1

The Role of Mesothelin Expression in Serous Ovarian Carcinoma: Impact on the Diagnosis, Prognosis and Therapeutic Target.

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Simple Summary

Ovarian cancer is the most lethal gynaecological malignancy, and a serous carcinoma is the most common subtype. The lack of symptoms and sensitive diagnostic tests in the early stage of development may explain why the diagnosis often occurs late, when the neoplasm has already spread outside the pelvis. Currently, the standard treatment of ovarian carcinomas requires cytoreductive surgery followed by platinum-based systematic chemotherapy, which does not reduce recurrences or mortality. Despite intense efforts to develop novel therapies that involve the use of new chemotherapeutic agents such as anti-angiogenesis agents and poly (ADP-ribose) polymerase inhibitors that improve patient outcomes, the five-year survival for this malignancy still remains low. Therefore, it is important to identify new targetable molecules for early diagnosis, disease monitoring and treatment for this malignancy. The aim of this review is to discuss the role of mesothelin in serous ovarian carcinomas, focusing on its diagnostic, prognostic and therapeutic perspectives.

Abstract

Mesothelin is a protein that is expressed in the mesothelial cell lining in the pleura, peritoneum and pericardium. The gene of mesothelin encodes a precursor protein that is processed to yield mesothelin, which is attached to the cell membrane by a glycophosphatidylinositol linkage and a shred fragment named the megakaryocytic-potentiating factor. The biological functions of this substance in normal cells are still unknown. Experimental studies on knockout mice suggest that this substance does not play an important role in development and reproduction.

In contrast, it has been observed that mesothelin is produced in abnormal amounts in several malignant neoplasms such as mesotheliomas and pancreatic adenocarcinomas.

Given that mesothelin is overexpressed in many solid tumours and has antigenic properties, this molecule could be considered a tumour marker or an antigenic target for many malignancies. Many molecular studies also have demonstrated that mesothelin is overexpressed in serous ovarian carcinomas and may bind to ovarian cancer antigen Ca-125, favouring the spread of the tumour in the abdominal cavity.

Here, we discuss the current knowledge of mesothelin and focus on its role in clinical and pathological diagnoses as well as its impact on the prognosis in serous ovarian carcinomas.

We also briefly discuss the latest progress of mesothelin-targeting therapies for this aggressive and lethal neoplasm.

Keywords: mesothelin; ovarian carcinoma; biomarker; mesothelin-targeting therapy

Introduction

Mesothelin (MSLN) is a glycoprotein that is located on the mesothelial lining of the body cavities and in many neoplasms [1]. It is anchored at the cell membrane by a glycosylphosphatidylinositol linkage. The mesothelin gene was first cloned by Chang and Pastan [1] and it encodes a precursor protein that is processed to yield a 40 kDa mesothelin protein and a 31 kDa soluble fragment. The soluble human fragment, named the megakaryocyte-potentiating factor, was reported to have a megakaryocyte-potentiating activity in mouse bone marrow [2].

In normal tissue, the physiological/biological function of MSLN is still uncertain. Studies in molecular biology have demonstrated that a lack of MSLN in an MSLN knockout mouse model did not affect development, growth or reproduction [3].

Conversely, MSLN is considered to be involved in several mechanisms of cancer pathogenesis. In ovarian carcinomas, it was demonstrated that binding with the partner MUC16 (CA125) could play a role in cell adhesion, facilitating intraperitoneal ovarian cancer metastasis [4-6].

There is evidence that mesothelin can be used as a new cancer biomarker [7] and as a target molecule for gene therapy [8].

Here, we discuss the current knowledge of MSLN, focusing on its role in clinical and pathological diagnoses as well as its impact on the prognosis in serous ovarian carcinomas. We also briefly discuss the latest progress

of mesothelin-targeting therapies for this aggressive and lethal neoplasm.

