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BRIEF REPORT

Mesothelin Expression in Patients with High-Grade Serous Ovarian Cancer Does Not Predict Clinical Outcome But Correlates with CD11c^{+} Expression in Tumor

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ABSTRACT

Introduction: Mesothelin (MSLN) is overexpressed in several tumors including ovarian cancer and is the target of current trials. There is limited and conflicting data on MSLN prognostic impact in ovarian cancer.

Methods: We performed a retrospective study on patients with high-grade serous ovarian cancer, analyzing MSLN expression by immunohistochemistry and examining the correlation of its expression to overall and progression-free survival. Correlations of expression of MSLN, CD8, and macrophage markers in different tumor compartments were also investigated.

Results: Positive MSLN expression was detected in 55.1% of primary tumors and 51.5% of the metastases. MSLN expression was not correlated with survival. We observed a significant positive correlation ($r = 0.34$, $p = 0.01$) between MSLN expression in the metastatic site and CD11c expression in total tumor area and perivascular area in the primary tumor.

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Conclusion: Our results show that MSLN expression does not correlate with clinical outcome. The impact of the correlation between MSLN and $CD11c^+$ cells on immunotherapy outcome should be further explored.

Keywords: Immunohistochemistry; Mesothelin; Ovarian cancer

Key Summary Points
Why carry out this study?
Mesothelin (MSLN) is overexpressed in several tumors including ovarian cancer and is the target of current trials.
There is limited and conflicting data on MSLN prognostic impact in ovarian cancer.
This study evaluated MSLN expression in patients with high-grade serous ovarian cancer and its association level with clinical parameters.
What was learned from the study?
Our data showed that MSLN expression did not correlate with clinical outcome (OS or PFS), and there was a positive correlation between MSLN expression in the metastatic site and CD11c expression in total tumor area and perivascular area in the primary tumor.
These results confirms that MSLN expression does not correlate with clinical outcome impact.
The correlation between MSLN and $CD11c^+$ cells should be further explored.

DIGITAL FEATURES

This article is published with digital features, including a summary slide, to facilitate understanding of the article. To view digital features for this article go to https://doi.org/10.6084/m9.figshare.13014242.

INTRODUCTION

Ovarian cancer is the most lethal gynecological malignancy [1] and high-grade serous (HGS) ovarian cancer is the most common among the subtypes. For the vast majority of patients, the disease is diagnosed at an advanced stage (60–70% in stage III–IV), and even though a majority of patients respond to primary platinum-containing chemotherapy regimens, a high percentage of them relapse and ultimately die from the disease. Hence, new therapeutic approaches and development of novel drugs are needed.

Mesothelin (MSLN) is a glycosylphosphatidylinositol (GPI)-anchored membrane glycoprotein. Its exact biological function remains unknown. A soluble form of MSLN (is generated by proteolytic cleavage or alternative splicing) is detectable in the sera of patients with tumors. While MSLN may be non-essential in normal cells, it plays a role in promoting tumor cell proliferation and chemoresistance [2]. MSLN is currently explored in early trials as an antigen for target therapy [3]. In patients with ovarian cancer, conflicting results have been published, showing that MSLN tumor expression correlated negatively [4–6], positively [7], or not [8, 9] with survival. High MSLN expression was shown in one report to correlate with serous epithelial ovarian cancer, but not other histological types such as endometroid, clear cell, or mucinous [4], while others have shown that higher MSLN expression in endometrioid compared to serous type [8].

A soluble form of MSLN (containing the GPI anchor) was suggested to bind to the mannose receptor CD206 [10] and play a role in macrophage polarization. Macrophage status in clinical samples is usually analyzed by detection of the CD68 marker. CD11c and CD80, associated with the M1-like phenotype [11], and CD163 associated with the M2-like phenotype [12] are markers that could provide a more specific detection of macrophage subtypes.

