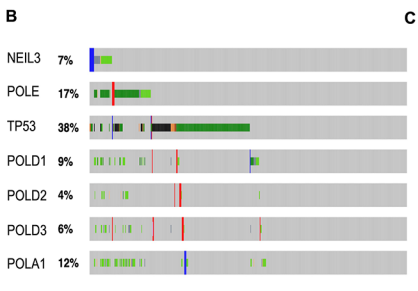


Fig. 2. NEIL3 associated with genomic instability in uterine endometrial cancer. (**A**) The mutation count of tumors with low and high NEIL3 expression; (**B**) Aneuploidy score of tumors with low and high NEIL3 expression; (**C**) Microsatellite instability score of tumors with low and high NEIL3 expression; (**D**) The tumor mutation burden (TBM) between tumor with low and high NEIL3 expression. Mann-Whitney-U test was used to compare the significance of the change. $p < 0.05$ was considered as statistically significant.





C

Genes	High-Neil3 (n=170)	Low-Neil3 (n=144)	P-value
POLE	34(20)	14 (9)	*
TP53	90 (52)	25 (17)	****
POLA1	25 (15)	10 (7)	*
POLD1	19 (11)	4 (2)	*
POLD2	7 (4)	1 (0.7)	ns
POLD3	11 (6)	2 (1)	*

Fig. 3. Co-occurrence of NEIL3 overexpression with molecular subtypes of uterine endometrial cancer. (**A**) The co-occurrence of altered NEIL3 expression in different molecular subtypes of EC; (**B**) The percentage of gene alteration in NEIL3 and other common tumor suppressor genes (TP53 mutation) and replicative polymerase (POLE, POLA1, POLD1, POLD2, POLD3) in EC; (**C**) The percent of co-occurrence of low and high NEIL3 expression in TP53, POLE, POLD1, POLD2, POLD3, and POLA1 mutated tumors. The Mann Whitney-U test was used to compare the low and high NEIL3 expressed tumors with TP53, POLE, POLD1, POLD2, POLD3, and POLA1 mutations.

