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CA125 in Ovarian Cancer

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Summary

Twenty five years after its discovery, circulating CA125 antigen is recommended for clinical use in the US for ovarian cancer (OC) screening of high risk women with ovaries despite its limited sensitivity and specificity. Recent findings suggest that CA125 might also serve as a predictive marker for pre-invasive OC. Methods to quantify circulating CA125 evolved towards sensitive and reliable double determinant ELISA assays. The CA125 gene, *MUC16*, was cloned 20 years after the protein discovery and revealed a very complex and unusual glycoprotein structure suggesting an immunological role. Recent evidence points toward CA125 function in the induction of materno-fetal tolerance through the alteration of NK phenotype. Two receptors for CA125 have been described: mesothelin and galectin-1. The specific location and functional proprieties of CA125 make it a therapeutic target of choice; clinical trials have demonstrated that anti-CA125 injections are well tolerated and suggest a potential survival benefit.

Keywords

CA 125; biomarker; detection; disease monitoring; ovarian cancer; pregnancy; Glycoprotein; NK; mesothelin; galectin-1

INTRODUCTION

CA125 is one of the earliest biomarkers for cancer

The discovery of OC125, an antibody that recognizes CA125, was made by Bob Bast and his colleagues in 1981 [1] only a year after prostate-specific antigen (PSA) was first measured quantitatively in the blood by Papsidero [2,3]. Recognition of CA125 as a circulating antigen opened the door to biomarker research for ovarian carcinoma, now a flourishing and potentially clinically significant field.

CA125 is expressed as a membrane-bound protein at the surface of cells that undergo metaplastic differentiation into a Müllerian-type epithelium [4,5], or released in soluble form in bodily fluids. CA125 concentration in bodily fluids parallels certain physical conditions. For example, CA125 is still the most extensively studied biomarker for possible use in the early detection of ovarian carcinoma (OC), and it has proved valuable in both detection and disease monitoring [6–11]. But there have also been reports of elevated levels of soluble CA125

in a number of other malignant conditions such as breast cancer, [12,13], mesothelioma, [14], non-Hodgkin lymphoma (NHL), [15–19], gastric cancer [20], and leiomyoma and leiomyosarcoma of gastrointestinal origin [21]. CA125 levels have also been found elevated in benign conditions [22,23] such as endometriosis [24–26], pregnancy [27–30], ovulatory cycles [31], liver diseases and congestive heart failure [32–37], as well as in infectious disease such as tuberculosis [38–41].

The complex structure of CA125 dominated by repeat sequences made it possible to create a first generation of diagnostic assays with only one mAb in the role of both capture and detection directed against the OC125-like epitope [1,42,43], but at the same time strongly challenged our structural comprehension. After 20 years of research, the structure of CA125 has been described [44–48] but its function remains a fascinating yet speculative field.

STRUCTURE AND FUNCTION OF CA125

CA125 Gene

Two research groups cloned the gene encoding CA125 protein [44,47–49] and revealed that CA125 is a membrane protein with some splice variants sharing the same intracellular and transmembrane regions. The CA125 gene was named *MUC16*, after the mucin-like nature of CA125. This feature includes a high serine, threonine, and proline content in an N-terminal region of nine partially conserved tandem repeats (156 amino acids each) and a C-terminal region non-tandem repeat sequence containing a possible transmembrane region and a potential tyrosine phosphorylation site. CA125 is conserved in evolution [50–53].

The biochemical analysis of CA125 revealed that it is a mucin-type *O*-linked glycoprotein [54,55] of high molecular mass estimated at 2.5 to 5 Mio Daltons under natural conditions [56], although smaller subunits have been reported [48,56,57]. CA125 is heterogeneous with regard to both size and charge. The oligosaccharides linked to CA125 present unusual features such as the expression of branched core 1 antennae in the core type 2 *O*-glycans, as well as a robust *N*-glycosylation, primarily high mannose and bisecting type *N*-linked glycans including Man5-Man9GlcNAc2 [45]. Several subspecies of CA125 were described [58], however it is not known whether any of the CA125 subspecies is linked to specific physical conditions.

The core CA125 subunit retains the capacity to bind both OC125 class antibodies and M 11 class antibodies. Denatured purified subspecies of the CA125 molecule appear to autoproteolyse, presumably due to an endogenous protease activity inherent to the molecule. Release or secretion of CA125 appears directly linked to the epithelial growth factor receptor signal transduction pathway. Prior to its release from cultured cells, CA125 is phosphorylated within its transmembrane domain at either/both serine and threonine which leads to cleavage by a prospective extracellular protease at the membrane surface [49].

The CA125 extracellular domain consists of the SEA domains repeated 7, 12, or 60 times, according to the variant. SEA domains consist of about 120 residues, of which about 80 residues are highly conserved and were first identified in Sea urchin sperm protein, Enterokinase, and Agrin. SEA domains exist in other molecules that are mostly membrane proteins but, in contrast with CA125, they usually have only one SEA domain. SEA domains are highly positively charged proteins, suggesting that they can bind negatively charged molecules such as nucleic acids or acidic sugars [59]. SEA domains are always located in the extracellular region and are often accompanied by an *O*-linked glycochain at the N-terminal side. They have been classified into several subfamilies, suggesting that each subfamily has a different function [46]. The SEA domains in *MUC16* are apparently more similar to each other than to any other SEA domains, suggesting that the multiplication of the SEA domains occurred after *MUC16* appeared [46].