Materials and Methods

We conducted a review of the current literature to report the current knowledge of MSLN in normal tissue and in ovarian serous carcinomas, focusing on its role in diagnosis, prognosis and therapy. The aim of this review was to report on the latest progress of mesothelin-targeting therapies in this neoplasm.

The medical subject heading terms used for the search in PubMed and Scopus were 'mesothelin' and 'ovarian carcinomas' as well as 'serous carcinoma', 'diagnosis' and 'therapy'.

The systematic review process was performed independently by two authors (E.F. and A.T.) and checked by another (G.G.).

The duplicates, e.g., articles that were not written in the English language and those evidently not relevant to the topic based on the title and a revision of the abstract, were excluded from the review. All works describing the MSLN expression and therapies in non-ovarian carcinomas were also excluded from our analysis.

Finally, we selected studies that focused on the role of MSLN in clinical and pathological diagnoses as well as its impact on the prognosis in serous ovarian carcinomas. We also briefly discussed the latest progress of MSLN-targeting therapies for ovarian carcinomas, especially the serous subtype.

Mesothelin as a New Cancer Biomarker for the Diagnosis and Prognosis of Ovarian Carcinomas

Among gynaecological neoplasms, ovarian carcinomas have the highest mortality because the diagnosis of this malignancy is often late, when the neoplasm is already in an advanced stage of development.

The early detection of this neoplasm is difficult due to the absence of physical symptoms and a lack of sensitive screening methods [9].

Mesothelin as a Serological Biomarker

Cancer antigen 125 (CA125) is currently the most common serological biomarker used for diagnosis and the management of patients with epithelial ovarian/fallopian tube or primary serous peritoneal cancers. Many studies suggest that CA125 can also be expressed at high levels in other types of cancers such as breast cancer [10, 11], mesotheliomas [12, 13], non-Hodgkin's lymphoma [14, 15] and leukaemia [16] as well as leiomyomas and leiomyosarcomas with a gastrointestinal origin [17]. CA125 was also found to be elevated in the sera of patients with benign conditions such as cirrhosis, ovarian cysts, endometriosis, pregnancy, congestive heart failure and musculoskeletal inflammatory disorders [18].

4

Only half the patients with early-stage ovarian carcinomas had elevated CA125 levels [19].

Thus, the sensitivity and specificity of CA125 for the detection of early stage of ovarian carcinomas are unfortunately low [20]. Therefore, it is extremely important to identify new molecules for an early diagnosis and disease monitoring of this lethal malignancy.

Concerning the use of MSLN as a biomarker for the diagnosis of ovarian carcinomas, many data in the literature suggest that this substance is expressed in different subtypes of ovarian carcinomas, especially serous [21]. A splice variant of soluble mesothelin, named the soluble megakaryocyte-potentiating factor (SMRP), was found in the sera of patients with ovarian carcinomas [22].

Studies have reported that SMRP serum was significantly higher in ovarian carcinomas than in ovarian benign lesions and heathy subjects. It was also observed that serum SMRP levels were related to FIGO pathological staging and grading of the neoplasms, demonstrating that high serum levels of mesothelin may be indicative of tumour progression and poor survival [23-25].

Mesothelin as a Biomarker in Other Fluids

Okla et al. observed that peritoneal fluid mesothelin levels did not differ significantly in patients with benign and malignant ovarian epithelial neoplasms. They also did not observe any differences of peritoneal fluid MSLN levels in different FIGO stages and histological types of neoplasms.

Thus, in contrast to the serum levels of MSLN, low levels of MSLN in the peritoneal fluid were not associated with a better prognosis [24].

Studies in the literature have reported that MSLN can also be detected in the urine samples of patients affected by ovarian carcinomas [26-29].

In particular, Badgwell et al. observed, for the first time, that the urinary levels of MSLN could be considered to have a greater sensitivity than the serum levels in the early stages of ovarian carcinomas [26].

