Pathology & Oncology Research (2020) 26:2265–2272
https://doi.org/10.1007/s12253-020-00817-z

ORIGINAL ARTICLE

Prognostic Role of Krüppel-Like Factors 5, 9, and 11 in Endometrial Endometrioid Cancer

Luigi Viola[1] · Ambrogio P Londero[2,3] · Serena Bertozzi[3,4] · Maria Orsaria[5] · Stefania Marzinotto[5] · Fulvio Antoniazzi[5] · Valentina Renda[5] · Jacqueline Cinel[6] · Arrigo Fruscalzo[7,8] · Ralph J Lellé[8] · Laura Mariuzzi[5]

Received: 15 November 2019 / Accepted: 28 April 2020 / Published online: 25 May 2020

Abstract

Background and Objective Krüppel-like factors (KLFs) are transcription factors with the ability to mediate cross-talk with signaling pathways involved in cell proliferation control, apoptosis, migration, and differentiation. They also appear to influence steroid hormone signaling through transcriptional networks involving steroid hormone receptors and members of the nuclear receptor family of transcription factors. Our study aims to evaluate the potential prognostic role of KLF5, KLF9, and KLF11 in endometrial cancer, and their correlation with hormonal receptor status and cellular proliferation.

Materials and Methods Retrospective observational study on cases of endometrioid endometrial adenocarcinoma collected in the period January 2000–December 2011 at the University of Udine. Formalin-fixed, paraffin-embedded tissue samples were all submitted to tissue microarray immunohistochemical study. A survival analysis was performed.

Results One hundred forty seven patients were included in the study with a mean age at surgery of 65.6 years (±10.2). 80.3% of endometrial malignancies were classified as stage FIGO I (118/147). Radiation therapy and chemotherapy were administered in 62.3% (91/146) and 6.2% (9/145) of patients respectively. Five-year overall survival and disease-free survival resulted 85.4% (95% CI, 79.8–91.4%) and 79.4% (95% CI, 73.0–86.4%) respectively. A high Ki-67, cytoplasmatic KLF5 (HR 4.72, CI.95 1.61–13.89, $p < 0.05$), and nuclear KLF11 (HR 3.04, CI.95 0.99–9.36, $p = 0.053$) scores correlated with a shorter overall survival. In addition, a high nuclear KLF11 (HR 2.59, CI.95 1.13–5.95, $p < 0.05$) score correlated with a shorter disease-free survival.

Luigi Viola and Ambrogio P Londero are equally contributed.
Where the study was carried out: DAME, University of Udine.
Financial support: University of Udine and Ennergi Research.
Presented at meeting: none.

Condensation
In patients affected by endometrioid endometrial carcinoma, higher expression levels of KLF5 and KLF11 correlated with a poorer prognosis.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12253-020-00817-z) contains supplementary material, which is available to authorized users.

✉ Ambrogio P Londero
ambrogio.londero@gmail.com

[1] Radiology Department, University of Campania "Luigi Vanvitelli", Naples 80138, Italy

[2] Clinic of Obstetrics and Gynecology, DAME, Academic Hospital of Udine, University of Udine, Piazzale Santa Maria della Misericordia, 15, 33100 Udine, Italy

[3] Ennergi Research (Non-Profit Organization), Lestizza 33050, Italy

[4] Breast Unit, DAME, Academic Hospital of Udine, University of Udine, 33100 Udine, Italy

[5] Institute of Pathology, DAME, Academic Hospital of Udine, University of Udine, Udine 33100, Italy

[6] Clinic of Surgery, Academic Hospital of Udine, University of Udine, 33100 Udine, Italy

[7] Clinic of Obstetrics and Gynecology, Christophorus-Kliniken, 48653 Coesfeld, Germany

[8] Clinic of Obstetrics and Gynecology, University Hospital of Münster, Albert-Schweitzer-Campus 1, Gebäude: A1, 48149 Münster, Germany

Conclusions In patients affected by endometrioid endometrial carcinoma, higher staining levels of KLF5 and KLF11 correlated with a poorer prognosis. However, further studies are required in order to better clarify the role of KLFs in the natural history of endometrial cancer.

Keywords Krüppel-like factors · KLF · KLF5 · KLF11 · KLF9 · Endometrioid endometrial cancer · Endometrial cancer

Abbreviations

CI	Confidence intervall
ESR	Estrogen receptor
FIGO	Fédération Internationale de Gynécologie Obstétrique (International Federation of Gynecology and Obstetrics)
H&E	Hematoxylin-eosin
HR	Hazard ratio
IQR	Interquartile range
KLFs	Krüppel-like factors
KLF5	Krüppel-like factor 5
KLF9	Krüppel-like factor 9
KLF11	Krüppel-like factor 11
m-RNA	Messenger RNA
PGR	Progesterone receptor
REA	Repressor of estrogen receptor activity
Real-Time PCR	Real-time polymerase chain reaction
RNA	Ribonucleic acid
TMA	Tissue micro array.

