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Original Article

Expression of apoptosis-related proteins in the pathogenesis of endometrial clear cell carcinoma

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Abstract: Background: The balance of pro- and anti-apoptotic proteins plays a critical role in the regulation of cell death, and a disruption of this delicate balance may eventuate in carcinogenesis through a net reduction in apoptosis. Numerous chemotherapeutic strategies directly or indirectly target apoptotic pathways. However, a thorough assessment of apoptosis-related proteins has not previously been performed in endometrial clear cell carcinoma (CCC). This study aims to determine the significance of 9 apoptosis-related proteins in the pathogenesis of CCC as compared to non-neoplastic endometrium (NNE), low- and high-grade endometrial endometrioid carcinomas (LG-EEC, HG-EEC), and endometrial serous carcinoma (ESC). Materials and methods: Expression of 6 anti-apoptotic proteins (Bcl-2, Bcl-xL, $cFLIP_L$, MCL-1, survivin, NFκB/p65) and three pro-apoptotic proteins (Bax, caspase-3, caspase-8) was assessed by immunohistochemistry on 49 CCC, 37 LG-EEC, 12 HG-ECC, 16 ESC, and 25 NNE in a tissue microarray. Objective IHC scores were assigned by an automated image capture system. Scores were then correlated with clinicopathologic values and each other. Results: Most notably, CCC showed significantly reduced expression of $cFLIP_L$ relative to ESC, LG-EEC, HG-EEC, and NNE. CCC also showed significantly reduced expression of both Caspase 8 and NF-κB/p65 relative to ESC, HG-EEC, and NNE, but not LG-EEC. Bcl-2 and Bcl-xL showed reduced expression in CCC relative to all groups except ESC for Bcl-2 and NNE for Bcl-xL. There was no significant correlation between the proteins regarding expression levels. Within the CCC group, none of the proteins showed any significant association with patient age, myometrial invasion, final stage, lymphovascular invasion, disease-free or overall survival. Conclusion: Our analysis of the expression and correlation patterns of a panel of apoptosis-related proteins suggests that the downregulation of $cFLIP_L$ in CCC is significant relative to almost all other tissues, NNE, HG-EEC, and ESC. Other proteins, including Caspase 8, NF-κB/p65, Bcl-2 and Bcl-xL may also be significant. The regulation of apoptosis-related proteins in CCC may be important and may provide insight into chemoresistance in this enigmatic histotype. However, the paradoxical downregulation of both pro- and anti-apoptotic mediators suggests that additional study is needed to clarify the role of apoptotic mechanisms in CCC.

Keywords: Apoptosis, endometrial clear cell carcinoma, endometrial serous carcinoma, endometrial endometrioid carcinoma, Bcl-2, Bcl-xL, cFLIPL, Mcl-1, surviving, NF-κB/p65, Bax, caspase-3, caspase-8

Introduction

Apoptosis, or programmed cell death, is essential for maintaining cell homeostasis and preventing tumorigenesis via DNA damage [1]. Initiation of apoptosis can be triggered by either mitochondrial leakage of pro-apoptotic mediators or ligand-mediated cell receptor activation, also known as intrinsic or extrinsic pathways, respectively (**Figure 1**). The intrinsic pathway is activated by multiple different mechanisms, such as DNA damage/p53 activation (for example, from irradiation), growth factor absence, oxidative stress, and/or intracellular calcium overload [2].

Such insults activate the pro-apoptotic Bcl-2 members Bax, Bak, and Bid, leading to increased mitochondrial membrane permeability and release of cytochrome c [3]. In the cytosol, cytochrome c combines with apaf-1 and caspase-9 to form an apoptosome, which is capable of activating the effector caspases, -3 and -7. The extrinsic apoptotic pathway involves activation of "death receptors", which belong to the tumor necrosis factor (TNF) receptor super-

