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Combined ASRGL1 and p53 immunohistochemistry as an independent predictor of survival in endometrioid endometrial carcinoma

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HIGHLIGHTS

- ASRGL1 is a promising biomarker in endometrioid endometrial cancer.
- An immunopanel consisting of p53 and ASRGL1 is a useful tool in EEC risk assessment.
- Different EEC subgroups can be characterized using sophisticated statistical methods.

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ABSTRACT

Objective. In clinical practise, prognostication of endometrial cancer is based on clinicopathological risk factors. The use of immunohistochemistry-based markers as prognostic tools is generally not recommended and a systematic analysis of their utility as a panel is lacking. We evaluated whether an immunohistochemical marker panel could reliably assess endometrioid endometrial cancer (EEC) outcome independent of clinicopathological information.

Methods. A cohort of 306 EEC specimens was profiled using tissue microarray (TMA). Cost- and time-efficient immunohistochemical analysis of well-established tissue biomarkers (ER, PR, HER2, Ki-67, MLH1 and p53) and two new biomarkers (L1CAM and ASRGL1) was carried out. Statistical modelling with embedded variable selection was applied on the staining results to identify minimal prognostic panels with maximal prognostic accuracy without compromising generalizability.

Results. A panel including p53 and ASRGL1 immunohistochemistry was identified as the most accurate predictor of relapse-free and disease-specific survival. Within this panel, patients were allocated into high- (5.9%), intermediate- (29.5%) and low- (64.6%) risk groups where high-risk patients had a 30-fold risk ($P < 0.001$) of dying of EEC compared to the low-risk group.

Conclusions. P53 and ASRGL1 immunoprofiling stratifies EEC patients into three risk groups with significantly different outcomes. This simple and easily applicable panel could provide a useful tool in EEC risk stratification and guiding the allocation of treatment modalities.

1. Introduction

Endometrial cancer (EC) is the most common gynaecologic cancer in developed countries. Approximately 80% of EC cases are of endometrioid (EEC) type. The majority of EEC cases are detected at an

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early stage (Figo stages I and II) when the prognosis is generally favourable. Still, over 10% of early stage EEC cancers relapse [1,2]. Currently, we are unable to assess accurately the outcome of early EEC patients. This either leads to unnecessary follow-up for the majority of patients, or to suboptimal treatment of the patients who eventually suffer from relapse. Following the principles of disease stratification as a step towards personalized treatment of EEC, biomarkers are needed to identify patient subgroups that would most benefit from additional adjuvant therapy. A number of tissue biomarkers have been introduced for EEC prognostication [3,4]. However, the integration of biomarkers into clinical guidelines has been slow.

In this study, our aim was to identify a prognostic panel of biomarkers based solely on immunohistochemical stainings, accompanied by sophisticated statistical modelling. More specifically, we wanted to test whether prognostication of EEC would be feasible using only tissue-derived parameters. For this purpose, we chose a set of well-established tissue markers that are known to have a prognostic value in EEC. These include ER and PR, whose prognostic role has been confirmed in several studies [5,6]. P53 was included, as its independent prognostic role in EC has repeatedly been shown [7–9]. Additional biomarkers included Ki-67, MLH1 and HER-2 [10–12]. Finally, two recently characterized promising biomarkers were included: L1-cell adhesion molecule (L1CAM) and L-asparaginase like 1 (ASRGL1). The prognostic value of L1CAM has been shown in various settings, both in type I and II EC [2,13–18] and it has attained an established role in EC biomarker panels both in retrospective and prospective studies. ASRGL1 is a novel biomarker candidate, which we recently demonstrated to have an independent prognostic value in two independent EEC cohorts [19] and the prognostic role of ASRGL1 was confirmed in a recent study [20]. Additionally, the *ASRGL1* gene has been identified as an important hub gene when comparing most differentially expressed genes between EEC and non-endometrioid carcinoma [21].

Although disease stratification by integration of different "omics" data is a promising future approach [22], both technological and financial constraints limit these methods to a few centres. For routine clinical diagnostics, there is still a dire need for robust, low-cost and widely applicable prognostic tools. In this study, sophisticated statistical modelling techniques were used to identify minimal panels of immunohistochemical markers that are capable of providing maximally predictive and practical tools for comprehensive prediction and modelling of EEC patient characteristics. Our main aim was to construct two separate panels; one useful in a pre-operative setting, which would predict clinicopathological risk-factors and aid in surgical treatment planning, and another to be used in a postoperative setting to identify patients who are at risk of disease relapse or death due to the disease.