Similarly, Hellstrom et al. demonstrated that, in women with a pelvic mass, assaying urine for human epididymis protein 4 (HE4) or mesothelin may detect early ovarian carcinomas more often than assaying the serum [27].

In their study, Hollevoet K et al. demonstrated that mesothelin levels in the urine depended on an impaired glomerular and tubular function, which could influence the interpretation of the mesothelin measurements and cause false-positive results [29]. Wu et al. considered SMRP serum to be a promising marker for the diagnosis

and monitoring of ovarian carcinomas, especially in combination with CA125, showing a sensitivity of 98.4% and specificity of 88.4 % [23].

Mesothelin as a Biomarker in Radioimmunoimaging

As mesothelin is a membrane antigen overexpressed in a variety of solid neoplasms including ovarian carcinomas, there are many studies in the literature that prove that analyses of radioimmunoimaging can be used for the non-invasive detection of mesothelin-overexpressing tumours [30-35].

Radioimmunoimaging is a type of molecular nuclear medicine imaging that applies specific antibodies of tumour-specific antigens labelled with radionuclides for imaging [36]. Thus, this molecular imaging allows the assessment of the tumour uptake and distribution in the primary and secondary tumour sites as well as the response to therapy. Different anti-mesothelin antibodies have been used in animal models.

In several studies, anti-mesothelin antibodies were used that could be detected by fluorescence imaging or magnetic resonance [31,32]. For detection by positron emission tomography (PET), other authors have demonstrated in ovarian models that 89Zr-labelled antibodies could be used to target MSLN, an antibody drug conjugate (ADC) that can provide information regarding both the organ distribution and drug dosing [32-35].

Mesothelin Detection in Neoplastic Tissue

In diagnostic pathology, the immunohistochemical expression of MSLN in several instances is a useful marker to distinguish between primary and metastatic ovarian carcinomas. In their paper, Kanner et al. demonstrated that MSLN expression could assist in differentiating Müllerian serous carcinomas from metastatic breast carcinomas (particularly those with a papillary morphology) and documented that none of the breast carcinomas were stained for mesothelin [37].

Ordóñez demonstrated that the carcinomas that most frequently exhibited a strong MSLN reactivity were non-mucinous carcinomas of the ovary; however, they observed that this marker was also expressed in other non-mucinous carcinomas such as clear cell carcinomas of the ovary, endodermal sinus tumours or renal cell carcinomas as well as clear cell type and transitional cell carcinomas of the ovary [21]. Weidemann et al., to identify tumours that might benefit from targeted cancer therapies, observed that the highest prevalence of MSLN positivity is was present in ovarian carcinomas (serous 97%) by the analysis of tissue microarrays for the MSLN expression of 122 different tumour types. Conversely, MSLN was rare in the cancers of the breast, kidney, thyroid gland, soft tissues and prostate [38].

The immunohistochemical expression of MSLN in the neoplastic section of serous ovarian carcinomas was

also investigated to establish its impact on the prognosis.

The literature suggests that there are limited and conflicting immunohistochemical data regarding MSLN expression and the prognostic impact on ovarian cancers.

According to the study of Chang et al., the immunohistochemical MSLN expression was related to the survival outcomes in patients with ovarian carcinomas. They observed that the neoplasms with a high expression of mesothelin showed a statistically worse prognosis than those with a low immunoreactivity [39].

Similarly, Yildiz et al. observed that a high expression of MSLN in advanced serous ovarian cancers was associated with a poor prognosis and with worse platinum sensitivity in the advanced stage [450]. Cheng et al. observed that a high MSLN expression, investigated by a molecular study using real-time quantitative reverse transcription PCR, was associated with chemoresistance and a poor survival in ovarian carcinomas [41].

By contrast, Yen et al., separating neoplasms with diffuse immunoreactivity from neoplasms with focal positivity, observed that a diffused MSLN expression correlated with prolonged patient survival in ovarian serous carcinomas [42].