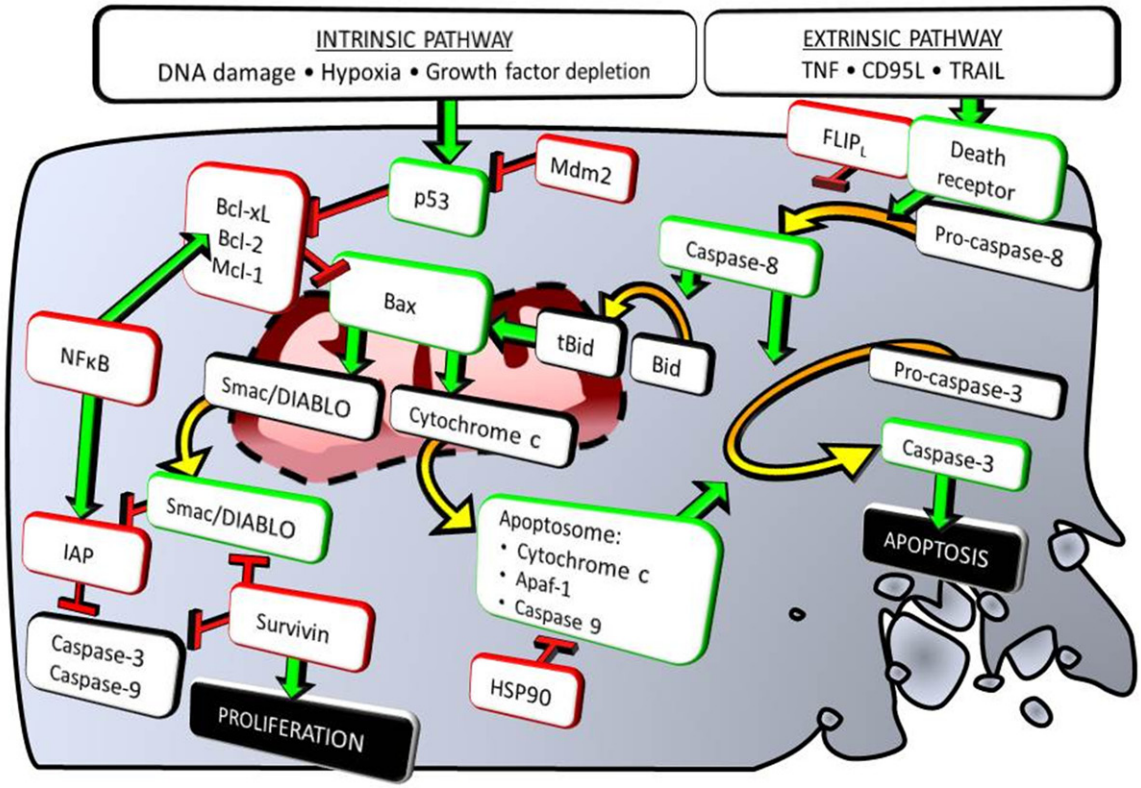

Figure 1. Apoptosis Pathways and mediators. Apoptosis is initiated by internal and external events, with overlap of downstream mediators. This illustration is simplified to emphasize mediators analyzed by immunohistochemistry. HSP90-heat shock protein-90. IAP-inhibitor of apoptosis protein. SMAC/DIABLO-second mitochondria-derived activator of caspase/direct IAP-binding protein with low pI.

family. Examples of death receptors include protein complexes such as TNF-R1, CD95, DR3, and TNF-related apoptosis-inducing ligand (TRAIL) receptors [4]. Death receptor ligands include TNF-β, TNF-α, CD95L, and TRAIL. Following death receptor activation, the extrinsic pathway leads to caspase-8 activation and subsequent effector caspase activation [4]. In both intrinsic and extrinsic pathways, effector caspases are ultimately responsible for the structural changes and nuclear fragmentation associated with apoptosis [5, 6] (**Figure 1**).

Defects in apoptosis are well-documented in tumorigenesis and described in all organ systems [4]. Many chemotherapeutics work by promoting apoptosis, with drugs available that support both extrinsic and intrinsic pathways. Drug-mediated cytotoxic effects indirectly lead to cell death, relying on intact apoptotic pathways to complete their desired effects [7, 8]. Drug-resistance may result from aberrant apoptotic pathways, such as death receptor down-regulation [9], over- or under-expression of Bcl-2-related proteins [10], or caspase dysfunction. To ensure maximum chemotherapy efficacy, it is important to understand whether and how a type of cancer avoids apoptosis. Limited data exist regarding apoptosis and endometrial carcinomas, with higher-risk carcinomas typically treated with carboplatin and paclitaxel, therapies largely unrelated to apoptosis [11]. The current study is an exploratory analysis of the role of apoptosis-related proteins in the pathogenesis of uterine carcinomas, with emphasis on the high-risk tumor endometrial clear cell carcinoma (CCC).

