

ORIGINAL RESEARCH

Expression of glucocorticoid receptor is associated with aggressive primary endometrial cancer with higher EMT phenotype and stemness

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Abstract

Background: Endometrial cancer (EC) is a gynecologic malignancy, with an increasing incidence and disease-associated mortality, worldwide. While the role of steroid hormone receptors in EC has been widely studied, the contribution of glucocorticoid receptor (GCR) to EC progression remains unclear. **Methods:** This study analyzed the gene expression of 507 primary EC samples from the Cancer Genome Atlas (TCGA) database. Samples were categorized into glucocorticoid receptor high (GCR^H) and Low (GCR^L) groups based on mean transcript expression levels. The study evaluated correlations with clinical parameters, immune profiling, epithelial-mesenchymal transition (EMT), cancer stemness, angiogenesis and survival. Additionally, druggable targets associated with GCR were identified. **Results:** High GCR expression was linked to an aggressive EC phenotype, characterized by enhanced EMT, increased cancer stemness and increased angiogenesis. Tumors in the GCR^H group exhibited a more immunosuppressive microenvironment, with enrichment of M2 macrophages, contributing to tumor progression and poorer clinical outcomes. Furthermore, 31 cancer driver genes and 25 druggable targets were identified. **Conclusions:** Our findings suggest that high GCR expression in EC is associated with poor prognosis due to its role in EMT, stemness and immune modulation. This highlights GCR as a potential biomarker for aggressive EC and a target for therapeutic intervention.

Keywords

Endometrial cancer; Gene expression; Immune cell M2; Druggability; Network analysis

1. Introduction

Endometrial cancer (EC) is the fourth most common malignancy and the most common cancer of female reproductive tissues. The EC incidence in women is ranked fourth, and the mortality rate is ranked sixth [1, 2]. The incidence and mortality rates from EC and its accompanying complications are increasing and survival is noticeably worse. In addition, the median survival time for women with metastatic stage is only about 7–12 months [3]. To address the poor prognosis for EC, more precise prognostic and predictive biomarkers should be found and used to track the progression of the disease and provide personalised treatment for each patient. In addition to

common clinical or pathological characteristics, various biological molecules, such as Phosphatase and Tensin Homolog (PTEN), Tumor Protein P53 (P53), Epidermal Growth Factor Receptor (EGFR), Kirsten Rat Sarcoma Viral Oncogene Homolog (KRAS), Fibroblast Growth Factor Receptor (FGFR), human epidermal growth factor receptor 2 (HER2), progesterone receptors (PR), oestrogen receptors (ER), *etc.*, have been proposed as predictive biomarkers in EC. Among these, the physiological roles played by steroid hormone receptors in female reproductive organs like the endometrium make them attractive targets. Several tissues have activated steroid hormone receptors simultaneously that can affect one another's functionality.

The uterus expresses the ER and the glucocorticoid receptor (GCR), and the effects of their ligands on uterine development can have opposed effects. The gene encoding GCR is Nuclear Receptor subfamily 3 group C member 1 (NR3C1). Of endometrial cancers, 80–90% are type I tumors express estrogen receptor (ER) and these EC are driven by hormone [4]. Estrogen causes increased uterine growth and its prolonged exposure can result in endometrial hyperplasia [5]. The role and expression of ER and PR have been extensively studied in EC and higher expression of these hormones are associated with good prognosis [6]. Among steroid hormone, only GCR is linked to poorer outcomes in EC [7]. Given that corticosteroids, which bind to and activate GCR, cause growth inhibition in the normal uterus, it is surprising that tumours with high GCR expression have a worse prognosis. This is due to the fact that, in endometrial hyperplasia and endometrial cancer cells, GCR-mediated growth inhibition is no longer achieved [8]. According to recent studies, patients with EC who expressed more of the GCR had worse outcomes. GCR acts as Estrogen Receptor alpha (ER α)’s antagonist in healthy endometrial tissue, preventing ER α activation and growth-inducing effects [9]. Clinical data indicate that the function of GCR is altered in EC, and is linked to disease aggressiveness and dismal prognosis [10]. GCR has become a crucial steroid nuclear receptor in hormone-dependent tumors, but there are few data to support its potential role.

In recent years, due to the rapid growth in array- and sequencing-based technology, EC has been further classified into four distinct molecular subgroups: (i) polymerase ϵ (POLE) ultra-mutated, (ii) microsatellite instability (MSI) hyper-mutated, (iii) copy-number low, and (iv) copy-number high [11]. Among these subgroups POLE mutated has an excellent prognosis, followed by MSI, copy-number low groups and copy-number high group has the worst prognosis [12]. This recent molecular genomic classification of EC has significantly improved the prognosis and led to the approval of new systemic therapies and development of precision medicine, resulting in therapeutic advancements and continual adjustments in EC patient’s management [12].

Despite new research on therapeutics that target immune checkpoint inhibitors and immune associated pathways, the response rates of these therapies are still modest. Despite of these advances, the poor outcomes are still associated with the high-grade, metastatic and recurrent EC. Consequently, there is a critical unmet demand to find gene signatures and focused treatment strategies for EC. To explore further the relationship between GCR expression and poor prognosis, we analyzed the higher GCR-expressing tumors and the lower GCR-expressing tumors separately. The aim of this study was to investigate expression of GCR in primary endometrial cancer lesions, and to assess its role in clinical characteristics and phenotype such as stemness, immune profile and epithelial to mesenchymal transition phenomenon. In addition, pathway enrichment and druggability analysis were also carried out to identify deregulated pathways and potential drug target for EC.

2. Materials and methods

2.1 Datasets

The gene expression profiles of Endometrial Cancer (EC) were retrieved from Genomic Data Commons (GDC, version 32) Portal (<https://portal.gdc.cancer.gov/>) using *r* package *TCGAbiolinks* and the structured clinical data were downloaded from cBioPortal (<https://www.cbioportal.org/>). These gene expression profiles were generated from the uterine adenocarcinoma tissues on gene expression array platforms. The details about the uterine sample types, sample sources, expression methods and statistical parameters were mentioned in the above-mentioned Genomic Data Commons Portal. The raw data was downloaded as raw counts which were generated through Spliced Transcripts Alignment to a Reference (STAR) pipeline [13]. Samples containing information about subtype, demographics and survival details were included in the analysis, being the only inclusion criteria used in this study.

2.2 Data processing

The raw counts of EC retrieved from GDC Portal were processed using *r* package DESeq2 [14]. The process included *estimateSizeFactors* followed by *estimateDispersions* and *nbinomWaldTest*. Further the data undergone variance stabilizing transformation using *vst* module in DESeq2. The expression value of *GCR* was extracted from the normalized count and divided the samples into “low” (GCR^L) and “high” (GCR^H) groups based on the median cut-off value. The differentially expressed genes were screened with absolute fold change of 2 and adjusted *p* value < 0.05 [15–21].