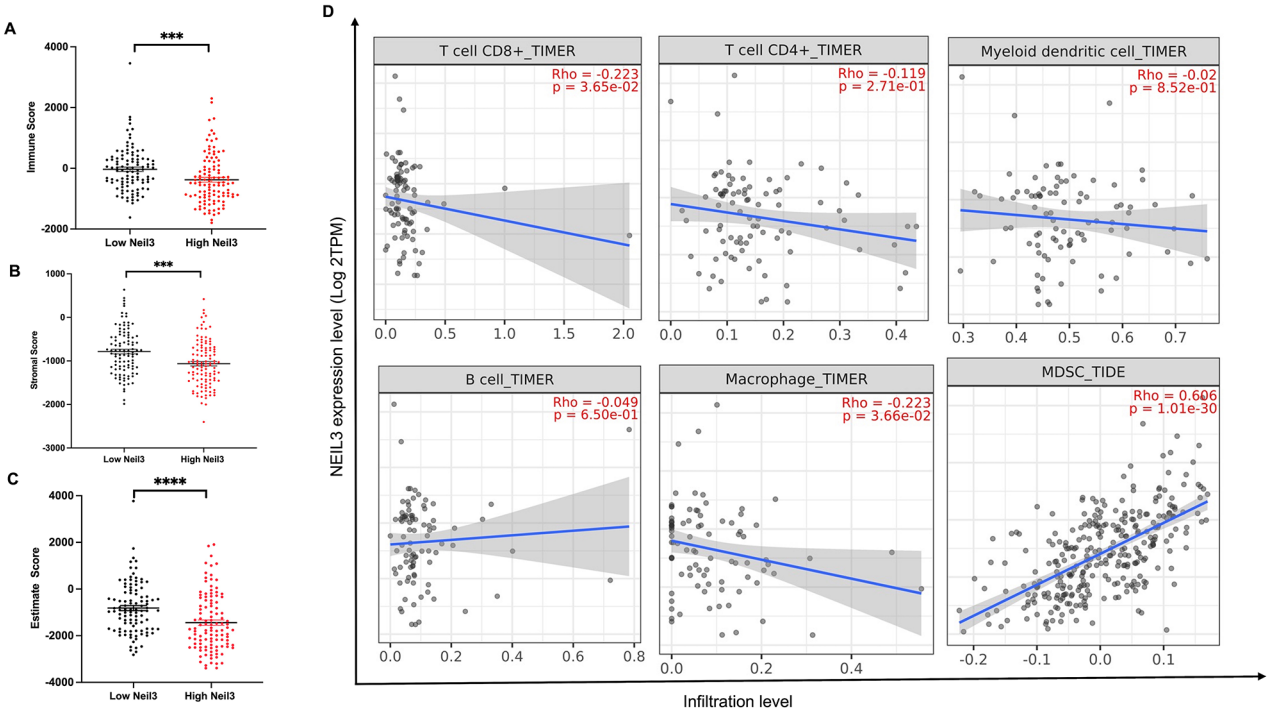


Fig. 4. Impact of NEIL3 overexpression on the tumor microenvironment of uterine endometrial cancer. (**A**) Immune, stromal (**B**) and Estimate score (**C**) of the tumor with low and high NEIL3 mRNA expression. The Mann Whitney-U test was used to compare the expression of low and high NEIL3 expression versus immune score, stromal score, and estimate score. (**D**) Overexpression of NEIL3 is associated with a low level of infiltration of immune cells to the tumor microenvironment. The correlations of NEIL3 expression versus immune cells (CD8 + T, CD4 + T, B cells, Macrophage, DC cells and MDSC) were analyzed using Spearman analysis.

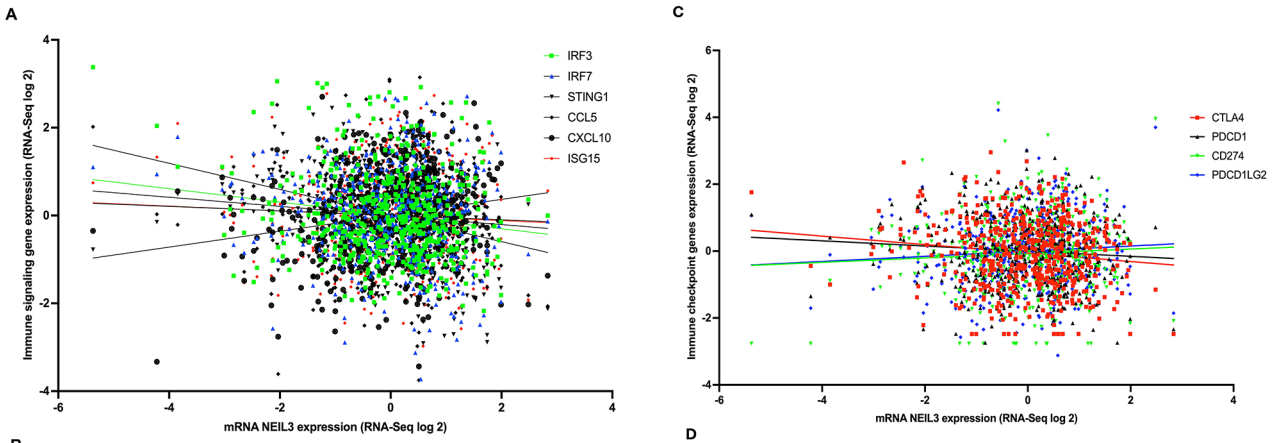
including TCGA (Pan cancer). We found that endometrial tumors with high NEIL3 expression have negative correlations with innate immune signaling genes (IRF3, IRF7, ISG15, STING1, CCL5) except CXCL10 (Fig. 5A**).** In particular, the expression of STING1, IRF3 and IRF7 were significantly low (Fig. 5B; $^{***}P < 0.001$). In contrast, our results indicate a weak significant positive correlation with the expression of CXCL10 (Fig. 5A **and B**, $^{***}P < 0.001$). Furthermore, our study examined whether NEIL3 expression in endometrial tumors is associated with altered expression of immune checkpoint genes (CTLA4, PDCD1, CD274 and PDCD1LG2) (Fig. 5C). The expression of NEIL3 is significantly negatively correlated with CTLA4 and PDCD1LG2 in endometrial tumors (Fig. 5C and D; $^{*}P < 0.5$). However, our results in Fig. 5D shows that the expression of NEIL3 did not show any significant negative or positive correlation with PDCD1 and CD274 respectively.