In this study, we analyzed MSLN expression in patients with HGS ovarian cancer, determined the association level of tumor MSLN expression with relevant clinical parameters, and explored the correlation of MSLN expression with the expression of CD8, CD11c, CD80, and CD163 immunomarkers.

METHODS

Patients and Methods

All patients diagnosed with ovarian, fallopian tube, or primary peritoneal carcinoma, or carcinoma of undesignated primary site, in Stockholm County between 2002 and 2006 were identified using the National Swedish Cancer Registry. Of the 401 patients screened, 135 fulfilled the study inclusion criteria (Fig. 1a). The study was approved by the Regional Ethics Committee (Dnr 2012/539-31/1). Platinum free interval (PFI) was defined as the time from the date of last course of platinum to progression, recurrence, or death of any cause (whichever came first). For eligibility criteria, definitions of survival endpoints, tissue microarray (TMA), immunohistochemistry IHC, MSLN expression evaluation, image analysis, and scoring detailed method, see the electronic supplementary material.

Statistical Analysis

Statistical differences in overall survival (OS) and in progression-free survival (PFS) were estimated using log rank tests. Correlations between markers and MSLN expression were determined with the Pearson correlation matrix. Calculations were made with the Statistica 13 software (TIBCO Software Inc.).

RESULTS

Patients

Among the 135 patients, adnexal (primary) tumor tissue was available in 113 cases, with paired metastatic tissue in 89 cases. For 13 patients, only tumors from the metastatic site (omentum) were available (Fig. 1a). Clinical data is summarized in Table 1. Median age at diagnosis was 64 years, patients had mostly stage IIIC disease (73%), and most underwent primary debulking surgery (79%). Macroscopic radical surgery was obtained in 28% of the patients. The 5- and 10-year survival rate was 23% and 8%, respectively (Fig. 1b).

MSLN Expression in Adnexa and Metastatic Site

MSLN expression was analyzed in primary adnexal site (n = 107) and/or metastatic site (n = 101), examples are shown in Fig. 1c, d. MSLN-positive tumor cells were detected in 55.1% of the primary adnexal tumors and 51.5% of metastases. In 74.5% of the patients where MSLN-positivity was detected in the primary tumor, the paired metastatic site was also MSLN positive. In 78.4% of the patients where the primary tumor was MSLN negative, the paired metastatic site was also MSLN negative. A positive correlation (r = 0.6157) between MSLN expression level in the primary adnexal site and metastatic site was observed (Supplementary Fig. 1). MSLN expression did not correlate with clinicopathological parameters (Supplementary Table 1).

OS and PFS

Positive MSLN expression in the adnexal site showed no significant correlation with OS when compared to negative MSLN (median OS 44 months versus 34 months, log rank p = 0.61, Fig. 1e). Positive MSLN expression in the metastatic site showed a non-significant trend for longer OS when compared to negative MSLN expression (median OS 40 months versus 34 months, log rank p = 0.19, Fig. 1f). No correlation between MSLN expression in adnexal site and PFS was found (log rank p = 0.4) (Supplementary Fig. 2).

MSLN Expression Related to Immunomarkers

We analyzed the correlation between MSLN expression and CD11c, CD80, CD163, and CD8 expression. MSLN expression and CD11c expression were analyzed in adnexal site and in biopsies from the metastatic site, whereas only adnexal tumors were included in the analysis of CD8 and additional macrophage-related markers (CD80 and CD163). MSLN expression in the metastatic site significantly correlated with CD11c expression in the perivascular area (PVA1, $r = 0.34$, $p = 0.011$) and total tumor area ($r = 0.28$, $p = 0.043$) in the primary adnexal site (Table 2). No other statistically significant correlation was found between MSLN expression and the other immunomarkers (Table 2).