The results of Yen et al. were not in accordance with those of other studies, in which a high expression of MSLN was associated with a poor survival in other malignant epithelial neoplasms such as lung adenocarcinomas and pancreatic ductal adenocarcinomas [43, 44].

The conflicting data on MSLN expression and its prognostic impact on patients with ovarian carcinomas may be due to many factors such as the different antibodies, protocols and criteria used to evaluate the immunoreactivity. Magalhaes et al., by an immunohistochemical analysis, demonstrated that MSLN expression in patients with high-grade serous carcinomas did not predict the clinical outcome but correlated with the CD11c+ positive-immune infiltrate in neoplasms. The MSLN expression also significantly correlated with CD11c+ in the metastatic sites and in the perivascular areas of the primary neoplasm. Thus, they concluded that these data could also have an important impact on the outcome of immune-related therapies [45].

Mesothelin as a therapeutic target

Currently, ovarian cancer treatment consists of surgical tumour debulking complemented by taxane-and platinum-based chemotherapy [46], occasionally associated with Avastin (bevacizumab, an anti-vascular endothelial growth factor therapy) [47]. In advanced or recurrent diseases, in patients with a BRCA mutation, maintenance therapy with a poly adenosine diphosphate (ADP-ribose) polymerase (PARP) inhibitor treatment

represented an effective treatment option [48].

However, radical treatment regimens and multiple chemotherapeutic treatments do not reduce the recurrences of the disease or the death of the patients.

Given that MSLN is overexpressed in many solid tumours and has antigenic properties, this molecule could be considered to be the antigenic target for immunotherapeutic strategies in ovarian carcinomas. The main immunotherapeutic strategies using different therapeutic agents include anti-mesothelin immunotoxin SS1P, MORAb-009 (chimeric anti-mesothelin mAb) and anti-mesothelin antibody drug conjugate (BAY-94 9343). Chimeric antigen receptor T cell (CAR T) therapy was also evaluated.

Anti-Mesothelin Immunotoxin SS1P

SS1P is an anti-MSLN immunotoxin, which was obtained from immunised mice fused to a truncated form of pseudomonas exotoxin A (PE38). SS1P binding to MSLN formed a complex that was internalised by endocytosis and PE, translocated in cytosol and killed cells catalysing protein synthesis, thus initiating programmed cell death [49]. Studies in vitro have demonstrated the cytotoxic effect of SS1P on the neoplastic cells of patients affected by ovarian carcinomas [50]. In a phase I clinical trial (NCT00066651), patients with ovarian carcinomas presented with a stable disease [51]. In line with cases of mesothelioma, SS1P could be used in combination with chemotherapy to obtain a major response [52].

MORAb-009 (Chimeric Anti-Mesothelin mAb)

MORAb-009 (chimeric anti-mesothelin mAb), also named amatuximab, represents the heavy and light chain variable regions of a mouse anti-mesothelin single chain Fv grafted onto a human IgG1 and k constant region. MORAb-009 has a high affinity with mesothelin and, in a preclinical evaluation, it was demonstrated that it could inhibit the adhesion between the cell lines expressing mesothelin and MUC 16 (Ca 125) as well as causing cell-mediated cytotoxicity on mesothelin-bearing tumour cells [53].

In clinical trials, it has been observed that patients treated with MORAb-009 showed a marked increase in CA125 serum, suggesting that it could block the binding between mesothelin and Ca-125. It was demonstrated that MORAb-009 could inhibit cellular adhesion during metastasis in cases of both ovarian carcinomas and mesotheliomas [54, 55]. Studies on in vivo animal models demonstrated that these effects were markedly increased in combination with chemotherapy agents such as gemcitabine and Taxol [55] or, in a phase II clinical trial, with other chemotherapeutic substances for cases of mesotheliomas (NCT00738582) [56].