2.3 Epithelial to mesenchymal transition assessment

Epithelial cells can change into mesenchymal cells through a complex and dynamic process. During embryogenesis, development, tissue repair, organ fibrosis and wound healing, the process of epithelial to mesenchymal transition (EMT) takes place [22]. Transition of epithelial to mesenchymal phenotype is caused by regulatory network complex. A pan-cancer EMT Signature derived from 11 different cancer types was used for analyzing the association with GCR groups (GCR^H and GCR^L) of EC tumor.

2.4 Cancer stemness, angiogenesis and IFNG score

Stemness score was calculated based on expression of 11 stem cell markers which were collected from the published literature: Cytochrome P450 family 19 subfamily A member 1 (CYP19A1), Paraoxonase 1 (PON1), Gastrin (GAST), Retinol Binding Protein 2 (RBP2), Lecithin–Cholesterol Acyltransferase (LCAT), Galanin and GMAP prepropeptide (GAL), Lipoprotein(a) (LPA), Somatostatin (SST), Alcohol Dehydrogenase 4 (ADH4), Caudal Type Homeobox 2 (CDX2) and UDP Glucuronosyltransferase Family 1 Member A8 (UGT1A8) [23]. The potential any cell to maintain its lineage, to produce differentiated cells, and to act together with its environment in order to balance quiescence, proliferation, and regeneration is designated as stemness. A tiny population within tumours known as cancer stem cells

possesses stem-like characteristics that support the growth of cancer, including advanced abilities for self-renewal, cloning, growing, metastasizing, homing and repopulating. We also calculated angiogenesis score as well as interferon-gamma (IFNG) score for GCR^H and GCR^L groups of EC tumors [24]. The development, invasion and metastasis of tumours are all significantly aided by angiogenesis. Angiogenesis is calculated using genes available from molecular signature database. Based on the distribution of expression across all samples, the IFNG expression is mean-centered and normalized by standard deviation to determine the IFNG score.

2.5 Identification of immune cell composition

The Cell type identification by estimating relative subsets of RNA transcripts (CIBERSORT) computational program was utilized for determining the ratios of 22 different immune cell populations in the EC transcriptome profile [25]. CIBERSORT application uses linear support vector (SVR) regression method for performing feature selection and deconvolution of the cell mixtures from the gene expression profile [26]. The CIBERSORT algorithm can find the enrichment of genes linked to 10 different types of adaptive immune cells and 12 different types of innate immune cells. The naive Cluster of Differentiation 4+ (CD4+) T cells, memory B cells, plasma cells, T follicular helper cells, CD8+ T cells, resting memory CD4+ T cells, active memory CD4+ T cells, regulatory T cells, and Gamma-delta T cells are among the adaptive immune cells. Natural Killer (NK) cells in the resting state, monocytes, macrophages M1, and macrophages M2 are the innate immune cell types that are predicted by the CIBERSORT. The program converts the gene expression matrix into the immune cell matrix and employs a 1000 permutation filter with a significant p value set at 0.05.

2.6 Identification of cancer drivers and druggability analysis

The cancer drivers were identified by mapping the differentially expressed genes (DEGs) to the 568 genes reported in the IntOGen database (<https://www.intogen.org/>). On the basis of mutational evidence from sequenced tumour samples, IntOGen, a framework, stores the data via automatic and thorough knowledge extraction. The framework pinpoints the putative mechanisms of action of cancer genes across various tumour types. The druggability of the cancer drivers were checked using the drug-gene interaction database (DGIdb) (<https://dgidb.org/>). DGIdb categorizes the druggable genes as Clinically Actionable, Druggable Genome, Kinase, Transcription Factor, External Side of Plasma Membrane, Cell Surface, Drug Resistance, Serine Threonine Kinase, Enzyme and Protein Phosphatase.

3. Results

3.1 Dataset information

The gene expression profiles of 589 specimens and among them only 529 specimens had clinical information, were collected from the TCGA database. We further filtered the datasets based on the molecular subtype classification. Finally, there were 507 samples with clinical, expression and subtype information. The median age of the patient was 64 and median follow-up was 30 months. Subtype frequencies of the EC samples are as follows; Copy-number (CN) HIGH: 163 (32%), MSI: 148 (29%), CN LOW: 147 (29%) and POLE: 49 (10%). The subtypes were clustered using a Principal Component Analysis (PCA) plot to observe a clear distinction between the subtypes especially between CN HIGH and CN LOW groups. The grade composition of the EC tumors is as follows; Grade 1: 19% (94), Grade 2: 22% (113), Grade 3: 57% (291) and High grade or Grade 4: 2% (9). The overall survival using Kaplan-Meier analyses performed on the subtype of EC has shown that CN HIGH has the worst prognosis ($p < 0.0001$). Comparison of CN HIGH with the other groups showed the significant difference as follows: CN HIGH vs. CN LOW (1.15×10^{-5}), CN HIGH vs. MSI (5.54×10^{-4}), CN HIGH vs. POLE (7.62×10^{-5}). Similar trend was identified in Disease Free Survival (DFS), Disease Specific Survival (DSS) and Proliferation Free Survival (PFS) statuses. Overview of the data characteristics in the current study are depicted in the Fig. 1.

3.2 Characteristics of low and high GCR endometrial tumors

The expression data of GCR was extracted from the normalized count, ranging from 6.51 to 12.22. EC tumor samples were further divided into “low GCR (GCR^L)” and “high GCR (GCR^H)” groups based on the median cut-off the GCR expression value. The distribution of GCR^H group of EC molecular subtypes is CN HIGH: 120 (74%), CN LOW: 53 (36%), POLE: 20 (41%) and MSI: 61 (41%). The GCR^L group of EC tumors distribution in CN HIGH: 43 (26%), CN LOW: 94 (64%), POLE: 29 (59%) and MSI: 87 (59%). This analysis has shown that GCR^H comprised of a greater number of samples from CN HIGH subtype whereas GCR^L were found high in CN LOW, POLE and MSI subtypes.

The grade distribution of GCR^H EC tumors was as follows; Grade 1: 34/94 (36%), Grade 2: 38/113 (34%), Grade 3: 174/291 (60%) and Grade 4: 8/9 (89%). In GCR^L EC tumors distribution across different grades were significantly different; Grade 1: 60 (64%), Grade 2: 75 (66%), Grade 3: 117 (40%) and Grade 4: 1 (11%). GCR^H has a higher proportion of Grade 3 and 4 tumor samples and GCR^L were enriched in Grade 1 and 2 tumors. The overall Kaplan-Meier survival curve of GCR groups has shown GCR^H with poor prognosis ($p < 0.0001$) across all molecular subtypes. We validated the role of GCR by performing its survival analysis only in CN HIGH (the worst prognosis group) EC tumor subtype and identified the same trend ($p = 0.015$). Overview of the GCR based data analysis are shown in Fig. 2.