Materials and methods

Patient selection

Forty-nine cases of CCC, 49 cases of endometrial endometrioid carcinoma (EEC), 16 cases of endometrial serous carcinoma (ESC), and 25 samples of non-neoplastic endometrium (NNE)

Table 1. Antibody specifications

Antibody	Clone	Company	Catalog #	AR/time	Dilution	Control Tissue/Cellular localization
Bcl-2	Mouse mAb bcl-2/100/D5	Leica	PA0117	ER2-20	RTU	Tonsil/cytoplasmic
Bcl-xL	Mouse mAb H-5	Santa Cruz	sc-8392	ER2-20	1:300	Colon/cytoplasmic
Mcl-1	Rabbit pAb	Santa Cruz	sc-819	ER2-20	1:500	Colon/cytoplasmic
$cFLIP_L$	Rabbit pAb	Santa Cruz	sc-8346	ER2-20	1:200	Colon/nuclear
Survivin	Rabbit pAb	abcam	ab24479	ER2-20	1:400	Tonsil/cytoplasmic and nuclear
NFκB/p65	Rabbit pAb	Cell Signaling	8242	ER2-20	1:1500	Breast/cytoplasmic and nuclear
Bax	Mouse mAb 2D2	Santa Cruz	sc-20067	ER2-20	1:250	Breast/cytoplasmic
Caspase-8	Mouse mAb 8CSP03	Santa Cruz	sc-56070	ER2-20	1:300	Colon/cytoplasmic
Caspase-3	Mouse mAb H-277	Santa Cruz	sc-7148	ER2-20	1:300	Colon/cytoplasmic

ER2: Bond Epitope Retrieval Solution 2. ER1: Bond Epitope Retrieval Solution 1. RTU: Ready to Use Antibody. mAb: monoclonal antibody. pAb: polyclonal antibody. AR: antigen retrieval (in minutes).

were studied. The larger CCC cohort from which these 49 cases were derived has previously been described in detail, and the cases therein were assembled from multiple institutions after multiple-layered review [12, 13].

EEC and NNE cohorts were composed of routine cases from one institution. The EEC group was stratified by FIGO grade, with FIGO grades 1-2 classified as low-grade and FIGO grade 3 tumors classified as high-grade. Of the EEC group, 37 low-grade (LG-EEC) and 12 high-grade (HG-EEC) tumors were evaluated. NNE included 10 proliferative phase, 10 secretory phase, and 5 atrophic endometria. The following clinicopathologic features were documented in the CCC group: age of patient, myometrial invasion, final stage, lymphovascular invasion, morphologic features, disease-free and overall survival.

Patient demographics, clinical data, and follow-up for the CCC group were collected from the electronic medical record.

Immunohistochemistry (IHC)

A tissue microarray (TMA) was constructed from the aforementioned 123 cases using conventional methods [14] on a manual arrayer (Beechar Instruments Inc., Sun Prairie, WI). Each core measured 1 mm in diameter and was arrayed in duplicate blocks. 5 μ-thick unstained sections were subsequently obtained for immunohistochemical assays. All steps apart from dehydration, clearing and coverslipping were performed on a Leica Bond Max autostainer. Following deparaffinization, heat-induced antigen retrieval was performed on the Leica autostainer using their Epitope Retrieval 2 solution. The sections were then incubated with the various primary antibodies, which included antibodies directed at 6 anti-apoptotic (Bcl-2, Bcl-xL, $cFLIP_L$, Mcl-1, survivin, NF-κB/p65) and three pro-apoptotic proteins (Bax, caspase-3, caspase-8). Antibody specifications are outlined in **Table 1**. The Bond Refine Polymer detection system was used for visualization.

Automated scoring of staining

Immunostained tissue microarray slides were imaged on an Ariol SL-50 automated slide scanner (Leica Biosystems). Tissue cores were imaged at 20× magnification to a resolution of 0.323 μm/pixel. Cells were identified utilizing standard Ariol analysis scripts (Leica). All cores were manually evaluated to determine the subcellular localization of the studied proteins. We devised an objective method for analyzing staining patterns. Upper and lower thresholds for color, saturation, intensity, size, roundness, and axis length were set for both blue hematoxylin staining of nuclei and for brown DAB reaction products. Thus, brown (DAB) positive cells could be distinguished from blue (hematoxylin only) negative cells. After manual preselection of lesional (exclusive of stroma) areas, the area of positive staining per core was calculated as a percent of the total analyzed area divided by area of brown (DAB-positive) pixels.