Introduction

Among the industrialized countries, endometrial carcinoma is the most common malignant tumor of the female reproductive organs and comprises 4% of all female cancers worldwide [1]. Type I estrogen-dependent tumors (endometrioid endometrial carcinomas) account for the majority of cases (80–85%) and type II non-estrogen-dependent (non-endometrioid endometrial carcinomas) for the remaining cases [2].

The Krüppel-like factor (KLF) family proteins belong to the Specificity-protein (Sp) family of transcription factors that currently includes 17 known members [3]. KLFs have been shown to be proteins relevant to human cancers with an important role in cell proliferation, apoptosis, migration, and differentiation [3]. The potential prognostic role for cancer outcome is demonstrated by many KLFs proteins that act as tumor oncogenes and/or suppressors under different cellular settings [3]. Moreover, KLFs proteins control cell proliferation by mediating the transcription of pro-proliferative genes and of anti-proliferative genes [4–7].

In addition, recent studies suggest that a number of KLFs may influence steroid hormone signaling through transcriptional networks involving steroid hormone receptors and members of the nuclear receptor family of transcription factors. In particular, KLF9, which results to be more expressed in the normal endometrium and well-differentiated endometrial tumors [8], interacts with both progesterone receptor (PGR), by regulating PGR-dependent gene transcription in uterine endometrial cells, and estrogen receptor (ESR). Less information on interactions with steroid hormone receptors is available regarding KLF5 and KLF11, although previous studies suggest their role in endometrial cancer behavior [9–11].

Our study aims to evaluate KLF5, KLF9, and KLF11 as possible prognostic factors in endometrioid endometrial carcinoma, and to evaluate possible correlations of KLF5, KLF9, and KLF11 with hormonal receptor status and cellular proliferation.

Materials and Methods

Study Design and Sample Selection

This is an observational study in which a retrospective review of the pathological archive and medical records was performed in order to identify cases of endometrioid endometrial adenocarcinoma. Tissue samples (from standard archived, formalin-fixed, paraffin-embedded tissues) and clinical data have been collected retrospectively at the Institute of Pathology and the Surgery Department of the Academic Hospital of Udine (Italy). In this study, only endometrioid endometrial adenocarcinoma were included, which were operated on between January 2000 and December 2011. All other endometrial carcinomas with histologic types other than endometrioid endometrial cancer were excluded from this study. This TMA immunohistochemical study considered the entire cohort of endometrioid endometrial adenocarcinoma cases with tissue stored in paraffin blocks at the time of the first-line surgical treatment. The present study was approved by the internal review board, was conducted in accordance with Helsinki Declaration and followed the dictates of the

general authorization for the processing of personal data for scientific research purposes issued by the Italian Data Protection Authority.

Table 1 Characteristics of the population, characteristics of the tumor and therapy. The reported values refer to mean (± standard deviation), median (IQR), or absolute values and percentage

Age at diagnosis (years)	65.6 (±10.2)
Parity	2 (1–2)
Obesity (BMI >30 kg/m^2)	50.8% (60/118)
Diabetes mellitus	21.9% (25/114)
Hypertension	65.1% (84/129)
Late menopause (after 55 years of age)	16.9% (22/130)
HRT	6.8% (9/133)
Tamoxifen	62.5% (5/8)
Familial history for carcinoma of the endometrium	4.5% (5/112)
Colon or breast associated neoplasms	
Not detected	21.8% (32/147)
No associated neoplasms	67.3% (99/147)
Colon neoplasia	8.2% (12/147)
Breast cancer	2.7% (4/147)
Blood levels of the CA125	
Preoperative	12 (9–23)
Postoperative	12 (8–18)
During follow up	8 (6–12)
At recurrence diagnosis	18 (13–35)
Characteristics of the tumor	
TNM/FIGO stadium	
I	80.3% (118/147)
II	8.2% (12/147)
III	9.5% (14/147)
IV	2% (3/147)
Tumor grading	
G1	39.6% (57/144)
G2	36.8% (53/144)
G3	23.6% (34/144)
Myometrial invasion >50%	44.6% (62/139)
Vascular invasion	57.1% (20/35)
Lymphatic invasion	23.9% (11/46)
Therapy	
Radiotherapy	62.3% (91/146)
External radiotherapy	59.3% (54/91)
Brachi-radiotherapy	1.1% (1/91)
Combined external radiotherapy and brcahitherapy	39.6% (36/91)
Adjuvant chemotherapy	6.2% (9/145)

Acronyms: *BMI* body mass index, *HRT* hormonal replacement therapy, *TNM* tumor, node, metastasis, *FIGO* Fédération Internationale de Gynécologie Obstétrique (International Federation of Gynecology and Obstetrics)

Sample Analysis

The expressions of KLF5, KLF9, KLF11, ESR, PGR, and Ki-67 were studied with immunohistochemistry among all cases including endometrioid endometrial adenocarcinoma. The cases were evaluated in terms of both staining percentage and intensity.