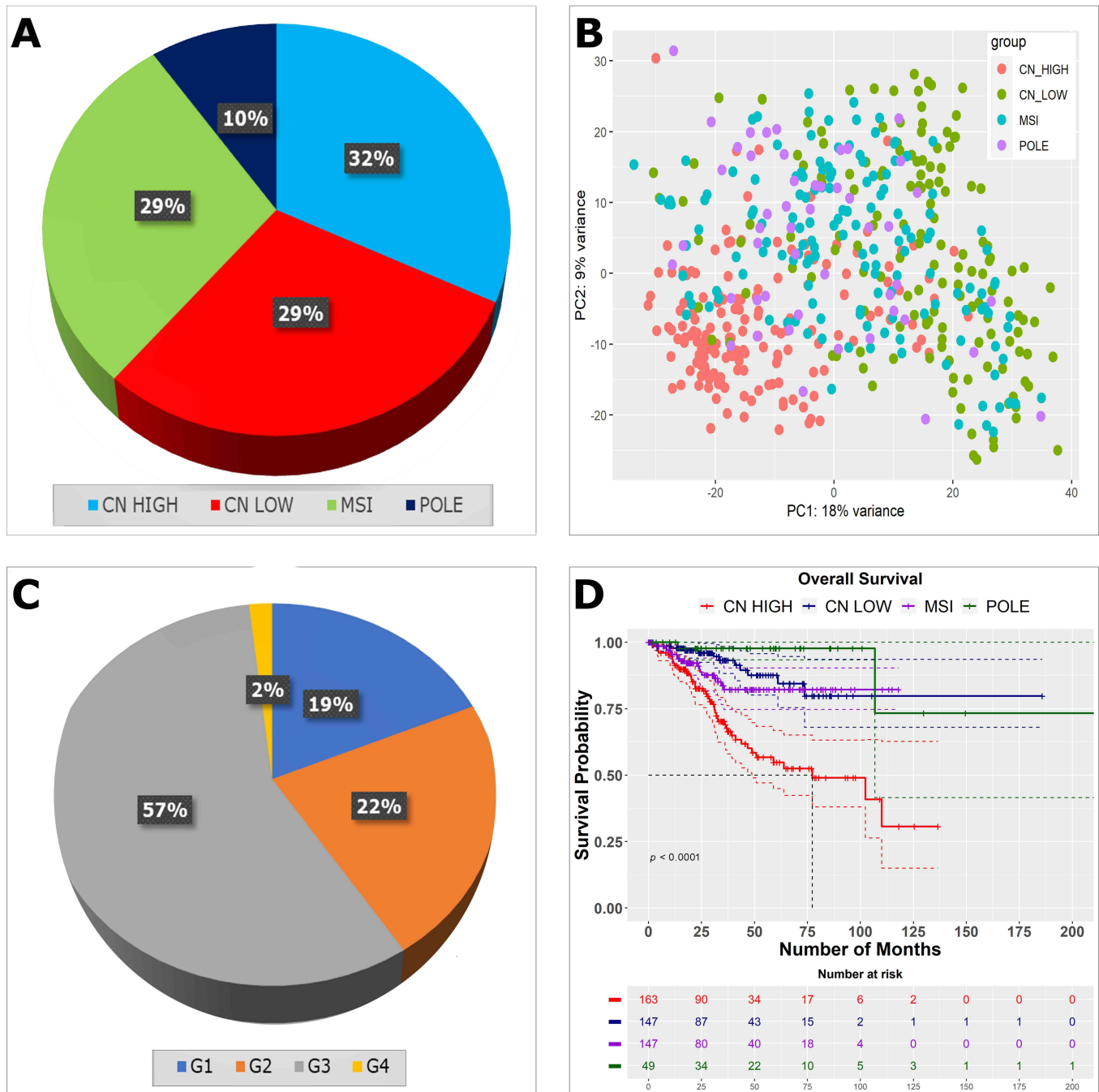


FIGURE 1. Summary of the EC sample data. (A) Molecular subtypes of EC samples. (B) The PCA plot of EC samples based on their molecular subtypes. (C) EC tumor grade distribution from low (G1) to high (G4). (D) Kaplan-Meier overall survival plot of EC patients showing significantly poor prognosis of the CN high molecular subtype patients. MSI: microsatellite instability; POLE: polymerase ϵ ; CN: Copy-number; PC: Principal Component.

3.3 Tumors with high expression of GCR is enriched for higher EMT and stemness

We evaluated the epithelial-to-mesenchymal transition (EMT) phenotype based on a pan-cancer EMT Signature [27]. The EMT analysis of EC tumors has shown that GCR^H is enriched with higher EMT ($p = 6.5 \times 10^{-14}$) (shown in Fig. 3A), indicating higher migratory power which can lead to metastasis and poor survival. Next, we evaluated the stemness property of the GCR^H and GCR^L groups of EC. The stemness analysis depicted a significant enrichment of in GCR^H group ($p = 9.7 \times 10^{-5}$) (shown in Fig. 3B–D). The evaluation of angiogenesis

and IFNG scores, key tumor promoters, has also shown higher significant enrichment in GCR^H group ($p = 4.6 \times 10^{-8}$ and $p = 2.8 \times 10^{-8}$ respectively).

3.4 Immune distribution of GCR^H and GCR^L groups

The immune cell composition landscape of EC in terms of GCR distribution has not yet been well investigated, especially for low abundant cell sub-populations. The CIBERSORT algorithm can detect gene enrichment in 10 types of adaptive immune cells and 12 types of innate immune cells. The