Statistical analysis

Scores generated by the automated image capture system were correlated with clinicopathologic values and each other using SAS

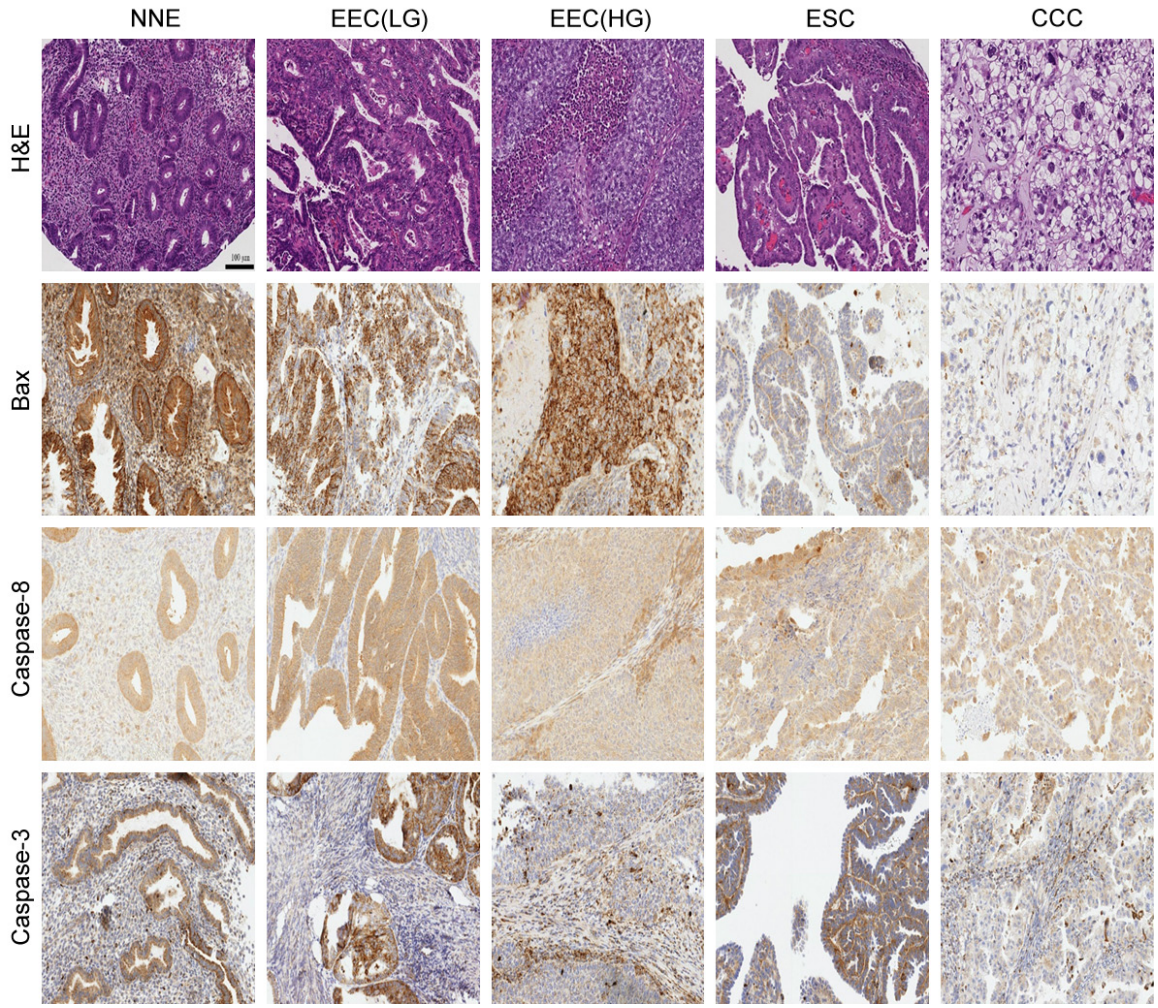

Figure 2. Representative H&E and immunohistochemical stains illustrating protein expression of pro-apoptotic proteins. NNE: non-neoplastic endometrium. EEC: endometrioid endometrial carcinoma (LG-low-grade; HG-high-grade). ESC: endometrial serous carcinoma. CCC: endometrial clear cell carcinoma (CCC). Original magnification 100×.

software, Version 9.4 (2014) of the SAS System for Windows 7 (SAS Institute Inc., Cary, NC, USA). Average automated scores were compared between each of the study subgroups, using the Student t test. Kendall's tau B was used to assess any correlation relationships between the proteins. Within the CCC group, Kaplan-Meier survival curves were generated for overall survival and progression-free survival, and comparisons between survival curves were performed using log-rank tests. Cox regression analyses were used to assess relationships between clinicopathologic factors using multivariate and univariate models.

This study was approved by the Human Research Protections Program at the University of California San Diego (Project #191204CX).

Results

Subcellular localization of the anti-apoptotic proteins were cytoplasmic for Bcl-2, Mcl-1, Bcl-xL, Bcl-2, Bcl-xL, nuclear for $FLIP_L$ and Mcl-1, and nuclear/cytoplasmic for survivin and NF-κB/p65 (See **Figures 2** and **3**, and **Table 2**). Most notable among these was the expression pattern of $cFLIP_L$ in CCC. CCC showed decreased expression of $cFLIP_L$ when compared to every other group that was assessed, including NNE, ESC, HG-EEC and LG-EEC. CCC also showed statistically significant reduced expression of NF-κB/p65 relative to ESC, HG-EEC, NNE, but not LG-EEC.

