Mutations in \textit{KIAA1109}, \textit{CACNA1C}, \textit{BSN}, \textit{AKAP13}, \textit{CELSR2}, and \textit{HELZ2} Are Associated With the Prognosis in Endometrial Cancer

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Endometrial cancer (EC) is one of the most common gynecologic malignancies. Emerging studies had demonstrated the mutations in genes could serve as diagnostic or prognostic markers for human cancers. In this study, we screened mutated genes in EC and found that the mutations in \textit{KIAA1109}, \textit{CACNA1C}, \textit{BSN}, \textit{AKAP13}, \textit{CELSR2}, and \textit{HELZ2} were correlated to the overall survival time in patients with EC. Bioinformatics analysis showed \textit{KIAA1109} was involved in regulating NIK/NF-kappaB signaling, \textit{CACNA1C} was found to regulate cell migration and proliferation, \textit{BSN} was found to regulate Wnt signaling pathway, \textit{CELSR2} was involved in regulating cell–cell adhesion, nuclear import, and protein folding, \textit{HELZ2} was found to regulate multiple immune related biological processes, and \textit{AKAP13} was involved in regulating translation, mRNA nonsense-mediated decay, rRNA processing, translational initiation, and mRNA splicing via spliceosome. The findings provided a novel therapeutic strategy in patients with EC.

\textbf{Keywords: endometrial cancer, bioinformatics analyses, mutation, overall survival time, biomarkers}

\section*{INTRODUCTION}

Endometrial cancer (EC) is one of the most common gynecologic malignancies (Attarha et al., 2011). Despite the prognosis of the early stage EC is good with a 5-year survival rate of 69–88\% (Gottwald et al., 2010). However, the prognosis of metastatic EC remained very poor, with a median survival of 7–12 months. Therefore, there is an urgent need to identify novel biomarkers for the prognosis of EC. Moreover, the mechanisms underlying the progression of EC remained largely unclear.

With the development of next-generation sequencing, multiple EC related mutations were identified. Emerging studies had demonstrated the mutations in genes could serve as diagnostic or prognostic markers for human cancers. For example, McConechy et al. identified a series of mutations in \textit{PTEN}, \textit{CTNNB1}, \textit{PIK3CA}, \textit{ARID1A}, \textit{ARID5B}, and \textit{KRAS} were associated with EC (Mcconechy et al., 2012). The mutations in \textit{FGFR2} were associated with poor outcomes in endometrioid endometrial cancer (Jeske et al., 2017). The genetic alterations in \textit{CTCF} could promote EC cell survival and alter cell polarity (Marshall et al., 2017). Jing et al. found that MUC16 mutations could improve patients’ prognosis by enhancing the infiltration of cytotoxic T lymphocytes in the EC microenvironment (Jing and Jing, 2014).

The present study identified prognosis related gene mutations in EC by analyzing TCGA databases (Collins, 2007). The mutations in 6 genes were correlated to the overall survival time in patients with EC. Bioinformatics analysis was used to predict the potential functions of these
genes. The purpose of this study was to evaluate the impact of somatic tumor mutation on recurrence-free survival in this patient population.

**MATERIALS AND METHODS**

**Data Mining With cBioPortal and TCGA Database**

In this study, we identified the gene mutations in EC using TCGA database (https://portal.gdc.cancer.gov/). All searches were performed according to cBioPortal’s online instructions (http://www.cbioportal.org/index.do) (Jianjiong et al., 2013). The survival analysis related to gene mutations was performed on the TCGA database (https://portal.gdc.cancer.gov/).

**Co-Expression Network Analysis**

In this study, the Pearson correlation coefficient was calculated according to the expression value between lncRNA–mRNA pair using cBioPortal’s online instructions (http://www.cbioportal.org/index.do). The top 500 co-expressing genes were selected as potential targets of mutated genes in EC.

**Bioinformatics Analysis**

GO and KEGG pathway enrichment analysis were performed to determine the biological significance of DEGs, using the Database for Annotation, Visualization, and Integrated Discovery (Dennis et al., 2003) (DAVID; version 6.8; http://david.ncifcrf.gov/).

**Patients’ Prognostic Analyses**

Survival curves were depicted using the Kaplan-Meier method and compared with log-rank test. Cox proportional hazards regression analysis was used for univariate and multivariate analyses to explore the association of clinical features, gene mutational status, and patients’ prognosis. All the prognostic analyses were conducted by survival R package.

**Statistical Analysis**

The two groups were compared using Student’s t-test. Overall survival time analyses were estimated using the Kaplan-Meier product-limit estimator, and then a log-rank test was conducted to compare wildtype and mutation status. Overall survival was measured from the date of surgery to the date of last contact or death. Patients alive were censored at the date of last contact or clinic visit. Stata v14.2 (College Station, TX) was used to conduct statistical analysis.