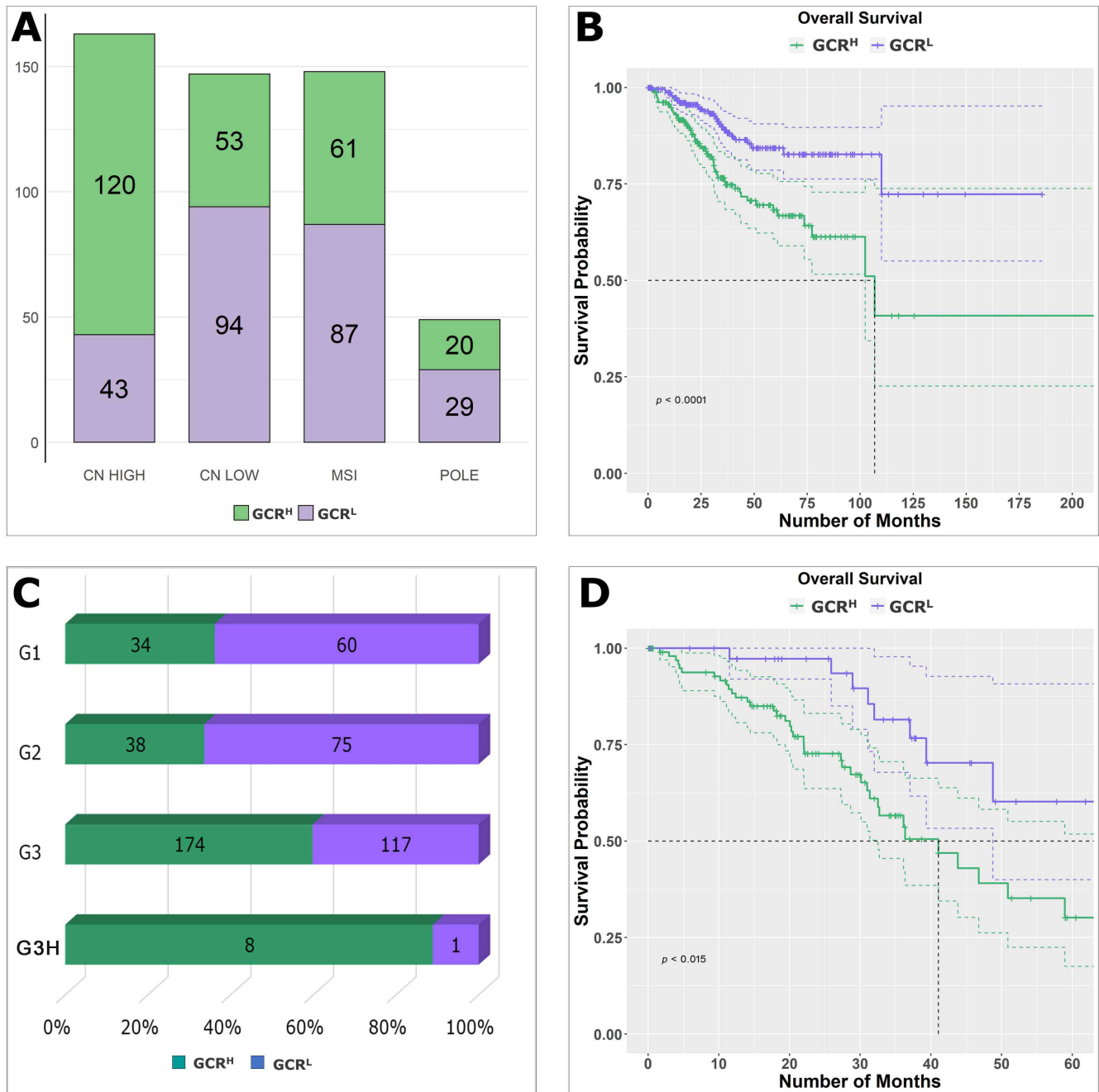


FIGURE 2. Characteristics of GCR low and high endometrial tumors. (A) Distribution of different molecular subtypes of EC tumors by GCR level. (B) Overall survival Kaplan-Meier plot of EC patients based on GCR level. (C) Distribution of different grades of EC tumors by GCR level. (D) The Kaplan-Meier survival curve of CN High molecular subtype EC patients with GCR levels. GCR^H: high glucocorticoid receptor; GCR^L: low glucocorticoid receptor; MSI: microsatellite instability; POLE: polymerase ϵ ; CN: Copy-number; G3H: Grade 3 and High grade/Grade 4.

immune cell proportions of innate and adaptive immune cells are shown in Fig. 4. The immune cell types like neutrophils, dendritic cells activated, resting dendritic cells, macrophages M0, activated NK cells, T cells Gamma-delta, T follicular helper cells, regulatory T cells and memory B cells, were not significantly enriched in the group comparison hence not analyzed further. The heatmap representing the proportion of the enriched immune cell types is presented in Fig. 4A. When comparing GCR^H samples to GCR^L samples, we found a larger relative fraction of genes enriched for macrophages M2 cell ($p = 0.00019$) (Fig. 4B). M2 macrophages frequently

tend to generate an immune suppressive characteristic that promotes tumour development and tissue repair. Additionally, M2 is expected to promote angiogenesis, neovascularization, stromal activation and remoulding, which can impact cancer progression and negatively affect patient prognosis. To validate the enrichment of M2 macrophages, we looked at the transcript expression of the *CD163* which is key marker of M2 macrophages (Fig. 4C). The analysis has shown significantly higher expression of *CD163* marker in GCR^H group (1.4×10^{-8}).

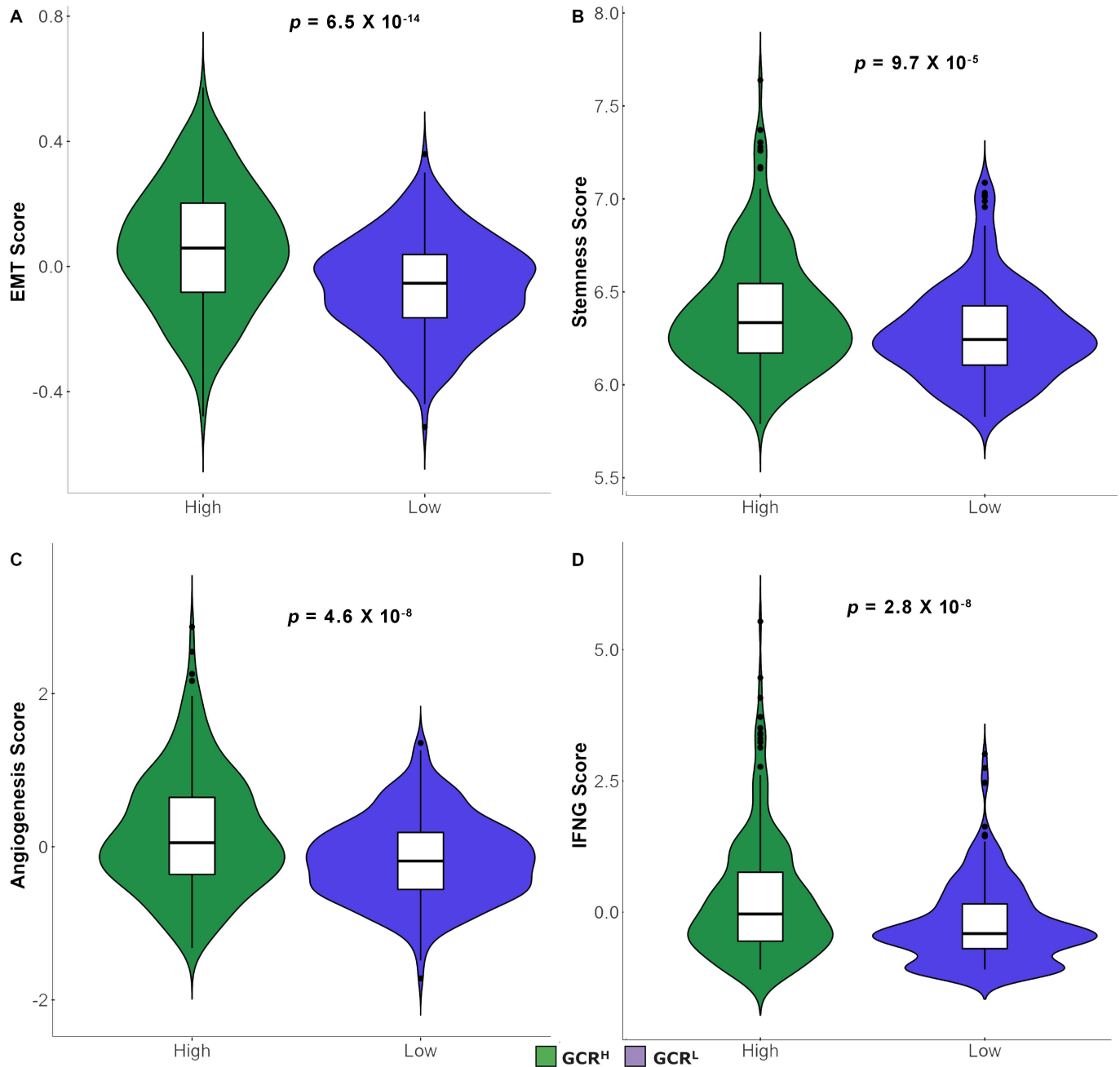


FIGURE 3. The pattern of EMT, stemness, angiogenesis and IFNG scores in GCR^H and GCR^L EC groups. (A) Epithelial-to-mesenchymal transition (EMT) phenotype. (B) Distribution of stemness. (C) Distribution of Angiogenesis score. (D) Distribution of IFNG score. EMT: epithelial-mesenchymal transition; GCR^H : high glucocorticoid receptor; GCR^L : low glucocorticoid receptor; GCR: glucocorticoid receptor.