Bcl-2 and Bcl-xL showed less uniformity of differential expression in CCC relative to the

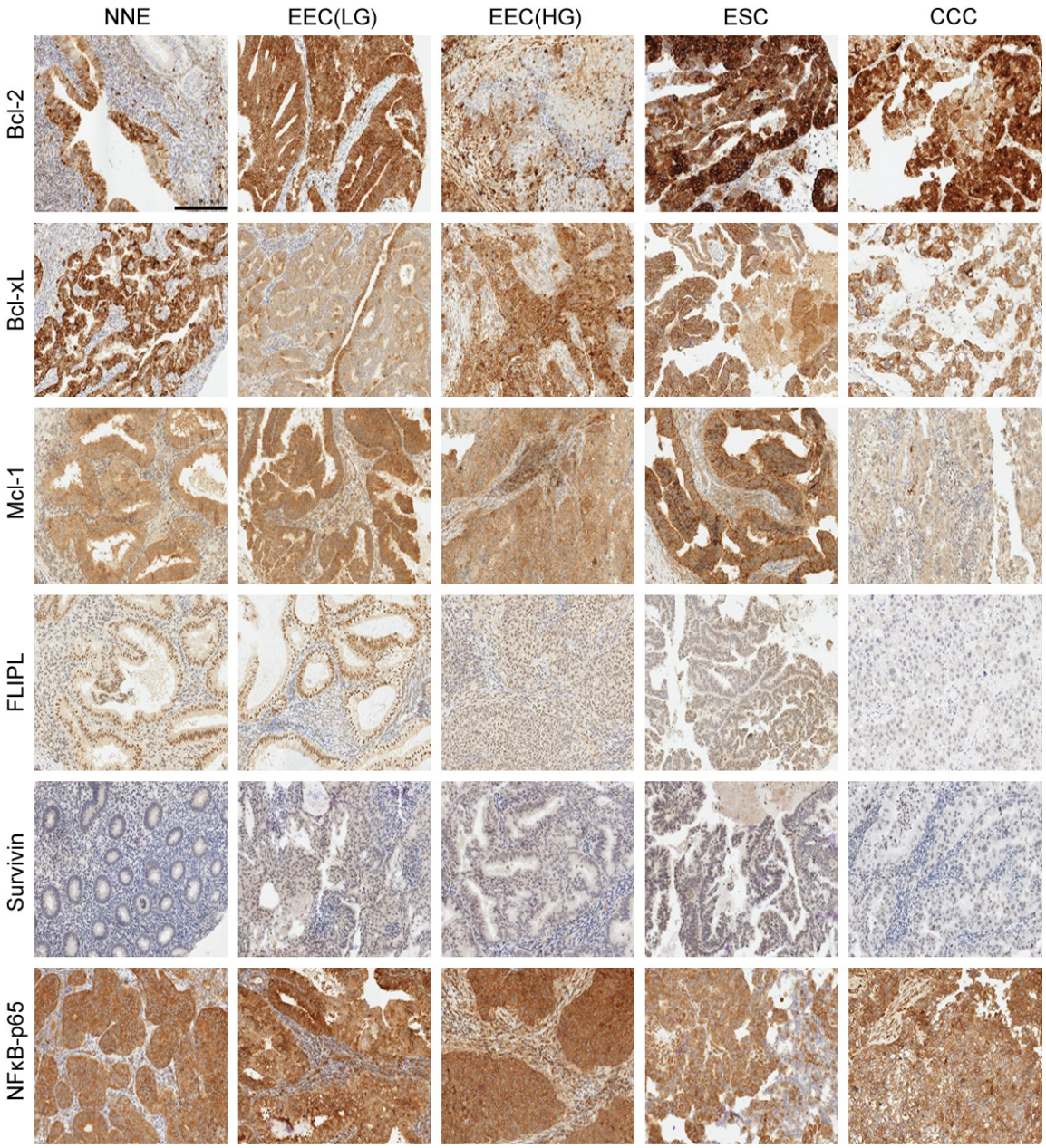

Figure 3. Representative immunohistochemical stains illustrating protein expression of anti-apoptotic markers. NNE: non-neoplastic endometrium. EEC: endometrioid endometrial carcinoma (LG-low-grade; HG-high-grade). ESC: endometrial serous carcinoma. CCC: endometrial clear cell carcinoma (CCC). Original magnification 200×.

other tissues (**Table 3**). Nonetheless, Bcl-2 and Bcl-xL showed reduced expression in CCC in relative to all groups except ESC for Bcl-2 and NNE for Bcl-xL. Mcl-1 and survivin expression showed no significant differences between the groups.

