Applications of CA125 in Ovarian Cancer

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Abstract. Ovarian cancer is currently the most fatal among gynecological malignancies with a low survival rate, especially in the case of type II tumors. Cancer antigen 125, the primary protein biomarker associated with ovarian cancer, has been researched for its involvement in the ovarian cancer pathogenesis and its potential applications in enhancing both accurate diagnostics and effective therapies. Research has investigated the connections between CA125 and several biological pathways, revealing their associations with cancer cell proliferation and migration. Further research aims to utilize CA125 for a more accurate diagnosis and potential treatments for ovarian cancer. Approaches have been established to address the low specificity of CA125 diagnostic tests, including altering the traditional CA125 value threshold used for ovarian cancer diagnosis and detecting CA125 alongside other antigens. CA125-specific cancer vaccines and immunotherapies are currently undergoing clinical trials and are being developed for future clinical use.

Keywords: Ovarian cancer; CA125; cancer immunotherapies.

1. Introduction

Ovarian cancer ranks as the 18th most common malignancies worldwide, yet it remains the most lethal gynecological malignancy. It is most frequently diagnosed in women aged 55-64. Despite its relatively low incidence rate of around 10.3 cases per 100,000 women each year in the United States, its 2013 to 2019 five-year relative survival rate was only 50.8% [1]. Ovarian cancer has two main types. Type I tumors such as low-grade serous (LGSC) and mucinous carcinomas are typically genetically stable and can often be detected at benign stages. The more aggressive type II tumors, which encompass but are not limited to high-grade serous carcinomas (HGSC) and undifferentiated carcinomas, typically exhibit genetic instability and are frequently linked to mutations in the TP53 gene or BRCA1/2 gene. The late-stage diagnosis of this cancer, especially Type II tumors, contributes significantly to the low survival rate.

Cancer antigen 125 (CA125), or MUC16, is a transmembrane mucinous protein with a substantial degree of glycosylation. It serves as a prominent biomarker, with increased expression identified in a large number of ovarian cancer patients. Over the past three decades, scientists have been exploring various ways to utilize CA125 in ovarian cancer patients. Ongoing extensive research aims to gain further understanding of the physiological functions of CA125 and to enhance its integration into ovarian cancer diagnostics and clinical therapeutic approaches for curing the disease.

2. CA125 Background

2.1. Structure and Functions

CA125/MUC16 originates from the embryonic amniotic layer and is consequently present in adult epithelial tissues, such as the fallopian tubes and endometrium epithelium. It is a kind of complex glycoprotein encoded by MUC16. Its backbone of a large molecular size, consist of an N-terminal region, a tandem repeat segment known as the SEA domain, and a cytoplasmic tail. The protein is also heavily glycosylated with O-linked glycochains in the extracellular domain. Multiple forms of oligosaccharides have been associated with CA125. The biochemical characteristics of CA125 remain a subject of controversy and have yet to be fully understood.

Despite extensive research on CA125 being conducted after it was first discovered, its physiological roles also remain unclear to this day. Researchers have found that CA125 enhances the

proliferation of MUC16+ cancer cells, regardless of the starting level of CA125. MUC16+ cancer cell viabilities and growths can be significantly reduced through MUC16 knock down. CA125 has also been identified as promoting ovarian cancer metastasis by inhibiting the Wnt pathway, which normally controls the migration of cancerous cells [2]. Cell surface MUC16 (csMUC16) has been found to have the ability to prevent cell-to-cell adhesion. Its anti-adhesive quality suppresses the recognition and binding of tumour cells by natural killer cells (NK cells) [3]. Without direct cell-to-cell contact, NK cells would be unable to initiate innate immune responses on the tumour.