3.5 Identification of DEGs and functional enrichment analysis

The DEGs were identified with absolute fold change of 2 and adjusted p value < 0.05 . The volcano plot representing the DEGs is represented in Fig. 5A. There were about 1163 differentially expressed genes, of which 991 and 172 were up- and down- regulated respectively (Fig. 5B). The top 10 up- and down- regulated genes are represented in Table 1. The enrichment of DEGs using ToppGene has shown up-regulation of pathways such as *Chemokine signaling* ($p = 0.0016$), *Jak-STAT signaling* ($p = 0.0338$), *Phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) Signaling in Cancer* (p

$= 0.0359$), *Toll-like receptor signaling* ($p = 0.0384$), *Th1/Th2 Differentiation* ($p = 0.0384$) and *Type II interferon signaling* ($p = 0.00821$). The *FGFR1 ligand binding and activation* ($p = 0.0338$) and *Phospholipase C-mediated cascade* ($p = 0.0338$) pathways were downregulated (Fig. 5C).

The enrichment analysis also collected gene ontologies associated with the DEGs; Biological Process (824 BP terms), and Molecular Functions (140 MF terms). The top enriched gene ontologies are represented in Table 2. We applied semantic or functional similarity to stratify the broad range of related terms into a clustered term as shown in Fig. 6. The clustered terms included cell activation, cell communication,

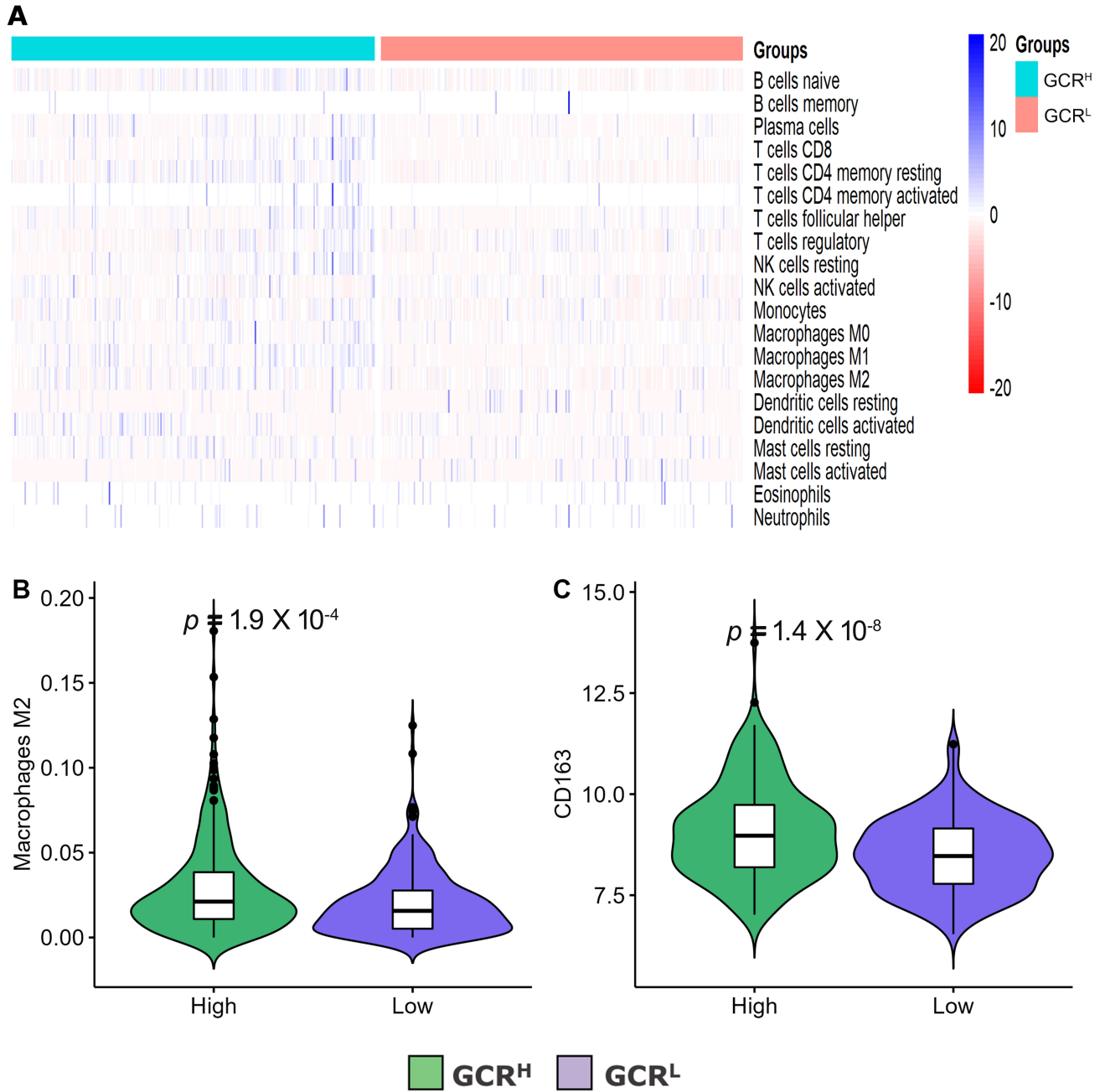


FIGURE 4. Immune cell composition identified by CIBERSORT algorithm. (A) Distribution of immune cell types in GCR^H and GCR^L groups. (B) The expression of M2 macrophages in GCR^H and GCR^L groups. (C) The expression of CD163, a M2 macrophage marker, in GCR^H and GCR^L groups. GCR^H : high glucocorticoid receptor; GCR^L : low glucocorticoid receptor; CD: Cluster of Differentiation; NK: Natural Killer.

chemotaxis, G protein-coupled receptor signaling pathway, immune system process, ion transport, regulation of cytokine production, myeloid leukocyte migration and mononuclear cell proliferation.

Overall, functional enrichment analysis demonstrated that the GCR^H tumors were enriched in immune associated processes, extra cellular matrix (ECM) degradation and tumor metastasis.