Subcellular localization of the pro-apoptotic proteins were cytoplasmic for Bax, Caspase-8, and caspase-3. CCC showed significantly decreased expression of Bax when compared to EEC ($P \leq 0.001$, all grades) but the differences between CCC and ESC, and between CCC and NNE were not significant. CCC expressed lower caspase-8 compared to NNE ($P=0.014$), ESC ($P=0.006$) and HG-EEC ($P=0.018$), but not LG-EEC ($p=0.056$). There was higher caspase 3 expression in CCC compared to NNE, but no significant differences between CCC and LG-EEC, HG-EEC and ESC were observed.

Table 2. Automated immunohistochemical scores

	Bcl-2	Bcl-xL	Mcl-1	$cFLIP_L$	Survivin	NFκB/p65	BAX	Caspase-8	Caspase-3
CCC									
n	49	47	49	49	49	49	49	49	48
Average	36.73459	35.33402	56.24396	21.87232	10.09864	62.46239	12.10315	14.22009	35.12183
SD	21.29707	23.24706	14.28244	24.28681	13.03695	22.58086	10.56438	16.26114	15.40287
NNE									
n	22	23	22	22	23	22	22	24	23
Average	66.66421	30.18894	52.12539	49.46612	6.786016	75.83879	7.681381	24.30429	18.64995
SD	17.10664	14.8687	15.81293	13.93054	8.958904	11.70076	9.476042	15.84833	9.948607
ESC									
n	12	14	11	12	16	12	12	13	16
Average	50.45641	60.86656	59.7402	42.14673	7.682844	81.10734	27.01943	34.09578	28.32675
SD	30.76985	18.85936	25.67506	22.37375	10.55558	14.74707	27.51298	20.78985	14.25785
HG-EEC									
n	12	12	12	12	12	12	12	12	12
Average	66.70706	47.69115	64.39719	49.73483	19.76015	74.48292	21.61412	31.33046	39.99545
SD	16.47189	16.38755	17.7308	17.80337	20.60562	13.76978	13.51178	20.8226	15.62642
LG-EEC									
n	35	36	35	35	37	36	35	37	37
Average	65.5864	49.34738	56.61315	41.66306	16.54912	67.09638	21.276	22.19019	36.39251
SD	22.02965	10.99665	15.85616	19.16667	19.17903	16.56681	15.39346	20.71782	16.33382

LG-EEC: FIGO Grade 1 and 2 endometrioid carcinoma; HG-EEC: FIGO Grade 3 endometrioid carcinoma; ESC: endometrial serous carcinoma; CCC: clear cell carcinoma; NNE: non-neoplastic endometrium; SD: Standard deviation; n: number of cases with evaluable/scoreable cores.

Correlation analysis failed to highlight significant relationships between markers. Within the CCC group, no markers showed any significant association with patient age, myometrial invasion, final stage, lymphovascular invasion, disease-free or overall survival.

Discussion

In this study, we evaluated the expression of several apoptosis-related proteins by immunohistochemistry in a cohort of endometrial cancers, with an emphasis on CCC, a rare and relatively aggressive form of endometrial cancer. The findings, in their totality suggest the involvement of apoptotic pathways in the pathogenesis of CCC. The interplay between the pro-apoptotic and anti-apoptotic members of the Bcl-2 family of proteins is essential in the induction of apoptosis in the intrinsic pathway of apoptosis. Pro-apoptotic mediators in the Bcl-2 family, including Bax, Bak, Bid, and Bok, facilitate the release of cytochrome c from the mitochondria into the cytosol to ultimately lead to apoptosis [15, 16]. Among the pro-apoptotic proteins of the Bcl-2 family, our panel only included Bax. We found CCC to show significantly reduced Bax expression compared to both low grade and high grade endometrioid carcinoma, but there were no significant differences between CCC and ESC regarding Bax expression. These findings are in contrast to those reported by Kokawa et al, who used a small cohort of 5 ESC and 3 CCC and found higher levels of Bax expression in ESC and CCC as compared with EEC [17]. This may be related to methodological differences between the two studies. Our cohort of CCC is the largest that has been evaluated thus far, and we utilized an objective immunohistochemical scoring method to minimize variability in scoring. Some authors have noted a progressive decrease in Bax expression in EEC relative to its precursors, whereas others have reported the opposite [18-20]; therefore, additional investigations are needed on this. Nonetheless, our findings are in keeping with prior experimental observations that loss of Bax protein expression is associated with resistance to apoptosis mediated by histone deacetylase inhibitors [21]. Among the anti-apoptotic markers in the Bcl-2 family, CCC showed reduced expression of Bcl-xL relative to all other subgroups except NNE.