**RESULTS**

**Screening of Mutated Genes in Endometrial Cancer**

The present study analyzed TCGA database to identify mutated genes in EC. As shown in Figure 1, the top 50 mutated genes in EC included TTN, MUC4, MUC16, PIK3CA, KMT2C, KMT2D, SYNE1, FLG, SYNE2, EP300, OBSCN, ADGRV1, RYR2, LRP1B, USH2A, MUC17, NEB, MDN1, MUC5B, CSMD1, PCLO, HUWE1, FBXW7, DMD, NSD1, NAV3, DNAH8, DST, PLEC, AHNAK2, LRP2, MKI67, DNAH2, TENM1, DNAH10, PRKDC, FAT1, TP53, HMCN1, ZFHX4, DNAH6, UBR4, NOTCH1, CREBBP, NIPBL, EYS, AHNAK, CSMD3, XRIP2, and MACF1. Among these genes, TTN, MUC4, and PIK3CA from the TCGA provisional data sets were 43.25% (125/289), 31.83% (92/289), and 29.41% (85/289), respectively.

**The Somatic Mutations of KIAA1109, CACNA1C, BSN, AKAP13, CELSR2, and HELZ2 Were Correlated to Overall Survival Time in Patients With EC**

Next, we screened somatic mutations associated with overall survival time in patients with EC. As shown in Figure 2, Log-rank test showed that mutations in KIAA1109, CACNA1C, BSN, AKAP13, and HELZ2 were significantly associated with the longer overall survival time in EC patients, however, mutations in CELSR2 were significantly associated with the shorter overall survival time in EC patients.

**Mutation Profiles in KIAA1109, CACNA1C, BSN, AKAP13, CELSR2, and HELZ2 in EC**

The mutation rates in KIAA1109, CACNA1C, BSN, AKAP13, CELSR2, and HELZ2 from the TCGA provisional data sets were 6.92% (20/289), 7.27% (21/289), 7.96% (23/289), 7.61% (22/289), 6.92% (20/289), and 7.27% (21/289), respectively. A, majority of mutations identified were missense and nonsense resulting in amino acid, changes and a truncation of these proteins. However, there was no evidence of a mutational hotspot in KIAA1109, CACNA1C, BSN, AKAP13, CELSR2, and HELZ2 in EC patients (Figure 4).

**The Effect of Mutations on mRNA Expressions of KIAA1109, CACNA1C, BSN, AKAP13, CELSR2, and HELZ2 in EC Patients**

Furthermore, we found the mutations in KIAA1109, CACNA1C, BSN, AKAP13, and CELSR2 and HELZ2 did not result in a significant alteration of their mRNA levels. However, we found that the mRNA levels in KIAA1109 and AKAP13 mutated EC samples were lower than that in KIAA1109 and AKAP13 wild type EC samples.

**Bioinformatics Analysis of KIAA1109, CACNA1C, BSN, AKAP13, CELSR2, and HELZ2 in EC Patients**

Furthermore, we performed bioinformatics analysis to reveal the potential functions of KIAA1109, CACNA1C, BSN, AKAP13, CELSR2, and HELZ2 using their co-expressing mRNAs in EC patients. The present study selected the top 500 correlated genes as the potential targets of KIAA1109, CACNA1C, BSN,
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AKAP13, CELSR2, and HELZ2. Bioinformatics analysis showed KIAA1109 was involved in regulating rRNA processing, translation, transcription, NIK/NF-kappaB signaling, and histone acetylation. The results were shown in Figure 6. CACNA1C was involved in regulating collagen fibril organization, cell-matrix adhesion, cellular response to amino acid stimulus, cell adhesion, and negative regulation of cell proliferation. BSN was involved in regulating epidermis development, cilium movement, smoothened signaling pathway, Wnt signaling pathway, planar cell polarity pathway, and cilium morphogenesis. AKAP13 was involved in regulating translation, mRNA nonsense-mediated decay, rRNA processing, translational initiation, and mRNA splicing via spliceosome. CELSR2 was involved in regulating cell–cell adhesion, keratinocyte differentiation, spliceosomal snRNP assembly, nuclear import, and protein folding. HELZ2 was involved in regulating type I interferon signaling pathway, innate immune response, immune response, inflammatory response, and T cell activation.

DISCUSSION

Endometrial cancer (EC) is one of the most common gynecologic malignancies. However, the mechanisms underlying EC progression remained unclear. Previous studies had showed the mutations in several genes were related to EC. For example, MUC16 mutations improve EC prognosis through enhancing the infiltration of cytotoxic T lymphocytes. PTEN and PIK3CA mutations played crucial roles in grade 3 EC (Jing and Jing, 2014). The present study screened mutated genes in EC. Our results showed TTN, MUC4, and PIK3CA were the most frequently mutated genes in the EC, which was consistent with previous studies. Moreover, we identified the mutations in 6 genes were associated with the prognosis of EC. The results showed that mutations in KIAA1109, CACNA1C, BSN, AKAP13, and HELZ2 were significantly associated with the longer overall survival time in EC patients. However, mutations in CELSR2 were significantly associated with the shorter overall survival time in EC patients. These results suggested the important roles of these genes in the progression and prognosis of EC.