3.6 Cancer drivers and druggability analysis

The differentially expressed genes between GCR^H and GCR^L group were mapped against cancer drivers of IntOGen

database. We identified 31 differentially expressed genes which fall under cancer driver's category (Fig. 7A). The druggability analysis has shown 25 of the 31 cancer drivers (80.65%) are druggable (Table 3). The 25 druggable genes were categorized as clinically actionable (18 targets), druggable genome (14 targets), kinase (8 targets), transcription factor (8 targets), external side of plasma membrane (4 targets), cell surface (3 targets), drug resistance (3 targets), serine threonine kinase, enzyme (2 targets) and protein phosphatase (2 targets) (Fig. 7B).

TABLE 1. The list of top differentially up- and down- regulated genes between GCR^H and GCR^L tumor groups.

Gene	log2FC	adj.pval	Gene name
<i>ZFP42</i>	3.61	1.64×10^{-17}	ZFP42 zinc finger protein
<i>ST8SIA3</i>	3.48	1.09×10^{-17}	Sialyltransferase St8Sia III
<i>MAGEA1</i>	3.26	1.95×10^{-10}	MAGE family member A1
<i>MAGEA4</i>	3.16	2.46×10^{-11}	MAGE family member A4
<i>MAGEA10</i>	3.15	2.58×10^{-12}	MAGE family member A10
<i>IGFBP1</i>	3.14	2.72×10^{-31}	Insulin like growth factor binding protein 1
<i>CENPVL3</i>	3.10	1.16×10^{-8}	Centromere protein V like 3
<i>NR1H4</i>	3.07	1.26×10^{-14}	Nuclear receptor subfamily 1 group H member 4
<i>GPR12</i>	3.03	1.02×10^{-12}	G protein-coupled receptor 12
<i>NLRP4</i>	2.99	2.56×10^{-20}	NLR family pyrin domain containing 4
<i>MT4</i>	-3.02	1.16×10^{-7}	Metallothionein 4
<i>KRT33B</i>	-2.50	1.45×10^{-10}	Keratin 33B
<i>KRTAP3-3</i>	-2.49	1.46×10^{-5}	Keratin associated protein 3-3
<i>DKK4</i>	-2.27	3.13×10^{-8}	Dickkopf WNT signaling pathway inhibitor 4
<i>DEFA5</i>	-2.12	3.78×10^{-3}	Defensin alpha 5
<i>MPC1L</i>	-2.02	3.61×10^{-16}	Mitochondrial pyruvate carrier 1 like
<i>KRTAP11-1</i>	-1.95	5.14×10^{-5}	Keratin associated protein 11-1
<i>KRTAP4-7</i>	-1.91	4.32×10^{-4}	Keratin associated protein 4-7
<i>MMP26</i>	-1.89	4.64×10^{-7}	Matrix metalloproteinase 26
<i>AZU1</i>	-1.80	1.29×10^{-14}	Azurocidin 1

log2FC: Fold Change in log2 scale; adj.pval: adjusted p value.

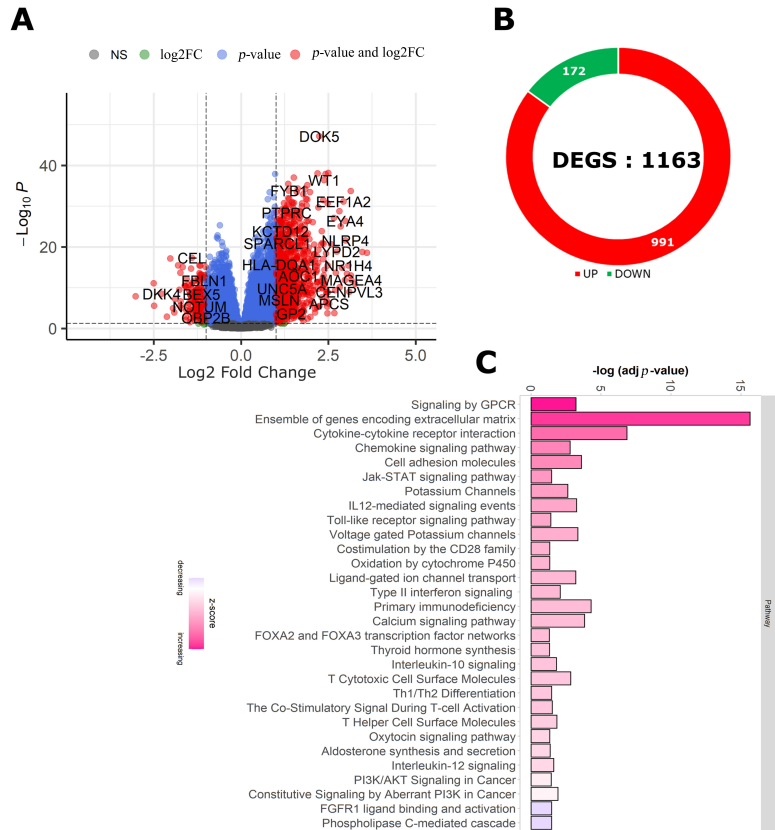


FIGURE 5. Differential expression and pathway enrichment analysis of GCR groups of endometrial tumors. (A) The volcano plot represents the distribution of differentially regulated genes. (B) Up- and down- regulated genes between GCR^H and GCR^L groups. (C) The significant pathways enriched by the differentially regulated genes.

TABLE 2. Highly enriched biological processes of GO annotations between GCR^H and GCR^L tumor groups.

Category	ID	Name	adj.pvalue	Gene count
GO:BP	GO:0006811	ion transport	4.79×10^{-15}	187
GO:BP	GO:0002684	positive regulation of immune system process	3.75×10^{-11}	125
GO:BP	GO:0098609	cell-cell adhesion	1.18×10^{-10}	120
GO:BP	GO:0045321	leukocyte activation	1.49×10^{-9}	128
GO:BP	GO:0046649	lymphocyte activation	5.36×10^{-9}	110
GO:BP	GO:0002682	regulation of immune system process	8.81×10^{-9}	163
GO:BP	GO:0001819	positive regulation of cytokine production	8.81×10^{-9}	73
GO:BP	GO:0043270	positive regulation of ion transport	8.81×10^{-9}	55
GO:BP	GO:0006954	inflammatory response	1.11×10^{-8}	108
GO:BP	GO:0042110	T cell activation	1.95×10^{-8}	81
GO:BP	GO:0001817	regulation of cytokine production	3.42×10^{-8}	102
GO:BP	GO:0010817	regulation of hormone levels	4.69×10^{-7}	76
GO:BP	GO:0030098	lymphocyte differentiation	5.59×10^{-7}	65
GO:BP	GO:0030217	T cell differentiation	8.75×10^{-7}	51
GO:BP	GO:0032943	mononuclear cell proliferation	9.57×10^{-7}	53
GO:BP	GO:1903131	mononuclear cell differentiation	9.73×10^{-7}	69
GO:BP	GO:0050870	positive regulation of T cell activation	2.96×10^{-6}	42
GO:BP	GO:0070661	leukocyte proliferation	4.55×10^{-6}	54
GO:BP	GO:0002521	leukocyte differentiation	5.03×10^{-6}	81
GO:BP	GO:0002443	leukocyte mediated immunity	6.75×10^{-6}	64

GO: Gene Ontology; BP: Biological Process; adj.pval: adjusted p value.