NEIL3 has a prognostic value for EC

ROC curve analysis was performed to assess the diagnostic value of NEIL3 in EC. After the generation of ROC curve, area under the curve (AUC) and 95%confidence interval (CI) were calculated. The area under the curve for NEIL3 gene was 82% (Figs. 6A and 95% CI: 0.72–0.93; $^{****}P < 0.0001$). Furthermore, we examined whether the co-occurrence of NEIL3 overexpression with TP53 or DNA POLE or POLA1 mutations increase the overall AUC score. We found that any of the co-occurrence of genes results minor change in AUC score (Fig. 6B- D).

Discussion

NEIL3 is a key enzyme for the protection of the genome against DNA damage *via* its role in BER and replication stress response[18]. In this work, we report that NEIL3 is overexpressed in a majority of EC and significantly in uterine serous endometrial cancer subtypes. The endometrial hypermutated subtype is characterized by a high mutational burden, primarily driven by POLE exonuclease domain mutations[7]. The microsatellite instability hypermutated subtype is associated with defects in DNA mismatch repair mechanisms, leading to frequent mutations in microsatellite regions[7]. The copy-number low subtype exhibits a relatively stable genome with fewer copy-number alterations, while the copy-number high subtype displays extensive genomic instability and frequent amplifications and deletions and worst prognosis[7]. Our data shows that NEIL3 overexpression in endometrial tumors is significantly associated with POLE hypermutated and chromosomal high copy number types of EC (Fig. 3). Importantly, our results show that NEIL3 overexpression have significantly decreased overall and progression survival outcomes in EC patients (Fig. 1). Our in-silico analysis shows that high NEIL3 expression is significantly associated with histologically high-grade tumors (Fig. 1) supporting that as



B

	IRF3	IRF7	STING1	CCL5	CXCL10	ISG15
r	-0.15	-0.1	-0.3	-0.05	0.18	-0.06
95% CI	-0.2 to -0.06	-0.19 to -0.018	-0.38 to -0.22	-0.14 to 0.03	0.1 to 0.3	-0.14 to 0.03
P-value	***	*	***	ns	***	ns

D

	CTLA4	PDCD1	CD274	PDCD1LG2
r	-0.1	-0.06	0.04	0.09
95% CI	-0.15 to -0.002	-0.05 to 0.12	-0.05 to 0.12	-0.002 to 0.2
P-value	*	ns	ns	*

Fig. 5. Immune signaling in NEIL3 overexpressed in uterine endometrial cancer; (**A**) The expression of innate immune genes (IRF3, IRF7, STING1, ISG15, CXCL10 and CCL5) in tumor with NEIL3 expression; (**B**) Correlation coefficient value (r) and statistical significant difference between NEIL3 expression versus innate immune genes; (**C**) The association of NEIL3 expression with immune checkpoint genes (CTLA4, PDCD1, CD274 and PDCD1LG2; (**D**) Correlation coefficient value (r) of NEIL3 expression versus checkpoint genes expression. The correlation coefficients were obtained through a spearman analysis represent $^{*}P<0.05$; $^{****}P<0.0001$ and ns represent no statistical significance difference.

an important feature of a specific EC with worse prognosis. Our data agrees with other in silico reports on hepatocellular carcinoma and lung adenocarcinoma[27,28].