Although most studies (NCT01521325, NCT01413451) on ovarian carcinomas focused on the efficacy of a

monotherapy with MORAb-009, these data suggest that a combination with different chemotherapeutic agents could provide satisfactory results.

Anti-Mesothelin Antibody Drug Conjugate (BAY-94 9343)

Anti-mesothelin antibody drug conjugate (BAY-94 9343), known as anetumab ravtansine, is an antibody drug conjugate (ADC) that is a complex consisting of a fully human immunoglobulin G1 anti-mesothelin monoclonal antibody conjugated to the maytansine derivative tubulin inhibitor DM4 through a reducible disulphide linker [57]. BAY-94 9343 has an anti-proliferative activity because, after binding to mesothelin on tumour cells, it is internalised and the disulphide linker is cleaved, releasing DM4. Subsequently, DM4 binding to tubulin disrupts the microtubule polymerisation, causing cell cycle arrest and apoptosis and consequently killing the dividing cells [58, 59].

Preclinical studies have shown that anetumab ravtansine was highly cytotoxic in MSLN-expressing mesotheliomas as well as pancreatic, non-small-cell lung and ovarian cancer cell lines.

Preclinical studies have shown that anetumab ravtansine was highly cytotoxic in MSLN-expressing mesotheliomas as well as pancreatic, non-small-cell lung and ovarian cancer cell lines [57].

In vivo, anetumab ravtansine had an antitumor activity in mesotheliomas as well as pancreatic and ovarian xenograft models [57].

The study of Quanz et al. demonstrated that in ovarian cancer cell lines and patient-derived xenografts, the combination of anetumab ravtansine with pegylated liposomal doxorubicin (PLD) or with carboplatin, copanlisib or bevacizumab showed an additive anti-proliferative activity both in vitro and in vivo compared with either agent used as a monotherapy [60].

Chimeric Antigen Receptor T cell (CAR T) Therapy

MSLN has also been regarded as an attractive target for chimeric antigen receptor T cell (CAR T) therapy because of an abundant expression in tumour cells and a limited expression in normal cells.

CAR T therapy is a type of treatment in which the T cells of a patient, obtained by apheresis, are changed in the laboratory by inserting into them a gene for a special receptor called a chimeric antigen receptor (CAR). The CAR T cells are grown in the laboratory and then given to the patient by infusion. The CAR T cells are able to bind to the antigens on the cancer cells and kill them. Once attached to the antigens present on the neoplastic cells, the CAR T cells become activated and stimulate the host immunosystem, which in turn attacks the MSLN-expressing cells [61]. The effectiveness of CAR T therapy has been observed in mouse models of

different solid neoplasms including ovarian carcinomas and mesotheliomas, in which the chimeric receptors recognise human MSLN, and the inflammatory cytokines secreted by the T cells (including IL-2, IL-6, tumour necrosis factor alpha and Interferon-y) produce cytotoxic effects for the cancer cells [62, 63].

For high-grade serous ovarian cancer (HGSC), investigating the co-expression of CA125, MSLN and folate receptor alpha (FOLRA) on individual tumour cells and their relationship with tumour-infiltrating T cells (TIL), Banville et al. provided insights into the design of logic-gated CAR T cell strategies with a greater number of antigens. They demonstrated that the most promising pairwise combination was CA125 and/or MSLN. Thus, a CAR T cell strategy against CA125 and MSLN would target the majority of tumour cells in most cases. The antigen expression and T cell infiltration demonstrated that this strategy was effective both in primary and recurrent diseases [64].

CONCLUSIONS

In conclusion, a typical expressing pattern of MSLN in normal and cancer tissues makes it a promising target for diagnosis and therapeutic applications. Although many clinical trials regarding MSLN-targeting therapies in ovarian carcinomas are underway, further studies are necessary to establish the effects on the health of patients and behavioural outcomes.

10

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Not applicable.

Conflicts of Interest

The authors declare no conflict of interest

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