KIAA1109, located on the chromosome 4, was reported to be associated with susceptibility to celiac disease. Of note, 2 recent studies indicated KIAA1109 was associated with the prognosis of human cancers. For example, Qing et al. reported mutations in KIAA1109, DNAH5 and KCNH7 were associated with poor survival of Chinese esophageal squamous cell carcinoma patients (Tao et al., 2017). Tindall et al. found genetic variation of KIAA1109 might be associated with prostate cancer susceptibility in men with a family history of the disease (Tindall et al., 2010). CACNA1C gene encodes an alpha-1 subunit of a voltage-dependent calcium channel (Fayi et al., 2016). The mutations in CACNA1C were observed in various types of human diseases, such as ventricular fibrillation, and schizophrenia (Charles et al., 2007). Previous studies showed CACNA1C was
down-regulated in multiple human cancers (Fastje et al., 2009), including brain tumors, kidney cancers and lung cancers, suggested its regulatory roles in cancer progression. BSN encoded a scaffolding protein involved in organizing the presynaptic cytoskeleton. BSN has been demonstrated to have chemo-preventive, antiproliferative, antifungal, and anti-carcinogenic activities. In addition, BSN has been reported to induce G1 phase arrest through increase of p21 and p27. In PCa, BSN was involved in regulating cell apoptosis in cancer cells (Xu et al., 2016). The dysregulation and mutation of AKAP13 were found to be associated with the progression of colorectal cancer and breast cancer. Bentin et al. showed AKAP13 is essential for the phosphorylation of ERαS305 (Toaldo et al., 2015), which leads to tamoxifen resistance in breast cancer. HELZ2 encoded a nuclear transcriptional co-activator for peroxisome proliferator activated receptor alpha (Jakobsson et al., 2010). However, its roles in human cancers remained largely unclear. CELSR2 was found to be dysregulated in breast cancer (Jiang et al., 2018). However, the potential functions of CELSR2 in EC remained unknown.
In the present study, we performed co-expression analysis to reveal the potential roles of these mutated genes in EC. The results showed KIAA1109 was involved in regulating NIK/NF-kappaB signaling. Of note, NF-kappaB signaling had been demonstrated to be a key regulator in cancers. Suppressing of NF-kappaB signaling could inhibit cell growth and invasion in multiple cancers. For example, NF-κB suppresses apoptosis and promotes the proliferation of bladder cancer cells. A recent study
showed liposomal curcumin targeting EC through the NF-κB Pathway. Bioinformatics analysis revealed CACNA1C played important roles in regulation of EC metastasis and proliferation. BSN was found to regulate Wnt signaling pathway. Mounting evidence has confirmed the activation of Wnt/β-catenin signaling was associated with multiple cancers, including EC. AKAP13 was predicted as a RNA processing regulator. CELSR2 was involved in regulating cell–cell adhesion, keratinocyte differentiation, spliceosomal snRNP assembly, nuclear import, and protein folding. HELZ2 was involved in regulating type I interferon signaling pathway, innate immune response, immune response, inflammatory response, and T cell activation. These results suggested these mutated genes played important roles in EC tumorigenesis and progression.

Despite that bioinformatics analyses were conducted to predict the potential functions of these mutated genes in EC, several limitations still existed in this study. First, the mutated sites of these genes should be further validated in EC clinical samples using Sanger sequencing. Second, the molecular function of these key mutated genes in EC remained unclear. Therefore, gain or loss of function assays should be further conducted to investigate their important roles in EC.
In conclusion, we screened mutated genes in EC and found that the mutations in KIAA1109, CACNA1C, BSN, AKAP13, CELSR2, and HELZ2 correlated with the overall survival time in patients with EC. Bioinformatics analysis showed KIAA1109 was involved in regulating NIK/NF-kappaB signaling, CACNA1C was found to regulate cell migration and proliferation, BSN was found to regulate Wnt signaling pathway, CELSR2 was involved in regulating cell-cell adhesion, nuclear import, and protein folding, and HELZ2 was found to regulate multiple immune related biological processes. The findings provided a novel therapeutic strategy in patients with EC.

DATA AVAILABILITY

All datasets analysed in this study can be found in the Genomic Data Commons Data Portal (https://portal.gdc.cancer.gov/). We screened the gene mutations in EC. We have downloaded these data from the database and the top 500 mutated genes in EC were listed in Supplementary Table 1.

AUTHOR CONTRIBUTIONS

JZ designed experiments; ZQ and YJ analyzed the data. All authors wrote and approved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2019.00909/full#supplementary-material

SUPPLEMENTARY TABLE 1 | The top 500 mutated genes in EC.
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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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