Tissue Micro Array (TMA) Preparation and Analysis

TMA preparation and analysis were performed as previously described [12]. Once the blocks containing the tumor tissue fixed in formalin and embedded in paraffin were selected, the stained sections of hematoxylin-eosin (H&E) were analyzed, and then the tissue core sampling for the TMA was performed taking care to include the tumor tissue (two core biopsies per primary tumor). Then, the receiver block was assembled. Subsequently, from the receiving block, a 4-μm cross section was obtained, which was stained in H&E. Later, further 4-μm cross sections were obtained to prepare slides for immunohistochemical staining and subsequent analysis. Immunohistochemical staining was performed according to standard protocol and manufacturer's instructions. For antigen retrieval and deparaffinization, slides were heated for 20 min at 98 °C in Target Retrieval Solution (low pH; Dako K8005, Glostrup, DK) with PT-link (Dako). The slides were then incubated at room temperature in H2O2 for 10 min to block endogenous peroxidase activity. The sections were rinsed in PBS and then incubated in a wet chamber at room temperature for 1 h with the following primary antibodies: KLF5 (Genetex International, diluted 1:800); KLF9 (Abcam Ltd., diluted 1:200); KLF11 (Abnova, diluted 1:100). A Dako REAL™ EnVision™ Dako Rabbit/Mouse (Dako, K5007, Glostrup, DK) was used as a second antibody. HRP activity was detected using Dako REAL™ DAB+Chromogen (Dako, K5007, Glostrup, DK) as substrate for 3 min in accordance with the

Table 2 Protein immunohistochemistry assessment. The reported values refer to median and IQR of H-scores

Ki-67 (%)	20 (5–40)
Estrogen receptor (H-score)	210 (70–270)
Progesterone receptor (H-score)	180 (90–270)
KLF5 (nuclear expression - H-score)	0 (0–0)
KLF5 (cytoplasmic expression H-score)	20 (0–100)
KLF9 (nuclear expression - H-score)	0 (0–0)
KLF9 (cytoplasmic expression H-score)	0 (0–100)
KLF11 (nuclear expression - H-score)	80 (61–93)
KLF11 (cytoplasmic expression H-score)	100 (80–100)

manufacturer's instructions. The sections were counterstained with hematoxylin. Sections incubated with non-immune rabbit serum instead of the primary antibody were used as negative controls.

In addition, immunohistochemical staining for ki-67, ESR, and PGR was performed automatically with Ventanas Benchmark® XT (Ventana Medical Systems, Tucson, AZ).

Immunohistochemical staining was evaluated in terms of H-score (the product of the actual percentage of positive-stained cells and intensity score - evaluated as strong 3, moderate 2 and weak 1 – giving a possible range of 0–300) except for Ki-67 which was evaluated as a percentage of positive nuclei. Scoring of the immunohistochemical staining was performed independently by two pathologists (M.O. and L.M.).

Statistical Analysis

Data was analyzed using R (version 3.5.1) and considering $p < 0.05$ significant. The normality of the variables was tested with the Kolmogorov-Smirnov test. Non-parametric data was presented with median and interquartile range (IQR), while the parametric data has been described by the mean and standard deviation. For bivariate analysis, the following statistical tests were used: Wilcoxon test, t-test, Spearman test, Kendall test, or Pearson test for continuous variables and Chi-square test or Fisher exact test for categorical variables. In addition, a Kaplan-Meier analysis was performed and the differences between groups were assessed by log-rank test. Univariate and multivariate Cox regression analyses were also performed.