Table 3. *p* values, comparing automated immunohistochemical scores for each subgroup

Bcl-2	CCC	NNE	ESC	HG-EEC	LG-EEC
CCC	1.0000	0.0000	0.1665	0.0000	0.0000
NNE	0.0000	1.0000	0.1124	0.9944	0.8676
ESC	0.1665	0.1124	1.0000	0.1254	0.1230
HG-EEC	0.0000	0.9944	0.1254	1.0000	0.8835
LG-EEC	0.0000	0.8676	0.1230	0.8835	1.0000
Bcl-xL	CCC	NNE	ESC	HG-EEC	LG-EEC
CCC	1.0000	0.2670	0.0003	0.0444	0.0005
NNE	0.2670	1.0000	0.0000	0.0056	0.0000
ESC	0.0003	0.0000	1.0000	0.0687	0.0399
HG-EEC	0.0444	0.0056	0.0687	1.0000	0.8095
LG-EEC	0.0005	0.0000	0.0399	0.8095	1.0000
Mcl-1	CCC	NNE	ESC	HG-EEC	LG-EEC
CCC	1.0000	0.3027	0.6705	0.1601	0.9130
NNE	0.3027	1.0000	0.3825	0.0586	0.1255
ESC	0.6705	0.3825	1.0000	0.6220	0.8905
HG-EEC	0.1601	0.0586	0.6220	1.0000	0.3206
LG-EEC	0.9130	0.1255	0.8905	0.3206	1.0000
$cFLIP_L$	CCC	NNE	ESC	HG-EEC	LG-EEC
CCC	1.0000	0.0000	0.0128	0.0002	0.0001
NNE	0.0000	1.0000	0.3187	0.9644	0.1629
ESC	0.0128	0.3187	1.0000	0.3684	0.8254
HG-EEC	0.0002	0.9644	0.3684	1.0000	0.3168
LG-EEC	0.0001	0.1629	0.8254	0.3168	1.0000
Survivin	CCC	NNE	ESC	HG-EEC	LG-EEC
CCC	1.0000	0.2140	0.4601	0.1447	0.0833
NNE	0.2140	1.0000	0.7835	0.0574	0.0023
ESC	0.4601	0.7835	1.0000	0.0828	0.0149
HG-EEC	0.1447	0.0574	0.0828	1.0000	0.7165
LG-EEC	0.0833	0.0023	0.0149	0.7165	1.0000
NFκB	CCC	NNE	ESC	HG-EEC	LG-EEC
CCC	1.0000	0.0016	0.0018	0.0263	0.2783
NNE	0.0016	1.0000	0.2992	0.7757	0.0480
ESC	0.0018	0.2992	1.0000	0.2677	0.0218
HG-EEC	0.0263	0.7757	0.2677	1.0000	0.2435
LG-EEC	0.2783	0.0480	0.0218	0.2435	1.0000
Bax	CCC	NNE	ESC	HG-EEC	LG-EEC
CCC	1.0000	0.0864	0.0902	0.0387	0.0035
NNE	0.0864	1.0000	0.0354	0.0056	0.0000
ESC	0.0902	0.0354	1.0000	0.5499	0.5042
HG-EEC	0.0387	0.0056	0.5499	1.0000	0.9556
LG-EEC	0.0035	0.0000	0.5042	0.9556	1.0000
Caspase- 8	CCC	NNE	ESC	HG-EEC	LG-EEC
CCC	1.0000	0.0142	0.0055	0.0183	0.0560
NNE	0.0142	1.0000	0.1544	0.3173	0.9776
ESC	0.0055	0.1544	1.0000	0.7429	0.1530
HG-EEC	0.0183	0.3173	0.7429	1.0000	0.3183