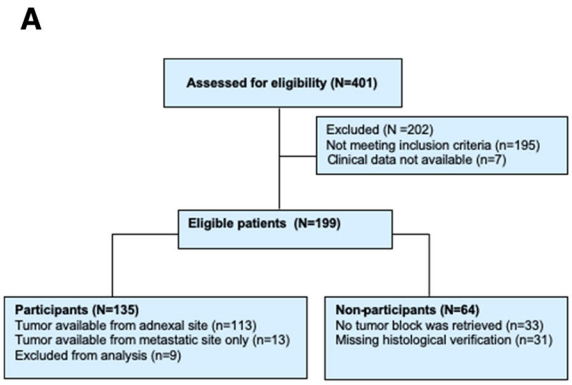
DISCUSSION

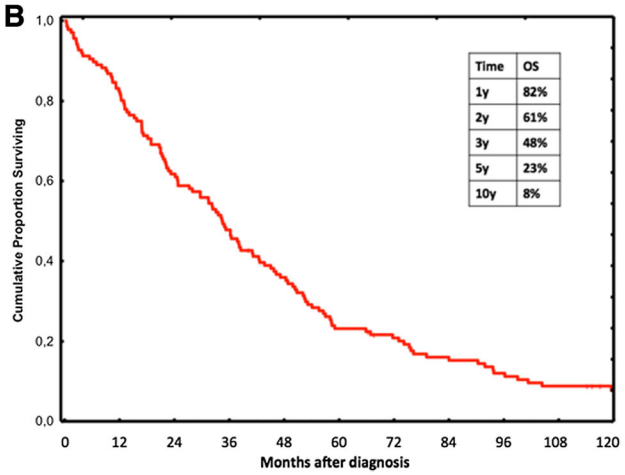
MSLN expression in HGS ovarian cancer in our cohort (55.1% in adnexal site) is in line with previous findings [7, 9] of MSLN in HGS ovarian cancer detected by IHC. Our analyses on a selected cohort of only patients with advanced HGS ovarian cancer show that MSLN expression does not correlate with clinical outcome (OS or PFS). While Köbel et al. showed that MSLN expression was not associated with disease-specific survival [9], Yen et al. reported that patients with diffuse MSLN expression (based on staining score) had a longer survival as compared to patients with no or low MSLN expression [7]. Only a limited number of reports have analyzed MSLN expression and survival in patients with ovarian cancer. Okla et al. reported that tumoral MSLN levels (determined by qPCR) did not correlate with survival in patients with epithelial ovarian cancer [8]. Hanaoka et al. showed in patients with epithelial ovarian carcinoma, with no distinction between high or low grade, that high MSLN expression (determined by IHC) associated with shorter PFS and OS [4]. Cheng et al. analyzed MSLN expression in patients with mixed types of epithelial ovarian carcinoma and showed that the OS of patients with high MSLN expression (determined by RT-PCR) was shorter as compared to patients with low MSLN expression [5]. Notably, when MSLN expression was analyzed in relation to OS, solely in the subgroup of patients with HGS, no statistical difference was observed ($p = 0.055$) between patients with high and low expression. This report also showed that MSLN expression was higher in chemoresistant patients as compared to chemosensitive patients. In our cohort, platinum resistance after 6 ($p = 0.66$) or 12 ($p = 0.68$) months was not correlated with MSLN expression in the adnexal site (Supplementary Fig. 3). Finally, to the best of our knowledge only one report, that by Yildiz et al., revealed a positive association between high MSLN expression by IHC and poor prognosis in patients with advanced serous ovarian cancer [6].