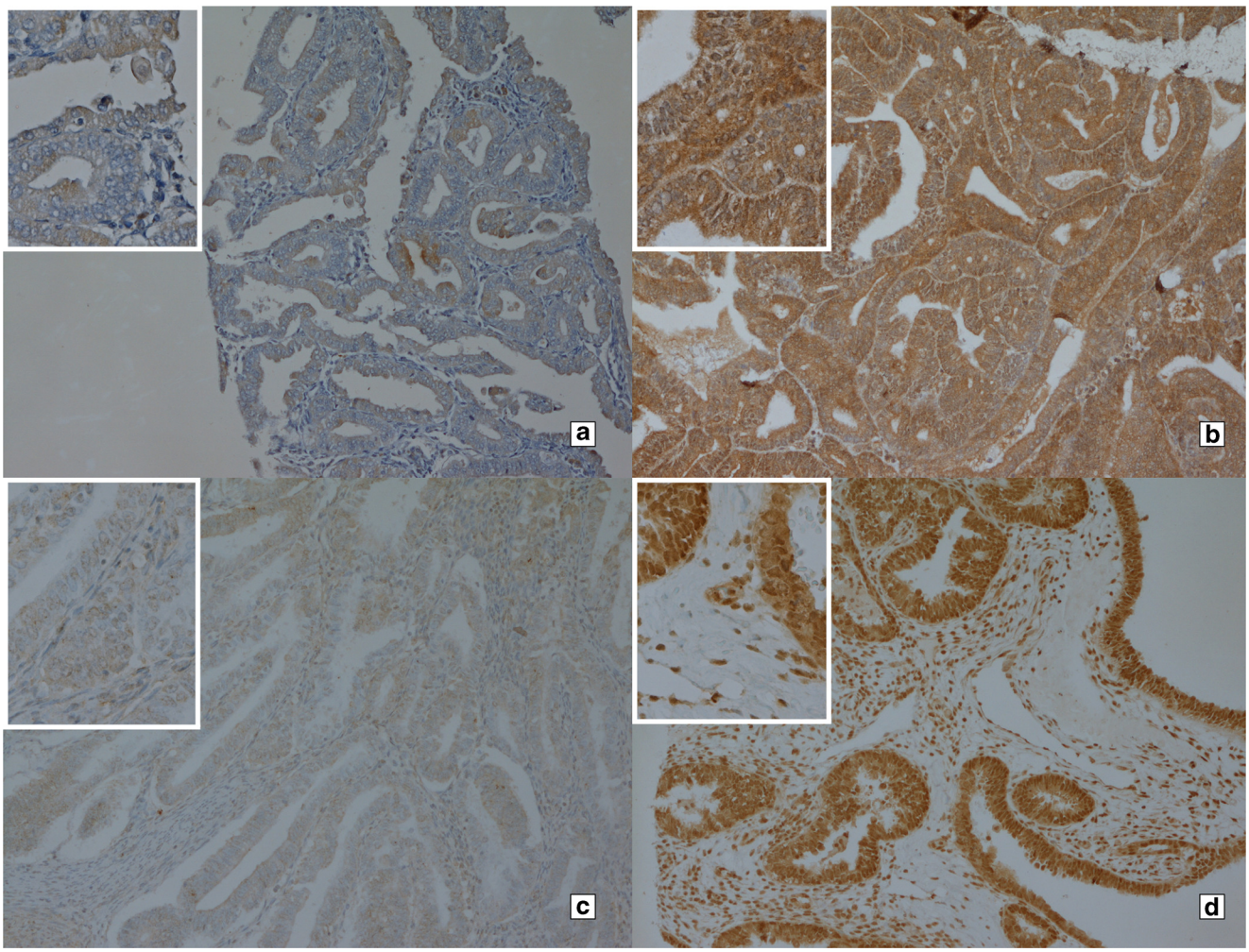


Fig. 1 Immunohistochemical stain of KLF5 and KLF11 in endometrioid endometrial cancer. Panel (**a**) Low KLF5 expression shown by immunohistochemistry (low H-score = below the 50th percentile of the distribution) (original magnification ×200 and in insert original magnification ×400). Panel (**b**) High KLF5 expression shown by immunohistochemistry (high H-score = above the 50th percentile of the distribution) (original magnification ×200 and in insert original magnification ×400). Panel (**c**) Low KLF11 expression shown by immunohistochemistry (low H-score = below the 50th percentile of the distribution) (original magnification ×200 and in insert original magnification ×400). Panel (**d**) High KLF11 expression shown by immunohistochemistry (high H-score = above the 50th percentile of the distribution) (original magnification ×200 and in insert original magnification ×400)

Results

Population characteristic are reported in Table 1. Mean age at surgery was 65.6 years (±10.2) and median parity was 2 deliveries (1–2) (Table 1). The majority of endometrial malignancies were classified as stage FIGO I (80.3%, 118/147). Table 1 also summarizes the adjuvant treatments which patients have undergone. Radiation therapy was performed in 62.3% of patients (91/146), whereas chemotherapy was administered in 6.2% (9/145) (Table 1). In our population, 5-year overall survival resulted 85.4% (95% CI, 79.8–91.4%), while disease-free survival was 79.4% (95% CI, 73.0–86.4%).

Immunohistochemical scores are reported in Table 2. Considering the immunohistochemical staining KLF5 and KLF11 had both nuclear and cytoplasmic localization, while KLF9 stained only in the cytoplasm (Fig. 1). We found cytoplasmic KLF5 and nuclear KLF11 expression to be higher in FIGO stage II than FIGO stage I, III, and IV. In addition, cytoplasmic KLF9 expression was slightly significantly higher in FIGO stage I than FIGO stage II or III-IV ($p < 0.05$). Table 3 reports the uni-variate Cox regression analysis and we found that a high Ki-67 score, KLF5 cytoplasmic H-score, and KLF11 nuclear H-score were significantly predictive of shorter overall survival. In the same Table other significant predictive factors were woman's age, FIGO stadium and tumor grading. Figure 2 shows the Kaplan Meier analysis for overall survival and disease-free survival and Fig. 1 shows the high and low immunohistochemical expression of KLF5 and KLF11. In addition, in multivariate Cox regression analyses KLF5 cytoplasmic H-score (HR 4.72, CI.95 1.61–13.89, $p < 0.05$) and KLF11 nuclear H-score (HR 3.04, CI.95 0.99–9.36, $p = 0.053$) were still predictive of poor overall survival outcome also after adjusting for woman's age, obesity, diabetes mellitus, FIGO stage, and tumor grading.