2.2. Contributions of CA125 to Ovarian Cancer Pathogenesis

With the slow development of discoveries regarding the fundamentals of CA125, scientists only recently began to investigate its role in ovarian cancer pathogenesis. Liu et al. discovered in their study that, β-catenin, a crucial mediator within the Wnt signaling pathway, contributes to ovarian cancer cell proliferation, particularly in SKOV-3 cells, through interactions with the C-terminus of CA125/MUC16 (MUC16C). Overexpression of MUC16C in SKOV-3 cells increased β-catenin levels in the nucleus while reducing them in the cytoplasm, indicating translocation. The ectopia of β-catenin activated the Wnt signaling pathway, leading to the upregulation of Wnt downstream genes. MTT assays demonstrated that cancer cell proliferation was associated with the overexpression of downstream Wnt genes. The observed reduction in ovarian cancer cell proliferation following the knockdown of the MUC16 gene reinforced this conclusion [4].

On the contrary, Yuan et al. found no significant A2780, or OVCAR-3 ovarian cancer cell proliferation induced by the elevated level of CA125. However, they found that CA125 has increasing effects on both A2780 and OVCAR-3 cell migrations in a dose-dependent manner, in consensus with Qi and colleagues' findings. Yuan et al. found that the suppressive effects of DKK-1, the antagonist of the Wnt pathway, can be reversed by serum CA125 [2]. The lack of inhibitory regulation leads to the hyperactivation of the Wnt pathway, promoting the migrations of ovarian cancer cells. Huo et al. arrived at similar results, determining that the downregulation of DKK-1 in ovarian cancer cells was facilitated through the strong binding between free CA125 and mesothelin. With a limited amount of existing research, understanding of how CA125 contributes to ovarian cancer pathogenesis is still in need of further studies.

3. Current Applications of CA125 in Ovarian Cancer

3.1. Diagnostic strategies

3.1.1 Sensitivity and Specificity

CA125 is a valuable biomarker in the diagnosis of ovarian cancer due to its elevated expression in affected tissues. Serum CA125 level in the bloodstream is used in clinical settings for pre-operative diagnosis of ovarian cancer. Nevertheless, the increase of CA125 also occurs in normal gynecological and benign conditions, resulting in a low diagnostic sensitivity for ovarian cancer of approximately 50% - 62%, a low specificity of around 73% - 77%, and a positive predictive value (PPV) of only approximately 1% [5]. When CA125 was first studied as a tool for early ovarian cancer detection by van der Burg et al., their research indicated that two consecutively elevated CA125 levels exceeding 35 U/mL could serve as an indicator of ovarian cancer progression [6]. This threshold of 35 U/mL has remained in use to the present day. When comparing this threshold with CA125 levels in patients with stages III and IV endometriosis, their CA125 levels were found to be elevated up to 66.5 ± 14.5 U/mL, surpassing the established threshold [7]. Other factors that may result in higher-than-normal CA125 concentrations include pelvic inflammatory disease and tubo-ovarian abscesses, with abscesses being another benign condition that typically exceeds this cut-off [8]. With the protein level affected by multiple factors, CA125 concentration alone is not enough to solidify an accurate ovarian cancer diagnosis.

3.1.2 Improvements to diagnosis

One proposed solution trying to mitigate the low specificity of CA125-determined diagnosis is to modify the CA125 threshold value. Musalhi et al. increased the threshold from 35U/mL to 82U/ml. This rise in the threshold resulted in a 24% increase in specificity but an 8% decrease in sensitivity. The cut-off of 162U/mL changed the specificity from 24% to 75.4%, at the cost of a decrease from 96% to 78% in sensitivity [9]. Other studies shared similar trends of bettered specificity and overall accuracy with a reduction in sensitivity.