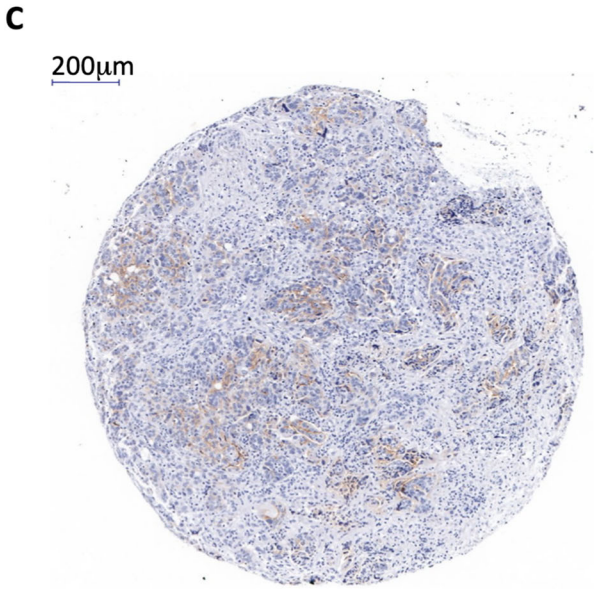
The engagement of soluble MSLN (via GPI anchor) to CD206 may impact macrophages polarization [10]. Increased tumor MSLN expression may correlate with increased soluble forms of MSLN (as shown previously [8]) which in turn could bind to macrophages and impact their differentiation. Our data revealed a positive correlation between MSLN expression in the metastatic site and CD11c in the primary site, in total tumor area and perivascular subcompartment. Further mechanistic studies are warranted to elucidate the biological mechanism underlying this association, and the potential impact of MSLN on immune cells. However, these results might suggest a possible role of MSLN-positive cells in promoting the differentiation of macrophages towards a M1 phenotype or, alternatively, imply a stimulating activity of M1-like macrophages towards MSLN expression on tumor cells.

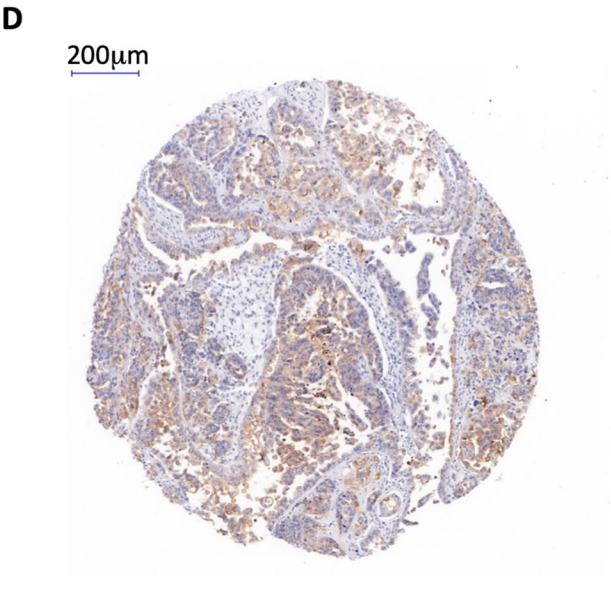
Our study has some limitations. It would be of interest to quantify MSLN in patients with ovarian cancer other than HGS and assess

Fig. 1 MSLN detection in patients with HGS ovarian cancer. **a** Flowchart of the study population. **b** Overall survival of 135 patients with HGS ovarian cancer. MSLN detection by IHC in TMA, showing **c** MSLN positive 50%, and **d** MSLN positive 90%. Kaplan–Meier 10-year overall survival for patients with HGS ovarian cancer based on MSLN expression in **e** adnexal site ($n = 103$), and **f** metastatic site ($n = 99$)


A
Assessed for eligibility (N=401)
Excluded (N =202)
Not meeting inclusion criteria (n=195)
Clinical data not available (n=7)
Eligible patients (N=199)
Participants (N=135)
Tumor available from adnexal site (n=113)
Tumor available from metastatic site only (n=13)
Excluded from analysis (n=9)
Non-participants (N=64)
No tumor block was retrieved (n=33)
Missing histological verification (n=31)


B
Time OS
1y 82%
2y 61%
3y 48%
5y 23%
10y 8%
Cumulative Proportion Surviving
Months after diagnosis