Table 3 Univariate regression according to Cox which analyzes the factors that influence overall survival. The reported values refer to hazard ratio (HR), 95% confidence interval (CI), and the corresponding *p* value

	HR (95% CI)	p
Ki-67 score >20	2.32 (1.02–5.32)	<0.05
Estrogen receptor H-score >210	0.54 (0.23–1.23)	0.143
Progesterone receptor H-score >180	0.57 (0.25–1.30)	0.183
KLF5 nuclear H-score >0	0.52 (0.07–3.89)	0.528
KLF5 cytoplasmic H-score >20	2.94 (1.22–7.09)	<0.05
KLF9 cytoplasmic H-score >9	1.33 (0.59–3.00)	0.491
KLF11 nuclear score >80	5.33 (1.8–15.75)	<0.05
KLF11 cytoplasmic score >100	2.41 (0.71–8.14)	0.157
Age	1.08 (1.04–1.13)	<0.05
Parity	1.21 (0.83–1.75)	0.321
Obesity	0.66 (0.28–1.55)	0.344
Diabetes Mellitus	0.88 (0.3–2.56)	0.808
Hypertension	3.09 (0.69–13.96)	0.142
Late menopause (after 55 years of age)	1.74 (0.63–4.85)	0.286
Tamoxifen use	1.20 (0.16–8.90)	0.860
TNM/FIGO stadium		
I	Reference	1.000
II	2.25 (0.65–7.83)	0.203
III	3.55 (1.28–9.87)	<0.05
IV	9.91 (2.23–44.05)	<0.05
Tumor grading (G3)	7.40 (3.22–16.99)	<0.05

Supplemental Table 2 shows the disease-free survival univariate Cox regression analysis and a high KLF11 nuclear H-score resulted to be significantly predictive of short disease-free survival (HR 3.48, CI.95 1.55–7.78, $p < 0.05$), adjusting in multivariate analysis for woman's age, FIGO stage and tumor grading the KLF11 nuclear H-score HR resulted 2.59 (CI.95 1.13–5.95, $p < 0.05$).

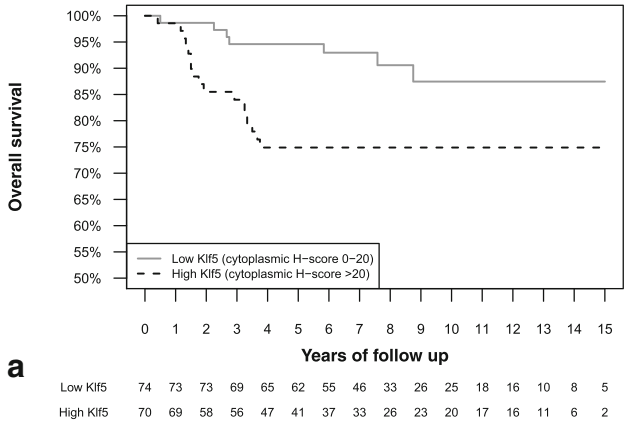
Correlations between immunohistochemical expression of the considered proteins among the full TMA were also assessed. A strong positive correlation between ESR and PGR (rho = 0.70, $p < 0.05$) was found. In addition, some fair correlations were found: first a positive correlation between nuclear and cytoplasmic KLF11 (rho = 0.34, $p < 0.05$); second a negative correlation between PGR and cytoplasmic KLF11 (rho = −0.33, $p < 0.05$); and third a negative correlation between PGR and nuclear KLF11 (rho = −0.31, $p = 0.062$).