Notably, in our previous study, we have shown that NEIL3 genes rarely mutated but commonly overexpressed in different types of cancer and is associated with accumulation of mutations or chromosomal instability[26]. In this in-silico study, NEIL3 overexpressed endometrial tumors harbor significantly high mutation frequency that likely contributes to tumor initiation or contribute to high tumor mutation burden to drive new peptide that elicit immune response. However, the mechanisms whether NEIL3 overexpression promotes tumor mutation loads is unknown. NEIL3 may likely provide the tumor cells more oncogenic like proliferation capacity and run off the replication or may play an epigenetic role to drive proliferation mediator gene expression[29]. It is also possible that excessive NEIL3 activity could unbalance the BER pathway by generating more repair intermediates than the cell can process, leading to stalled replication forks and error-prone repair mechanisms. This accumulation of toxic BER intermediates could contribute to MSI and higher mutational loads. Additionally, in vivo study has revealed that NEIL3 mutation was linked to impaired B cell function and severe autoimmunity[30]. By contrast, in vitro studies elucidated that NEIL3 expression promotes genomic stability *via* the repair of interstrand cross link and replication associated DNA damage[8].

It is known that the tumor immune microenvironment has great implications for EC progression and susceptibility to immunotherapy[31]. Recently, we have shown that defective BER induces innate immune signaling and Type I interferon response on other types of cancer[32]. Moreover, interferon-induced signaling pathways has also been associated with resistance to immune checkpoint inhibitors through upregulation of program death ligand 1 (PD-L1), program death ligand 2 (PD-L2), CTLA-4, CIITA, IDO1, CXCL12, and nitric oxide production in tumor cells[33–35]. We found that antitumor cells including $CD8^+$ T cells and $CD4^+$ T cells were less abundant in the endometrial tumor samples, while several types of immunosuppressive cells including MDSC were more abundant in NEIL3 overexpressed tumors (Fig. 3). Overexpression of NEIL3 is associated with low immune cell infiltration and low expression of Type I interferon response genes that are responsible for cytokine-driven immune cell recruitment and inflammation. Several studies have shown that Type I interferon responses result in upregulation of immune checkpoint proteins such as PD-L1[36], resulting in inhibition of T-cell-mediated cancer cell killing[37,38]. Furthermore, CTLA-4 (CD152) is a receptor expressed by both $CD4^+$ T and $CD8^+$ T cells, which inhibit T-cell activation, negatively correlated NEIL3 overexpression. PDCD1 expression, which is a cell surface receptor on T cells and B cells that has a role in regulating the immune system negatively correlated with NEIL3 overexpressed tumors. By contrast, the tumors had low expression of PD-L1 and PD-L2 associated with NEIL3 overexpressed tumors suggesting that the NEIL3 overexpression likely contributes to immunosuppressive tumor microenvironments. Although myeloid-derived suppressor cells (MDSCs) have been found in tumor tissues, tumor-associated macrophages (TAMs) have consistently been identified as major contributors to a pro-tumorigenic environment[39–41]. In line with this report, we have found that NEIL3 overexpressed tumor exhibit significantly high infiltration of MDSC (Fig. 3) that likely contribute to tumorigenesis.

To check the diagnostic value of NEIL3 overexpression with other molecular categorizations of EC, a ROC curve analysis was performed. An ROC curve for NEIL3, NEIL3 with a p53 mutation, and NEIL3 with a POLE