2. Material and methods

2.1. Patient characteristics

During the years 2001–2007, 327 women with endometrial cancer were operated in Turku University Hospital. Both full patient records and paraffin-embedded material were available. From this cohort, we excluded 14 patients with a non-endometrioid or mixed-type histology, four patients who received any form of pre-operative treatment and three due to unsatisfactory tissue material. The remaining 306 EEC patients were included in this study. All the patients were restaged (AA, JH) based on patient record information in accordance with the FIGO 2009 staging guidelines [23]. The study was approved by the Ethical Committee of the Southwestern Finland Hospital District and the Finnish National Authority for Medicolegal Affairs.

The histopathology of each tumor was re-classified by two expert gynaecopathologists (LT, OC) according to the FIGO guidelines [23,24]. Demographic, clinical, and pathologic information and follow-up data for relapse or death was obtained from the hospital records. The patients were followed until September 2014 or death. The survival data were obtained from the hospital records and the Population Registry, and the cause of death from Statistics Finland. Disease relapse and death due to disease were registered as the end-point events for postoperative modelling. No patients were lost to follow-up.

The clinical and pathologic features of the 306 EEC patients included in this study are summarized in Table 1A. All patients were surgically treated; 74% underwent surgical staging, of which 58.2% underwent pelvic and 15.4% both pelvic and para-aortic lymphadenectomy. Adjuvant therapy was primarily given according to the hospital guidelines, provided that there was no patient-related impairment.

During a median follow-up of 7.2 years (range 0.15–13.02), 40 (13.1%) of the patients experienced a relapse. The majority of these (87.5%) were distant relapses. During a median 7.4 years follow-up, 10.1% died of EEC.

2.2. Tissue microarrays (TMAs) and immunohistochemistry (IHC)

Generation of TMAs, basic IHC techniques, and slide scanning were performed as previously described [19,25] at the Swedish Science for Life Laboratory (SciLifeLab) Tissue Profiling Facility at Uppsala University (Sweden), in accordance with the standards used in The Human Protein Atlas project (www.proteinatlas.org) [26]. The study was performed in accordance with recommended biomarker reporting guidelines (REMARK). Detailed information on the antibodies used in this study is presented in supplementary data.

The immunohistochemically stained slides were scanned and independently evaluated by two pathologists (OC and JH). Both tissue cores for each case were analysed and an average was determined and used for further analysis. If staining result was assessed as a continuous variable, an average of the two cores was calculated. In cases with discrepancies in evaluation, the slides were re-evaluated until an agreement was reached.

Immunoreactivity for ER, PR, ASRGL1 and Ki-67 was quantitatively scored based on positive staining of tumor cells. The frequency was assessed semi-quantitatively in 6 classes (0%; 1–10%; 11–25%; 25–50%; 51–75% and >75% thresholds). P53 staining was considered aberrant if cancerous cells were completely negative, or if moderate-to-strong nuclear staining was present in over 75% of the tumor cells. HER-2 staining was considered positive if membranous staining in >10% of the tumor cells of strong intensity were present. MLH1 was considered negative if there was no evident staining in the cancer cells but stromal cells showed positive staining. L1CAM was considered negative if <10% of tumor cells were positive, as on the threshold if approximately 10% of cells were negative, and as positive if >10% were positive

Table 1
Clinicopathological characteristics of the presented 306 EEC cases (A) and their association with risk assessing immunoprofile (B, $n = 305$).

A			B		
			Low-risk[b]	Intermediate-risk[c]	High-risk[d]
Age[a]		66 (59–73)	66 (59–73)	66 (58–74)	70 (65–76)
Figo stage	I	247 (80.7%)	171 (69.5%)	64 (26.0%)	11 (4.5%)
	II	9 (2.9%)	7 (77.8%)	1 (11.1%)	1 (11.1%)
	III	42 (13.7%)	16 (38.1%)	23 (54.8%)	3 (7.1%)
	IV	8 (2.6%)	3 (37.5%)	2 (25.0%)	3 (37.5%)
Grade	1	166 (54.2%)	131 (78.9%)	32 (19.3%)	3 (1.8%)
	2	87 (28.4%)	52 (59.8%)	30 (34.5%)	5 (5.7%)
	3	53 (17.3%)	14 (26.9%)	28 (53.8%)	10 (19.2%)
MI	<50%	206 (67.3%)	144 (70.2%)	52 (25.4%)	9 (4.4%)
	≥50%	100 (32.7%)	53 (53.0%)	38 (38.0%)	9 (9.0%)
LVI	No	203 (66.3%)	131 (64.9%)	59 (29.2%)	12 (5.9%)
	Yes	37 (12.1%)	15 (40.5%)	18 (48.6%)	4 (10.8%)
	Missing	66 (21.6%)			

N(%)
[a] Median (IQR).
[b] Low-risk: p53 wild-type, ASRGL1 > 75%.
[c] Intermediate-risk: p53 wild-type, ASRGL1 ≤ 75% or p53 aberrant and ASRGL1 > 75%.
[d] High-risk: p53 aberrant, ASRGL1 ≤ 75%.

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