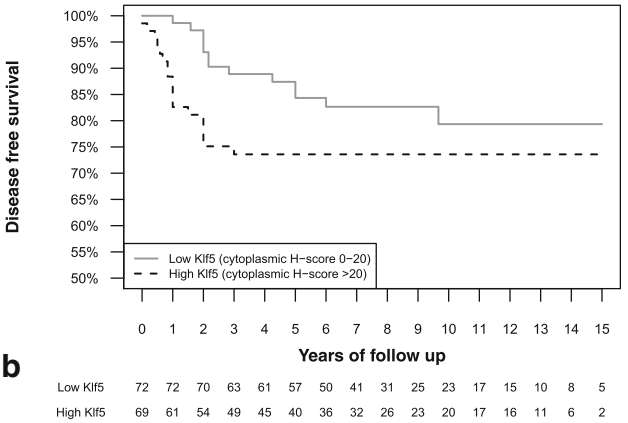
Discussion

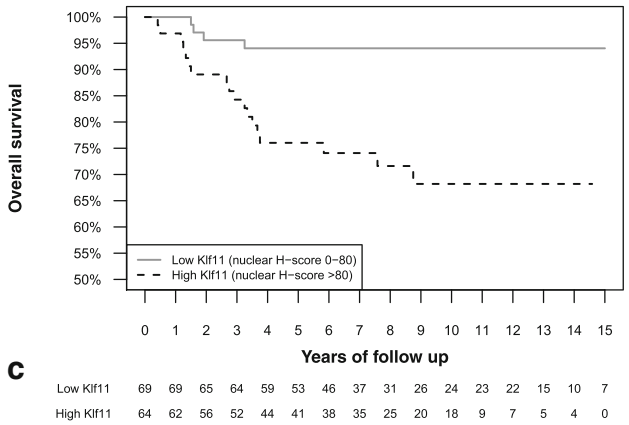
Our study suggests a correlation between higher staining levels of KLF5 and KLF11 and a poorer prognosis of endometrioid endometrial cancer. In particular, a higher Ki-67 score, a higher cytoplasmatic KLF5 score, and a higher nuclear KLF11 score resulted correlated with a shorter survival.

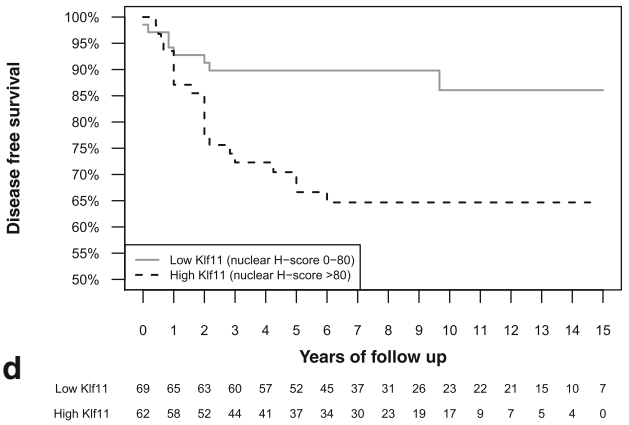
For what concerns the role of KLFs in endometrial cancer, since the expression of these proteins has not yet been localized to specific cell types, it is highly questionable whether the deregulated expression of these KLFs in tumors is directed from the stromal compartment or the epithelium. Previous published gene array data by Mutter and colleagues on KLFs expression in human normal endometrium (proliferative and secretory phases of the menstrual cycle) and in endometrial carcinoma tissues indicated that the transcript levels of most KLFs were unaffected by malignant status [9]. However, there were some exceptions such as KLF6, KLF9, and KLF5, whose transcript levels were reduced and tended to increase in endometrial tumors [9].

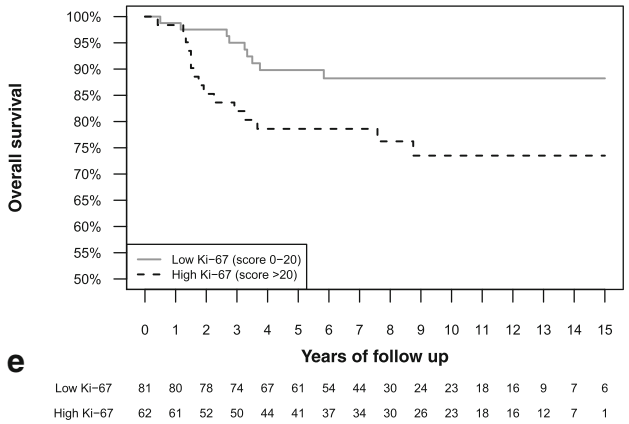
In a recent study, researchers found a significant increase in KLF9 transcript levels, determined by quantitative real-time