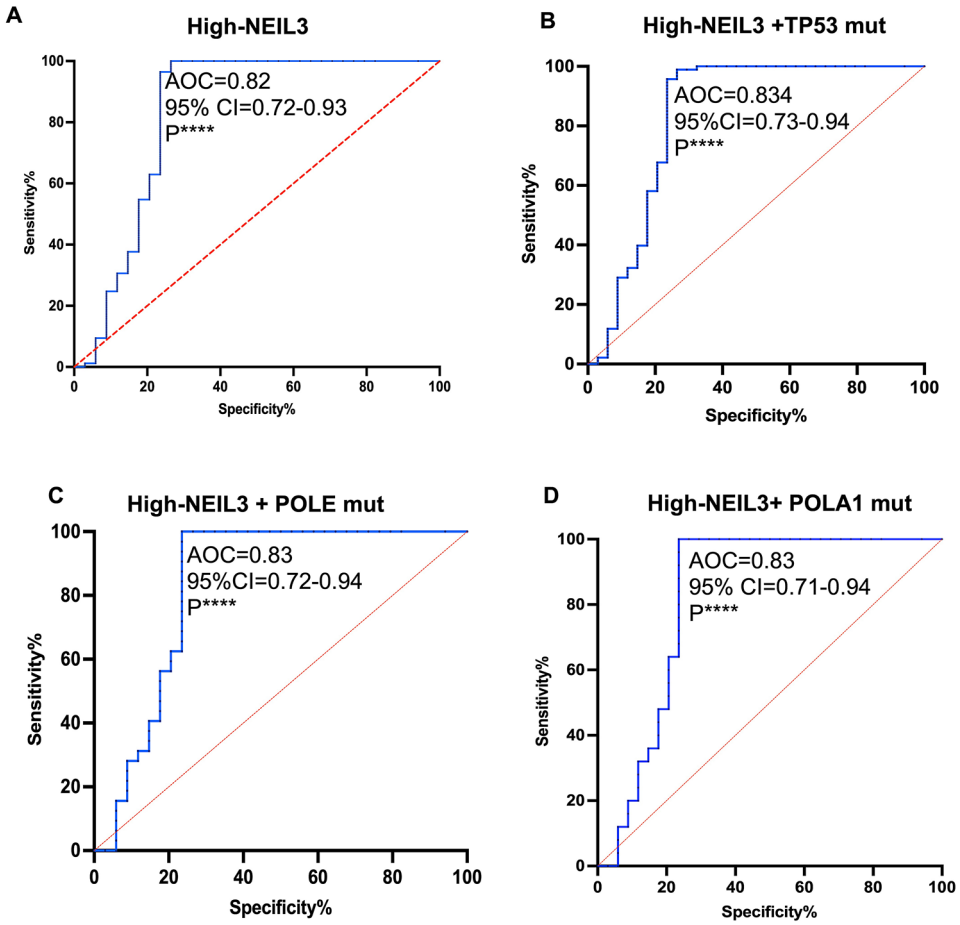


Fig. 6. Prognostic value of NEIL3 gene expression. (**A**) Diagnostic ROC curves of NEIL3 overexpression from TCGA database; (**B**) Diagnostic ROC values of NEIL3 overexpression with TP53 mutation, (**C**) ROC analysis of NEIL3 overexpression with POLE mutation; (**D**) ROC analysis of NEIL3 overexpression with POLA1 mutation. The data was analyzed with Wilson/Brown methods using GraphPad prism. AUC: represent area under the curve, *** represent statical significant $P < 0.0001$.

mutation showed that the AUC value was nearly 82 and 83% respectively, indicating that the expression level of NEIL3 with or without p53 mutation or DNA replicative polymerase (POLE, POLA1 and POLD1) mutations might be used to diagnosis of endometrial cancer.