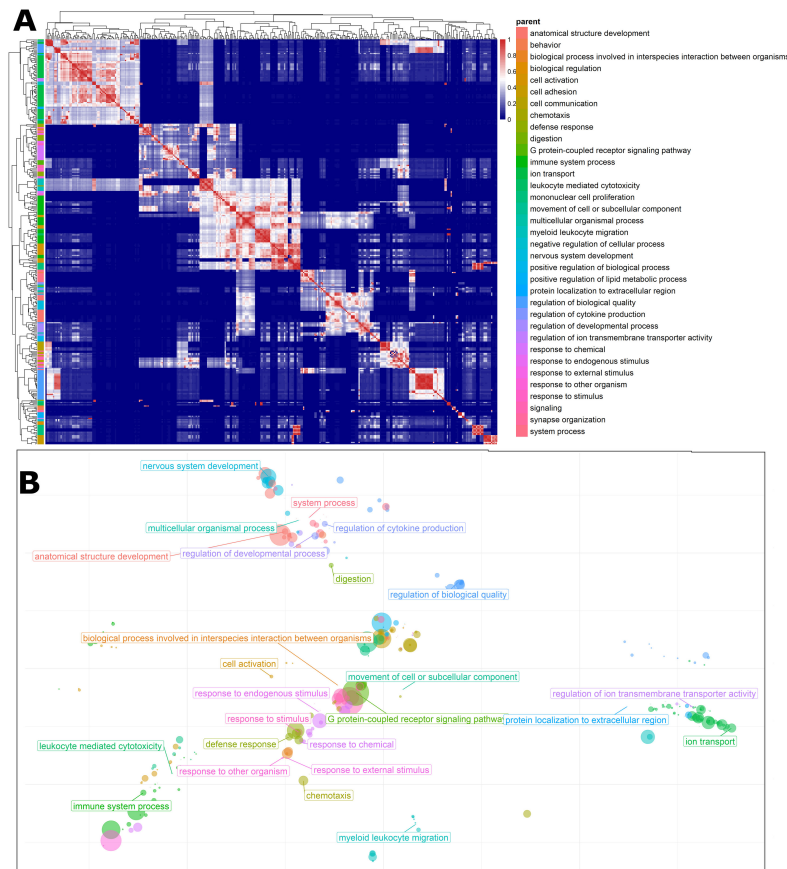


FIGURE 6. Representation of gene ontology analysis. (A) The semantic similarity of gene ontology represented as a correlation map. (B) The clustered gene ontologies associated with the differentially regulated genes of GCR^H when compared to GCR^L groups.

TABLE 3. The list of druggable cancer driver genes in EC.

Gene	FC	adj.pval	Gene name
<i>WT1</i>	5.21	1.08×10^{-33}	WT1 transcription factor
<i>IRF4</i>	3.44	1.24×10^{-30}	interferon regulatory factor 4
<i>HOXD13</i>	3.36	7.95×10^{-8}	Homeobox D13
<i>ZBTB16</i>	3.20	1.25×10^{-22}	Zinc finger and BTB domain containing 16
<i>DCC</i>	3.04	9.73×10^{-17}	DCC netrin 1 receptor
<i>NRK</i>	2.80	1.63×10^{-14}	Nik related kinase
<i>PTPRT</i>	2.77	1.95×10^{-7}	Protein tyrosine phosphatase receptor type t
<i>FAT3</i>	2.74	3.85×10^{-13}	FAT atypical cadherin 3
<i>CARD11</i>	2.60	6.47×10^{-24}	Caspase Recruitment Domain Family Member 11
<i>PLAG1</i>	2.56	4.00×10^{-15}	PLAG1 zinc finger
<i>TLL1</i>	2.55	5.58×10^{-19}	Tolloid like 1
<i>PDCD1LG2</i>	2.55	1.08×10^{-29}	Programmed cell death 1 ligand 2
<i>MYH11</i>	2.51	2.23×10^{-12}	Myosin heavy chain 11
<i>PTPRC</i>	2.47	1.57×10^{-26}	Protein tyrosine phosphatase receptor type C
<i>P2RY8</i>	2.43	3.07×10^{-24}	P2Y receptor family member 8
<i>BCL11B</i>	2.37	6.76×10^{-21}	BAF chromatin remodeling complex subunit BCL11B
<i>IL7R</i>	2.35	5.36×10^{-22}	Interleukin 7 Receptor
<i>CD79B</i>	2.33	2.02×10^{-19}	CD79b molecule
<i>PRKCB</i>	2.29	3.52×10^{-21}	Protein kinase c beta
<i>SALL4</i>	2.06	5.67×10^{-9}	Spalt like transcription factor 4
<i>FLT3</i>	2.05	2.33×10^{-12}	Fms related receptor tyrosine kinase 3
<i>ZBTB20</i>	2.03	9.55×10^{-15}	Zinc finger and btb domain containing 20
<i>AKT3</i>	2.03	1.28×10^{-26}	AKT serine/threonine kinase 3
<i>IKZF1</i>	2.01	9.45×10^{-21}	IKAROS family zinc finger 1
<i>FBLN1</i>	-2.07	3.57×10^{-11}	Fibulin 1

FC: Fold Change; adj.pval: adjusted *p* value.

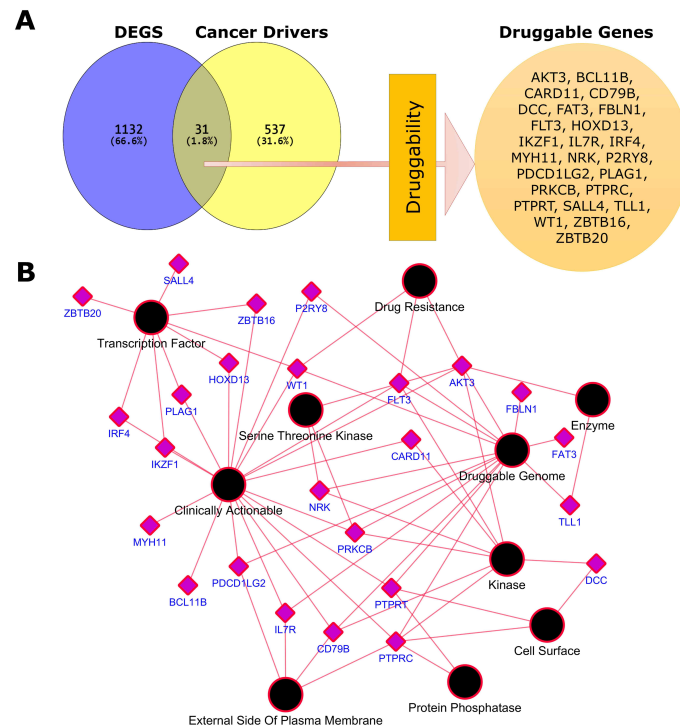


FIGURE 7. Identification of cancer drivers and druggable targets. (A) Druggable differentially expressed Cancer driver genes in EC. **(B)** Druggable genes and their clinically actionable categories.