To further increase the specificity of the diagnosis test without forfeiting the sensitivity, researchers distinguished between patients with elevated CA125 levels using additional antigens. Using antibody-lectin ELISA assay, Wang et al. found that the lectin VVA, which has a high binding affinity to Thomsen-nouveau antigen (Tn antigen), has the best ability to differentiate benign conditions or borderline tumours and ovarian cancer among all lectin tested. Tn antigen is a simple O-glycan, found with increased expression in malignant conditions. Using CA125-Tn instead of purely CA125 for ovarian cancer diagnosis, Wang and colleagues significantly improved the specificity from 35.1% to 75.7% among patients 45 and older with a fix sensitivity of 90%. The overall specificity of diagnosing patients of all ages was increased from 20.1% to 58.3% [10]. The method used in their study is considered applicable in clinical settings as it requires a limited amount of serum CA125 for glycosylation analysis.

It is also proposed that CA125 levels in premenopausal and postmenopausal women should be regarded distinguishably. Van Calster et al. discovered that CA125 levels were higher in benign tumors among premenopausal women, while postmenopausal patients with malignant tumors exhibited elevated CA125 levels. Their study also developed likelihood tables of gynecological conditions by employing CA125 concentrations as predictive factors, separately for premenopausal and postmenopausal individuals, based on statistical analysis of historical patient data [8]. Instead of relying on a static threshold value, they suggested that serum CA125 blood test results should only be regarded as a predicting index.

3.2. Therapeutical application of CA125

Ovarian cancer is clinically treated with chemotherapy medication such as paclitaxel. Nevertheless, with the current frontline treatment approaches, ovarian cancer demonstrates a substantial relapse rate, while the low remission rate contributes to the high mortality of this cancer. Researchers aim to develop new strategies, such as immunotherapies and combination therapies to reduce the risk of recurrence and increase the survival rate.

3.2.1 Anti-Id ACA125 vaccines

A murine monoclonal antibody (mAb) called ACA125 imitates CA125 function and can induce cellular and humoral immunity responses. The phase I clinical trial conducted by Reinartz and their team, involving a limited cohort of seven patients with advanced ovarian carcinoma, demonstrated a significant rise in intracellular Th1 cytokine production following ACA125 vaccination therapy. In one particular patient, IFN-γ amplified from 12.7% production to 61.2%, and IL-2 from 2.0% to 21.0% [11]. Similar trends of improved cytokine production could be found in other patients, which reaffirms the cellular and humoral immunity development.

Reinartz and colleagues completed a phase I/IIb trial for the evaluation on the impact of anti-idiotypic antibody (Ab2) vaccine ACA125, in continuation of their previous study. 68% of the patients displayed induced anti-anti-idiotypic (Ab3) antibodies. Using Ab3 antibodies as a surrogate marker, patients who are identified as Ab3 responders have a remarkably prolonged median survival time of 23.4 months, in comparison to the median survival time of 4.9 months observed in Ab3 non-responders. The improvement in survival is consistent across all ages. 50.4% of the patients in their study developed CA125-specific antibody (Ab1') or Ab1' immunocomplexes. Similarly, patients with positive Ab1' responses had statistically significant longer survival time [12]. Further studies and clinical trials are encouraged for this ACA125 vaccination therapy as confounding variables such as

simultaneous antitumor therapies are present in this study. In the study by Sabbatini et al., they further confirmed the increasing Ab3 titers post injections of ACA125, now developed to be called Abagovomab. After the abagovomab immunization, Ab3 and human antimurine antibody (HAMA) responses were significantly amplified at week 10 and were maintained. In both ACA125 clinical trials mentioned above, the vaccination of ACA125/Abagovomab, was found to be safe and had not caused any adverse events in the course of the treatments [13].