Taken together, NEIL3 overexpression associated with enhanced expression of immunosuppressive molecules, and immunosuppressive cell recruitment to the tumor microenvironment. Further, our in silico-data-driven observations suggest that tumors with aberrant NEIL3 expression may suppress tumor immunogenicity that likely raise the possibility of dampening immune checkpoint blockade therapy response. It is expected that high tumor mutation burden leads to relatively more antigen derived immunogenic tumor, However, in NEIL3 overexpressed tumor harbor low expression of genes involved in innate immune signaling and low infiltration of immune cells. This scenario likely arises due to low antigen processing capacity of the tumor cells that cause poor infiltration of anti-tumor immune cells (Supplement Table 1). In addition, using NEIL3 overexpression may likely contribute to identify reliable biomarkers to stratify patients for appropriate immune-based treatment. Nevertheless, there is a clear need to understand the mechanisms through which aberrant NEIL3 contributes to the immunogenicity of the tumor either through increased tumor mutation burden or concurrent activation of the innate immune signaling *via* STING pathway that may lead to enhanced T-cell-mediated cancer cell death. Overall, the data analysis from the TCGA dataset suggests dysregulated expression of NEIL3 in tumors appear to avoid host immune-mediated elimination through suppression of anti-tumor immune cell infiltration and innate immune signaling genes. Furthermore, our data suggest that a systemic understanding of the DNA repair landscape in a tumor may help assess the tumor's susceptibility to immunotherapy. We recognized that this study has limitations due to the exclusion of tumors from patients with a NEIL3 *z*-score ranging between − 0.5 and 0.5, which may impact the interpretation of the results. Future in vitro and in vivo studies are needed to explore the molecular mechanism by which NEIL3 regulates the immunogenicity of endometrial tumors.

Methods

Data acquisition

RNA sequencing (RNA-Seq) and clinical information (histological grade, survival status, and tumor subtypes, of EC ($n = 507$) were retrieved from the Cancer Genome Atlas (TCGA pan cancer data sets: cBioProtal (http://cbioportal.org). Patients' data were categorized as NEIL3 high and low expression to analyze the tumor data including the tumor stage, lymph node status, histological grade, and patient characteristics. In accordance with the journal's guidelines, we will provide our data for independent analysis by a team selected by the Editorial Team for the purposes of additional data analysis or for the reproducibility of this study at other centers if such is requested.

Exclusion and inclusion criteria

NEIL3 is the primary source of interest for this analysis, only individuals with valid RNA Seq V2 RSEM data for NEIL3 were included. Individuals with a z score of < -0.5 were placed into the low expressing group while individuals with a z score of > 0.5 were placed into the high expressing group. Individuals falling between -0.5 and 0.5 were excluded from the analysis.

Immune cell infiltration in EC

The TIMER algorithm was used to calculate the tumor abundance of six infiltrating immune cells ($CD4^+$ T cells, $CD8^+$ T cells, B cells, neutrophils, macrophages, and dendritic cells) based on RNA-Seq expression profiles data. The correlations between NEIL3 expression and immune cell infiltration were calculated by Spearman correlation analysis using TIMER 2.0.

Estimation of stromal and immune cells in tumor tissues

The ESTIMATE algorithm-generated matrix, immune scores and stromal scores were used to estimate the level of infiltrating matrix and immune cells in EC tissue and tumor purity through expression profiles. Results for the tumor-infiltrating immune component were yielded with data extracted from the TCGA database, which was analyzed by the CIBERSORT algorithm.

Mutation count and CNA

The mutation counts for each patient data were acquired from the TCGA data set for each selected individual. Values were grouped according to low and high *NEIL3* expression for a given cancer type and an unpaired t test was used to assess the statistical differences between the two groups. The CNA data for each patient data were pulled from the cBioPortal website and merged with the TCGA data set of a given cancer line. For correlation pairing, we used correlation analysis with Spearman correlation coefficient to determine the association between *NEIL3* and infiltration of immune cells, innate immune signaling and immune checkpoint involved genes.

Statistical analysis

Group comparisons for continuous data were conducted using t tests, and quantitative variables were analyzed with the Wilcoxon signed-rank test or the Spearman rank correlation test. Kaplan–Meier analysis was used to assess overall survival and progression free survival. Uni-variable associations between NEIL3 overexpression and clinicopathologic variables were tested using nonparametric tests. Statistical significance was set at $P < 0.05$.

Data availability

All extracted data from cBioPortal (www.cBioPortal.org) and The Cancer Genome Atlas data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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C.B. data collection and data analysis, A.S. performed manuscript writing; D.K. performed data analysis, interpretation, and manuscript writing. All authors read and approved the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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