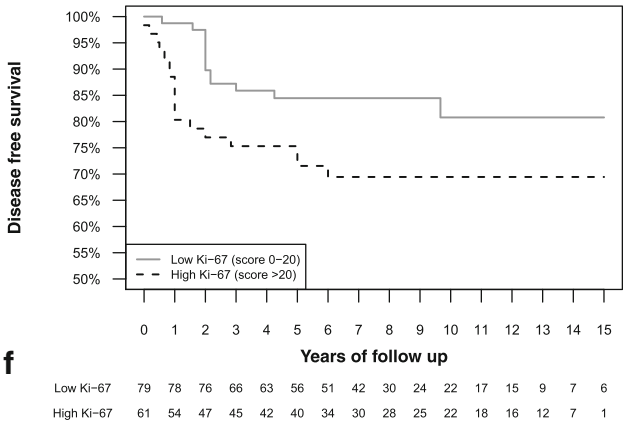


Fig. 2 Panel (**a**) Cytoplasmic H-score of KLF5 and overall survival ($p < 0.05$ – Log-rank test). The H-score was divided into two categories based on the distribution median. Panel (**b**) Cytoplasmic H-score of KLF5 and disease-free survival ($p = 0.131$ – Log-rank test). The H-score was divided into two categories based on the distribution median. Panel (**c**) Nuclear H-score of KLF11 and overall survival ($p < 0.05$ – Log-rank test). The H-score was divided into two categories based on the distribution median. Panel (**b**) Nuclear H-score of KLF11 and disease-free survival ($p < 0.05$ – Log-rank test). The H-score was divided into two categories based on the distribution median. Panel (**c**) Ki-67 nuclear score and overall survival ($p < 0.05$ – Log-rank test). The score was divided into two categories based on the distribution median. Panel (**d**) Nuclear score of Ki-67 and disease-free survival ($p < 0.05$ – Log-rank test). The score was divided into two categories based on the distribution median

PCR analyses, in normal endometrium and stage I endometrial tumors compared to advanced stages endometrial tumors (stages II, III, and IV) [8]. In our TMA an increased level of KLF9 in stage I compared to other stages was also observed. In addition, it was shown that a null mutation of KLF9 in mice affects proliferation and apoptosis in all endometrial cell

types, suggesting an important role for KLF9 in uterine growth regulation [13].

Early experimental data indicated that KLFs may attenuate or promote endocrine-responsive tumors leading to the hypothesis that there were interactions among KLF, ESR, and PGR [14]. Definitely, in vitro cell culture studies and in vivo mouse mutant models have confirmed that KLFs family members play a role as ESR and PGR co-activators [3]. To date, major evidence comes from PGR and KLF9 interactions to regulate endometrial cells PGR-dependent gene transcription [3]. Comparable to its role in PGR signaling pathway, KLF9 also has a role in ESR signaling pathway [3, 13, 15, 16]. Supportive evidence of this is provided by the following in vitro and in vivo experiments: (a) inverse correlation between ESR and KLF9 mRNA transcripts in endometrial cancer [3]; (b) in Ishikawa endometrial cancer cells, KLF9 mediates transcriptional down regulation of ESR signaling pathways by ligand-dependent down regulation [16]; (c) loss of susceptibility, in KLF9 null mice, to E2-activated endometrial cells proliferation, conceivably mediated by loss of KLF9-dependent inhibition of Repressor of Estrogen Receptor Activity expression [15]; and (d) increased ESR expression in endometrial stromal cells during peri-implantation period of KLF9 knockout mice [13]. In our TMA low KLF9 expression and no correlation with PGR or ESR were found. In general, only some week correlations between ESR or PGR and the tested KLFs family members were found, making these results difficult to interpret. Taking KLF11 into account, its possible cancer suppressor role in ovarian cancer and pancreatic cancer has been observed recently [10, 11]. Thus, a possible explanation of KLF11 prognostic role may place in KLFs potential influence on steroid hormone signaling through transcriptional networks involving both steroid hormone receptors and members of the nuclear receptor family of transcription factors.

Although no data are available regarding the participation of KLF5 in steroid hormone signaling, perturbations in its expression under a pathological E2-dominated environment (endometrial carcinoma) suggest potential linkages. However, given that the null mutation of KLF5 results in embryonic or perinatal lethality [17], it is currently not possible to utilize knockout mice for the evaluation of the respective uterine and mammary gland phenotypes; such studies await the generation of mammary- and uterine-targeted gene mutations. In our study KLF5 was found to be predictive of poor outcome, but no significant correlation with ESR and PGR expression were found, therefore the prognostic effect of KLF5 could be independent of ESR or PGR.

The main limitation of this study is its retrospective nature, which has prevented us from accurately assessing the disease-free survival. Conversely, the strength of our study is the large number of cases assessed in the TMA by immunohistochemistry.

In conclusion, our data support a role of KLF5 and KLF11 in the prognosis of endometrioid endometrial carcinoma, the higher expression levels being associated with a poorer prognosis. However, further studies are needed to better understand the role of KLFs in gynecological cancer, starting from their possible prognostic role.

Acknowledgements The authors would like to thank the whole staff who collaborated in the clinical practice and in the study, especially during data collection. We are particularly grateful to Matteo De Luca and Patrizia Savini for the technical support, and to Dr. Raluca I. Necula for the linguistic revision.

Contribution to Authorship Substantial contributions to conception and design or data acquisition or to data analysis and interpretation (LV, APL, MO, SM, FuA, VR, JC, AFr, RJL, LM). Drafting the article or critically revising it for important intellectual content (LV, APL, MO, SM, FuA, VR, JC, AFr, RJL, LM). All authors have read and approved the final manuscript.

Financial Support University of Udine and Ennergi Research.

Compliance with Ethical Standards

Disclosure of Interests The authors state that they have no potential conflicts of interest relevant to this article. This study received no financial support.