C
200µm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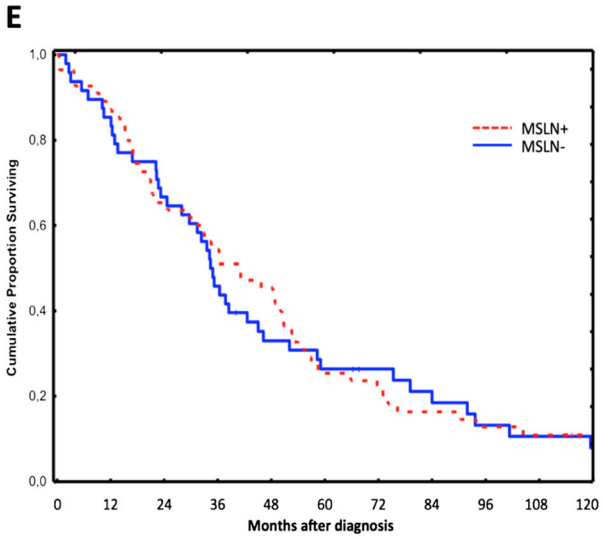
E
MSLN+
MSLN-
Cumulative Proportion Surviving
Months after diagnosis

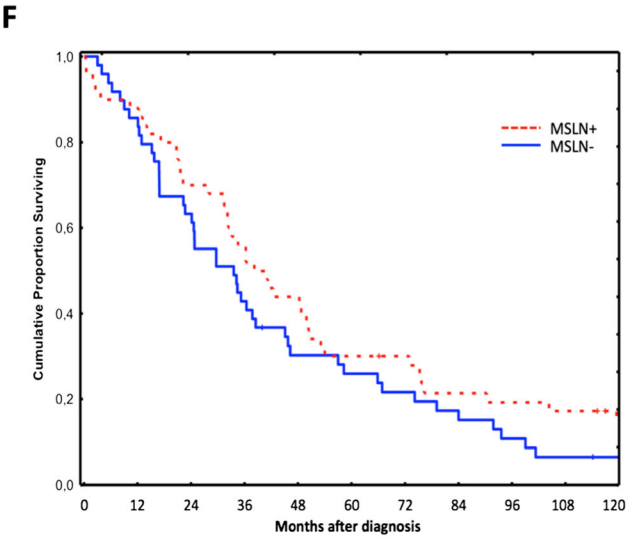
F
MSLN+
MSLN-
Cumulative Proportion Surviving
Months after diagnosis

Table 1 Patient characteristics

Characteristic	Patient cohort $N = 126$
Median age at diagnosis, years (range)	64 (36.5–84.2)
Diagnosis	
Ovarian cancer	88 (69.8%)
Fallopian tube cancer	13 (10.3%)
Peritoneal cancer	22 (17.5%)
Undesignated site	3 (2.4%)
Missing	0
FIGO stage	
IIC	2 (1.6%)
IIIA	1 (0.8%)
IIIB	6 (4.0%)
IIIC	92 (73.0%)
IV	25 (19.8%)
Missing	0
Type of surgery	
Primary surgery	99 (78.6%)
Delayed primary/interval	18 (14.3%)
No surgery	9 (7.1%)
Missing	0
Macroscopic residual disease after surgery	
Absent	33 (28.2%)
Present	84 (71.8%)
Missing	0
Chemotherapy first line	
Platinum based	116 (92.0%)
No platinum	1 (0.8%)
No chemo	8 (6.3%)
Missing	1 (0.8%)
Response at EOT	
CR	69 (59.0%)
PR	26 (22.2%)
SD	3 (2.6%)
PD	16 (13.7%)
Missing	3 (2.6%)
Survival	
Alive with no evidence of disease	4 (3.2%)
Alive with evidence of disease	5 (4.0%)
Dead from ovarian cancer	111 (88.1%)
Dead from other causes	3 (2.4%)
Lost at follow-up	3 (2.4%)
Median follow-up	36.4 months (0.4–171.9)
Missing	0
Time from EOT to recurrence/progression	
$\geq$ 6 months (platinum sensitive)	70 (60.3%)
$<$ 6 months (platinum resistant)	46 (39.7%)
Missing	0