3.2.2 Oregovomab

Oregovomab (MAb-B43.13) is a modified monoclonal antibody originated from mouse. Due to its high affinity for CA125, current ongoing clinical trials aim to further explore its ability to elicit CA125-specific immune responses. A prompt decrease in serum level of CA125 in patients after they received their first injection of oregovomab during their phase I trial was observed. After multiple injections, a three-times increase in the anti-CA125 antibodies level and a notable elevation in T-cell responses were shown in 43% and 53% of the patients respectively [14]. The phase II trial by Brewer and colleagues examined the simultaneous administration of carboplatin–paclitaxel chemotherapy and oregovomab infusion. Their study revealed a median progression-free survival (PFS) time as 41.8 months in patient who received oregovomab, in comparison to the median PFS of 12.2 months in the those treated with solely chemotherapy [15]. Similar trends regarding PFS were observed in Berek and colleagues' phase II clinical trial. Their study also detected an elevation in Ab2 and HAMA response in most patients by their third oregovomab injection, although the concentrations of Ab2 and HAMA varied largely between patients. Notably, there was no significant disparity in time to recurrence (TTR) between the oregovomab group and the control. In all of the mentioned clinical trials, MAb-B43.13 immunizations were found to be safe and could be well tolerated. Similar research has confirmed the great potential for oregovomab in the combined application with carboplatin/paclitaxel chemotherapy to be promising for eliciting a humoral immune response and inducing Ab2 and HAMA in recurrent ovarian cancer as well.

MAb-B43.13 has been proven to increase PFS and overall survival when used in combination with chemotherapy. However, in Brewer and colleagues' phase III trial using oregovomab as a mono-immunotherapy, they failed to find improvements from the placebo group. The PFS showed comparable outcomes between the oregovomab group and the placebo group. In comparison with their previous study when oregovomab was used along with chemotherapy, the HAMA concentrations after each injection were all significantly lower in this trial.

3.2.3 Chimeric antigen receptor therapy

CA125, MSLN, and FOLRA are the three antigens identified as exhibiting elevated expressions in individuals with ovarian cancer, especially in those with high-grade serous carcinomas (HGSC), making them promising targets for chimeric antigen receptor (CAR) T-cell therapy. Logic-gated CAR T therapies that target MSLN and FOLRA are current under clinical trials. However, new studies propose that expressions of these antigens exhibit heterogeneity according to the location within and of the tumour sampled. Thus, Banville et al. suggested that it would be best to use OR-gated CARs, which expand the target range by recognizing more than one antigen. Their study indicated that over 90% of HGSC patients express CA125 and/or MSLN in their cancer tissues. The common co-occurrence of CA125 and MSLN in HGSC can be exploited effectively in OR-gated CAR T cell therapies [16]. CAR T-cell therapy itself is a highly promising cancer immunotherapy that has been extensively developed in recent years. It significantly reduces the risk of toxicity and harm to healthy cells compared to other cancer treatments like chemotherapy. The discovery by Banville and colleagues illuminates a novel approach that has the potential to treat HGSC and other advanced ovarian cancer types by harnessing antigen expression heterogeneity.

4. Conclusion

Among all gynecological malignancies, ovarian cancer stands as the primary cause of mortality. Late-stage diagnoses of aggressive type-II tumors contribute the most of ovarian cancer-related deaths. CA125, the role of highly glycosylated mucinous protein and how it can help facilitate an earlier diagnosis and more effective treatment has been investigated in the development of ovarian cancer. Researchers have conflicted over whether CA125 leads to ovarian cancer cell proliferation but agreed upon its influence in promoting cancer cell metastasis, although focusing on different pathways. When used for ovarian cancer diagnosis, the CA125 test has a low specificity and a low sensitivity. Ways to increase specificity include increasing the traditional cut-off value of 35U/mL, which increases specificity at the cost of sensitivity, and using additional Tn antigen along with CA125 to increase specificity without decreasing the sensitivity. Among all therapeutical applications of CA125 in treatments of ovarian cancer, ACA125 (abagovomab) vaccinations and chemo-immunotherapy with oregovomab have both shown promising results in inducing immune responses and increasing survival time. However, oregovomab has failed as a mono-immuntherapy with effects undifferentiable from the placebo treatment. Further research is needed to study their clinical applicability and efficiency before applying CA125 as therapeutical approach for ovarian cancer as CA125 remains relatively poorly understood.

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