References

1. Allen NE, Key TJ, Dossus L, Rinaldi S, Cust A, Lukanova A, Peeters PH, Onland-Moret NC, Lahmann PH, Berrino F, Panico S, Larranaga N, Pera G, Tormo MJ, Sanchez MJ, Ramon Quiros J, Ardanaz E, Tjonneland A, Olsen A, Chang-Claude J, Linseisen J, Schulz M, Boeing H, Lundin E, Palli D, Overvad K, Clavel-Chapelon F, Boutron-Ruault MC, Bingham S, Khaw KT, Bas Bueno-de-Mesquita H, Trichopoulou A, Trichopoulos D, Naska A, Tumino R, Riboli E, Kaaks R (2008) Endogenous sex hormones and endometrial cancer risk in women in the European prospective investigation into cancer and nutrition (EPIC). Endocr Relat Cancer 15:485–497
2. Lacey JV, Yang H, Gaudet MM, Dunning A, Lissowska J, Sherman ME et al (2011) Endometrial cancer and genetic variation in PTEN, PIK3CA, AKT1, MLH1, and MSH2 within a population-based case-control study. Gynecol Oncol 120:167–173
3. Simmen RCM, Pabona JMP, Velarde MC, Simmons C, Rahal O, Simmen FA (2010) The emerging role of Krüppel-like factors in endocrine-responsive cancers of female reproductive tissues. J Endocrinol 204:223–231
4. Simmen RCM, Zhang XL, Michel FJ, Min SH, Zhao G, Simmen FA (2002) Molecular markers of endometrial epithelial cell mitogenesis mediated by the Sp/Krüppel-like factor BTEB1. DNA Cell Biol 21:115–128
5. Yoon HS, Chen X, Yang VW (2003) Kruppel-like factor 4 mediates p53-dependent G1/S cell cycle arrest in response to DNA damage. J Biol Chem 278:2101–2105
6. Rowland BD, Bernards R, Peeper DS (2005) The KLF4 tumour suppressor is a transcriptional repressor of p53 that acts as a context-dependent oncogene. Nat Cell Biol 7:1074–1082
7. Wang Z, Spittau B, Behrendt M, Peters B, Krieglstein K (2007) Human TIEG2/KLF11 induces oligodendroglial cell death by downregulation of Bcl-XL expression. J Neural Transm (Vienna) 114:867–875
8. Simmen FA, Su Y, Xiao R, Zeng Z, Simmen RCM (2008) The Krüppel-like factor 9 (KLF9) network in HEC-1-A endometrial

carcinoma cells suggests the carcinogenic potential of dys-regulated KLF9 expression. Reprod Biol Endocrinol 6:41
9. Mutter GL, Baak JP, Fitzgerald JT, Gray R, Neuberg D, Kust GA et al (2001) Global expression changes of constitutive and hormonally regulated genes during endometrial neoplastic transformation. Gynecol Oncol 83:177–185
10. Wang G, Li X, Tian W, Wang Y, Wu D, Sun Z, Zhao E (2015) Promoter DNA methylation is associated with KLF11 expression in epithelial ovarian cancer. Genes Chromosomes Cancer 54:453–462
11. Buck A, Buchholz M, Wagner M, Adler G, Gress T, Ellenrieder V (2006) The tumor suppressor KLF11 mediates a novel mechanism in transforming growth factor beta-induced growth inhibition that is inactivated in pancreatic cancer. Mol Cancer Res 4:861–872
12. Londero AP, Orsaria M, Tell G, Marzinotto S, Capodicasa V, Poletto M, Vascotto C, Sacco C, Mariuzzi L (2014) Expression and prognostic significance of APE1/Ref-1 and NPM1 proteins in high-grade ovarian serous cancer. Am J Clin Pathol 141:404–414
13. Velarde MC, Geng Y, Eason RR, Simmen FA, Simmen RCM (2005) Null mutation of Kruppel-like factor9/basic transcription element binding protein-1 alters peri-implantation uterine development in mice. Biol Reprod 73:472–481
14. Zhang D, Zhang XL, Michel FJ, Blum JL, Simmen FA, Simmen RCM (2002) Direct interaction of the Krüppel-like family (KLF) member, BTEB1, and PR mediates progesterone-responsive gene expression in endometrial epithelial cells. Endocrinology 143:62–73
15. Pabona JMP, Velarde MC, Zeng Z, Simmen FA, Simmen RCM (2009) Nuclear receptor co-regulator Krüppel-like factor 9 and prohibitin 2 expression in estrogen-induced epithelial cell proliferation in the mouse uterus. J Endocrinol 200:63–73
16. Velarde MC, Zeng Z, McQuown JR, Simmen FA, Simmen RCM (2007) Kruppel-like factor 9 is a negative regulator of ligand-dependent estrogen receptor alpha signaling in Ishikawa endometrial adenocarcinoma cells. Mol Endocrinol 21:2988–3001
17. Pearson R, Fleetwood J, Eaton S, Crossley M, Bao S (2008) Krüppel-like transcription factors: a functional family. Int J Biochem Cell Biol 40:1996–2001