FIGO International Federation of Gynecology and Obstetrics, *NACT* neoadjuvant chemotherapy, *EOT* end of treatment (including patients that received platinum-based therapy), *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease

correlation or lack of with clinical parameters. We could not measure soluble MSLN and assess whether there is a correlation between soluble MSLN levels, MSLN expression (detected by IHC), and clinical outcome.

CONCLUSION

This study confirms that in patients with HGS ovarian cancer, MSLN expression does not predict clinical outcome. Our data show a correlation between MSLN expression and the presence of a CD11c-positive immune infiltrate that needs to be further analyzed and explored

Table 2 Correlation between MSLN expression and immune markers

		CD11c								
		PVA1	PVA2	tot. tum	epith	stroma	tot. tum	PVA1	stroma	epith
		Adnexal site					Metastatic site[a]			
MSLN adn	*r*	0.108	0.060	− 0.008	0.056	0.058	0.074	0.036	− 0.048	− 0.113
	p	0.434	0.662	0.954	0.686	0.676	0.54	0.762	0.659	0.302
MSLN met	*r*	0.344*	0.246	0.276*	0.166	0.170	− 0.123	− 0.051	0.008	0.046
	p	0.011	0.072	0.043	0.229	0.218	0.303	0.67	0.938	0.674

	CD80					CD163					CD8		
	PVA1	PVA2	tot. tum	stroma	epith	PVA1	PVA2	tot. tum	stroma	epith	epith	stroma	stroma 2–3
MSLN adn	− 0.009	0.012	− 0.131	− 0.121	− 0.030	− 0.080	− 0.130	− 0.136	− 0.109	0.008	0.160	0.050	0.100
	0.947	0.927	0.343	0.382	0.826	0.563	0.347	0.324	0.430	0.952	0.247	0.719	0.471
MSLN met	0.188	0.103	0.006	− 0.043	0.049	0.055	0.006	− 0.033	− 0.002	0.112	0.095	0.078	0.099
	0.173	0.459	0.962	0.755	0.725	0.688	0.962	0.811	0.987	0.417	0.494	0.571	0.476

adn adnexal site, *met* metastatic site, *tot. tum* total tumor area, *epith* epithelial, *PVA1* perivascular area 1 density (area of 15 μm closest to the CD34-positive region), *PVA2* perivascular area 2 density (area of 15 μm PV-A1 surrounding PV-A1 area)

*Correlation with $p < 0.05$

[a] The majority of metastatic site was the omentum

for its possible impact on the outcome of immune-related therapies.

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Authorship Contributions. Isabelle Magalhaes, Daria Glaessgen, Jonas Mattsson and Hanna Dahlstrand conceived and designed the study. Josefin Fernebro, Daria Glaessgen, Sara Corvigno and Hanna Dahlstrand provided materials and patient data. Isabelle Magalhaes, Josefin Fernebro, Sulaf Abd Own, Sara Corvigno, Mats Remberger and Hanna Dahlstrand did the data analysis and interpretation. Isabelle Magalhaes, Josefin Fernebro, Sara Corvigno, Jonas Mattsson and Hanna Dahlstrand wrote the paper, and all authors approved the final version of the paper.

Disclosures. None of the authors (Isabelle Magalhaes, Josefin Fernebro, Sulaf Abd Own, Daria Glaessgen, Sara Corvigno, Mats Remberger, Jonas Mattsson and Hanna Dahlstrand) have anything to disclose.

Compliance with Ethics Guidelines. The study was approved by the Regional Ethics Committee (Dnr 2012/539-31/1).

Data Availability. The data sets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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