Caspase-8 is another pro-apoptotic mediator whose expression pattern in CCC was found to be noteworthy. CCC expressed lower caspase-8 levels compared to NNE (P=0.014), ESC (P=0.006) and HG-EEC (P=0.018). CCC also showed apparently decreased expression when compared to LG-EEC but the difference was not significant (P=0.056). Caspase-8 is a cysteine protease that is thought to be a key factor in apoptosis, and its inactivation is a necessity for death receptor-associated programed cell death [22-24]. Active caspase-8 can cleave several proteins that execute the apoptotic pathway as well as activate other apoptosis-related mediators [22-24]. Caspase-8 may be epigenetically (or genetically) silenced in some tumors and upregulated in others, possibly depending on tumor type, location and/or tumor microenvironment [22, 25]. Similar to other apoptosis-related proteins such as CD95/Fas and TNFα RI, caspase-8 is also involved in non-apoptosis related pathways, and its basic apoptosis-related functions may be modified into unrelated cellular activities that ultimately promote tumorigenesis and/or chemoresistance [22-24]. Therefore, understanding the precise factors that result in some tumors not expressing caspase 8 or expressing caspase-8 at significant lesser levels, would be of clinical utility. In endometrial cancer, the expression of caspase-8 has mostly been studied in cell lines [26, 27]. We demonstrate in this study that the reduced expression of Caspase-8 in CCC is not simply a function of it being a high grade cancer, since it was found to be expressed at lower levels in CCC relative to other high grade cancers (ESC and HG-EEC). In our CCC cohort, the expression of caspase-8 was not significantly associated with clinicopathologic factors, including disease-free or overall survival. Reduced caspase-8 expression has previously been found to be a poor prognostic factor in a few cancers, including ovarian cancer [22, 28].

Among the anti-apoptotic mediators, the differential patterns of expression

LG-EEC	0.0560	0.9776	0.1530	0.3183	1.0000
Caspase-3	CCC	NNE	ESC	HG-EEC	LG-EEC
CCC	1.0000	0.0000	0.1171	0.3463	0.7165
NNE	0.0000	1.0000	0.0272	0.0006	0.0000
ESC	0.1171	0.0272	1.0000	0.0543	0.0437
HG-EEC	0.3463	0.0006	0.0543	1.0000	0.5979
LG-EEC	0.7165	0.0000	0.0437	0.5979	1.0000

LG-EEC: FIGO Grade 1 and 2 endometrioid carcinoma; HG-EEC: FIGO Grade 3 endometrioid carcinoma; ESC: endometrial serous carcinoma; CCC: clear cell carcinoma; NNE: non-neoplastic endometrium; SD: Standard deviation.

of $cFLIP_L$, NFκB/p65, and the aforementioned Bcl-xL were found to be most significant in CCC. The cFLIP (cellular FLICE-like) inhibitory protein family of proteins are major modulators of caspase-8 activation. The three isoforms of cFLIP in human beings are $c\text{-}FLIP_L$ (long), c-FLIPS (short), and c-FLIPR (splice) [29-33]. The expression of $c\text{-}FLIP_L$ may have a dual role (anti-apoptotic or proapoptotic), depending on unclear factors, possibly the specific tissue context and/or the levels of protein expression [29-32]. cFLIP is upregulated in many cancers [30]. In our cohort of CCC, however, $c\text{-}FLIP_L$ expression was significantly reduced relative to all other tissues that were evaluated, including ESC, HG-EEC, LG-EEC and NNE and was not found to be a significant prognostic factor. Caspase-8 can activate the NF-κB expression, and cFLIP and NF-κB may each be associated with the cellular accumulation of the other [34-37]. Similar to caspase 8, there was significantly reduced expression of NF-κB/p65 in CCC relative to ESC, HG-EEC, and NNE, but not LG-EEC. However, there was no direct correlation between the expression levels of caspase 8 and NF-κB.

Overall, these findings highlight the complexity of apoptotic pathways, especially in the context of cancer. The seemingly paradoxical downregulation of both pro apoptotic (caspase-8) and anti-apoptotic ($cFLIP_L$ and NFκB/p65) mediators may reflect the aforementioned complexity of apoptotic pathways in cancers, the fact that some apoptotic mediators show pro-apoptotic or anti-apoptotic function depending on the context, the fact that CCC is a histotype biologically distinct from other histotypes of endometrial cancer, or a combination of the above.

Our findings are limited by the relatively small size of our CCC cohort. However, the current study is the largest reported to date of apoptosis-related proteins in CCC. Furthermore, the present analysis did not include phosphorylated forms of the proteins that were assessed. The expression patterns of the phosphorylated forms of the studied proteins may have provided additional insights into the roles of these proteins in apoptotic pathways in CCC. Nonetheless, our findings do indicate that the expression patterns of some apoptosis related proteins in CCC are different from those of most other histotypes of endometrial cancer. Given the emerging use of targeted therapies related to apoptosis pathways in cancers, evaluation of larger cohorts of CCC are needed, to help clarify the role of these pathways in the pathogenesis and treatment of this enigmatic cancer.

Disclosure of conflict of interest

None.

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