4. Discussion

In solid tumours, emerging evidence indicates that signaling through the glucocorticoid receptor (GCR; encoded by NR3C1 gene), can accelerate the growth and metastasis and many drugs targeting it are in different stages of clinical trials. For these reasons, GCR may be useful in predicting the outcome when diagnosed or to predict whether they are responder or non-responders to specific drugs which target this receptor [28]. Previous research has demonstrated that primary EC with GCR expression has aggressive behavior and has a poor prognosis.

We confirmed that the poorer prognosis is substantially correlated with higher expression of GCR in primary EC. In subgroup analyses, increased GCR expression is linked to poor survival regardless of the molecular EC subtypes POLE, MSI, CN HIGH or CN LOW. We examined the pathologic differences between GCR^H and GCR^L endometrial cancer types. EMT is a phenomenon characterized by phenotypic and cellular modifications that enable plasticity to undergo transformations, which are essential for migration, immune evasion, distant organ seeding, and ultimately metastasis [29]. The GCR^H groups possess high EMT features rendering them capable of collective cell migration (possibly metastasis), immune evasion, higher tumour initiating capability which are strongly linked with a worse survival prognosis.

Stemness is defined as a cell's ability to preserve its lineage, give rise to differentiated cells, and interact with its environment in order to maintain a balance between proliferation, quiescence and regeneration. Cancer stemness, or the stem-cell-like phenotype of cancer cells, has been identified as playing essential roles in various facets of cancer. Stemness properties of cancer stem cells which include improved abilities for self-renewal, growth, homing, metastasizing and re proliferation to support tumors. As they may instruct nearby cells to deliver nutrition and work together to evade the immune surveillance system, they exhibit remarkable organizational abilities and promote the formation of tumors [30, 31]. The GCR^H groups possess higher stemness promoting the tumor progression. These findings imply that expression of GCR enhances EC tumour aggressiveness.

Angiogenesis, also known as germination, is the process by which new blood vessels are created from pre-existing ones. Under physiological conditions, it mostly contributes to wound healing and embryonic development [32]. Pathological angiogenesis is one of the hallmarks of the tumour since it is impossible for tumours to develop larger than 1–2 mm without vascular supply [33, 34]. Rapid development of new vascular networks, fueled by angiogenic agents, is necessary to support the high metabolic and proliferative rates of cancer cells [35]. These newly developed blood arteries not only supply the oxygen and nutrients needed to support the rapid development and multiplication of tumour cells, but they also offer a potential entry point for metastasizing tumour cells into the circulatory system [36]. With reference to the angiogenesis process, the GCR^H groups possess high expression of angiogenesis markers thereby promoting enough nutrient and oxygen supply for the continued tumor progression.

In addition, we have checked the expression of Interferon-

gamma (IFNG) in the EC samples. As a crucial coordinator of antitumor immune responses in the elimination phase of the immunoediting paradigm, IFNG has long been identified as a key molecule in tumor developments [37]. But growing evidence indicates that IFNG may also play significant pro-tumor roles in the equilibrium and escape phases through its regulatory effects on immune-evasive mechanisms that support tumorigenesis [38]. According to published research, the IFNG is a cytokine with both pro-tumor and anti-tumor properties, suggesting that it may serve as a link for immunotherapy responsiveness. Many cancer therapies stimulate the production of IFNG by activated T cells and natural killer cells. Patients who are resistant to these medications typically have genetic abnormalities in the IFNG signaling pathway or have resistance molecules mediated by IFNG [39]. In the present study, IFNG score is significantly higher in GCR^H group, supporting their crucial role in poor prognosis in this EC patient category.

The tumor microenvironment analysis has shown higher enrichment of pro-tumorigenic immune cell type, M2 macrophage. Indicators of a bad prognosis include tumour metastasis. Degradation and damage to the basement membrane of endothelial cells in tumour tissue are the primary causes of tumour cell migration and metastasis. The direct production of soluble substances by activated tumour associated macrophages (TAM) has been shown to have a direct impact on increase in metastasis [40]. By secreted matrix metalloproteinases (MMPs), serine proteases, cathepsins and other enzymes, M2 macrophages can damage the matrix membrane of endothelial cells and break down different collagen and extracellular matrix components, which facilitates the migration of tumour cells and tumour stromal cells. The root of tumour metastasis, which is prevalent in the EC GCR^H group, is epithelial-mesenchymal transition [41]. This transition enables tumor cells to develop the capability to migrate and grants them with the characteristics of cancer stem cells. Additionally, the development of TAMs is aided by cytokines produced by tumour cells, creating a positive feedback loop between TAMs and EMT.

The immune environment has been reported to play a pivotal role in EC proliferation, progression and tumorigenesis [42]. On other hand, ECM remodeling is an important mechanism involved in tumor growth, differentiation, migration and invasion [43]. Dysregulated ECM is characterized by increased laminin and fibronectin fragments along with increased collagen and MMPs [43]. ECM components are chemotactic and attract endothelial and immune cells such as macrophages and neutrophils [44]. Alteration in these pathways in EC paves the way for tumor cell invasion which is the first key step in the metastatic cascade of tumors.

Molecularly targeted drugs such as Bevacizumab, Olaparib, Nivolumab and Pembrolizumab are used alone or in conjunction with cytotoxic agents in current treatments have varied degrees of response rates and side effects [45]. It is obvious that finding new molecular targets that are highly effective against endometrial cancer cells is necessary. We identified 25 potential tractable targets which can be used either as a biomarker or as new indication for molecules targeting these druggable genes for endometrial tumor with poor prognosis.

5. Conclusions

Advancements in understanding the molecular landscape of ECs have led to the development of potential biomarkers and drug targets. ECs with high expression of GCR are found to be aggressive with poor prognosis, and their progression is associated with various features like EMT, stemness, angiogenesis and pro-tumorigenic M2 macrophages. A better understanding of correlation of these phenotypes in EC for tumor progression can guide specific therapy targeting the GCR^H subgroup with poor survival in the future. The limitations of the computational method used to identify immune cell fractions infiltrating tissues are acknowledged, and the use of gene expression patterns with functional enrichment and druggability is proposed to overcome this limitation. Overall, our research analysis has presented the effectiveness of linking gene expression with their phenotype association in the identification of subgroup of EC with poor prognosis and aggressive nature.

AVAILABILITY OF DATA AND MATERIALS

All datasets analyzed for this study are mentioned in the article. They are identifiable by GSE accession numbers mentioned in the methodology part.

AUTHOR CONTRIBUTIONS

NNS, NAS, ZA, RE and SAE—conceptualization and study design. KKN, MajA, RHA, FAM, NAS, SAE—data curation. NNS—formal analysis; funding acquisition; project administration. AAE, AHG, MajA, BB, ZA, RE, NAS—methodology. BB, SAE, MahA, AHG—software; visualization. RE, NAS, ZA—supervision. BB, NAS, RHA, KKN, NNS, MajA, MahA, AHG—writing—original draft and review. NAS and BB—contributed equally and share joint senior authorship.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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