CA125 Function

Although a large body of work has been undertaken to analyze its function, the role of CA125 in health and in disease remains poorly understood. The unusual features of the oligosaccharides linked to CA125 suggest a role for CA125 in cell-mediated immune response [45]. There is evidence that CA125 attenuates complement lysis of antibody-sensitized cells [60]. In addition, CA125 bisecting type biantennary oligosaccharide can inhibit the cytotoxic responses of human natural killer (NK) [61,62] and this inhibition correlates with a severe reduction in CD16 (FcγRIII) expression on the NK cell surface [63,64]. *In vitro* inhibition of cytotoxic responses can be obtained at concentrations of native purified CA125 that are expected to be significantly lower (10,000–100,000 U/ml) than those observed in the tumor microenvironment [63], which suggests that tumor-derived CA125 acts as a suppressor of the anti-tumor immune response.

CA125 could play a role in altering the phenotype of NK cells, maybe through direct binding to these or other immune cells [64]. The binding of CA125 to the NK cells was also observed in pregnant women [64]. The attachment mechanism of CA125 to NK is not well understood yet, but several leads are under investigation [64] including binding *via* galectin-1, a member of the family of β-galactoside binding proteins that has growth regulatory and immunomodulatory activities [65–68], *via* autoantibodies against CA125 forming complexes with soluble CA125 that may be retrieved by CD16, or *via* CA125 terminal sialic acid residues [45] that may be recognized by Siglec-9, an NK inhibitory receptor [69]. Taken altogether, the NK suppressive effect of CA125 [63], its increased titers during pregnancy [27–30], its binding to NK of pregnant women [64], its gene overexpression in the proliferative phase of the human endometrium, just prior to the detection of NK cell specific genes in this tissue [70] and the well established role of regulatory NK cells in the maintenance of pregnancy [71] suggest a role of CA125 in the prevention of immunological rejection of the fetus.

CA125 also binds to mesothelin, [72–74] a 40 kDa protein expressed by ovarian, lung and pancreas cancer cells but also by normal mesothelial cells [74–77]. The interaction between mesothelin and CA125 proteins could play an important role in the peritoneal implantation of ovarian tumor cells by promoting cell attachment between CA125-expressing tumor cells and the peritoneal lining that constitutively expresses a membrane-bound form of mesothelin [78–80].

CA125 AS A BIOMARKER FOR OVARIAN CANCER

Ovarian cancer is the second most common and the most lethal gynecologic malignancy in the U.S. Epithelial OC comprises the majority of malignant ovarian tumors in adult women. Over 70% of women with OC are diagnosed with advanced stage disease [81] when 5-year relative survival is 30%. Five-year survival is 90% when disease is confined to the ovaries but overall survival is poor because only 25% of cases are found in this early stage. Surgery and chemotherapy are initially effective for 80% of patients but the disease recurs and becomes increasingly difficult to treat in most women with advanced disease at the time of diagnosis. Incidence rates remain high and mortality rates are virtually unchanged over the last 30 years despite rising rates of oral contraceptive use and prophylactic surgery in high-risk (HR) women with a family history suggesting a mutation in BRCA1 or BRCA2. Until recently the natural history of OC has been only poorly understood, but increasingly careful examination of tissue from women undergoing prophylactic surgery has been enlightening. In women with a documented mutation in BRCA1 or BRCA2, occult malignancy of serous histology accompanied by intraepithelial carcinoma or dysplasia is frequently found in the fimbrial end of the fallopian tube (FT) at the time of prophylactic surgery [82–85]. These findings suggest that serous OC may originate in the FT in mutation carriers, and that tubal intraepithelial carcinoma (TIC) and/or tubal dysplasia may represent precursor lesions for this disease. It is

likely based on recent reports [84] that some serous OC originates in the FT in sporadic cases as well.

The quantification of soluble CA125 levels is currently performed with a second generation of assays based on double determinant ELISA tests that use two monoclonal antibodies (mAb) directed against the epitope groups M11 and OC125 [86–88].

Anti-CA125 antibodies are divided into three groups, OC125-like (group A), M11-like (group B), and Ov197-like which recognize domains of nonoverlapping epitopes [44,89,90]. The OC125-like antibodies can be subgrouped into 4 groups [91]. All three types of antibodies can recognize either native or denatured CA125 [49]; however, antibodies of the group A4 and B are best to detect denatured CA125 immobilized on a membrane [90]. The epitope site of the M11 antibody is a peptide between two conserved cysteine residues in the SEA domain [49]. Although some antibodies are able to bind differentially to CA125 of high or low molecular weights [58], and OC125 exhibits a reduced binding after treatment with PNGase F [92], currently available anti-CA125 antibodies do not permit fine discrimination among various CA125 species.

Commercial kits, now supplied by various manufacturers (and in different versions, e.g. IRMA, EIA, etc.) are currently widely applied in the following clinical situations: (i) Monitoring of disease: Doubling or halving of CA125 serum values correlated in 87% of all cases with ovarian tumor progression or regression, respectively; (ii) Early prediction of outcome: Deviation from the ideal CA125 regression curve predicts poor outcome within 3 months of cytostatic treatment; (iii) Tumor status after completion of therapy: Most patients with CA125 > 35 U/ml have tumor present at second look surgery while half of the patients with CA125 < 35 U/ml have only minimal residual disease; (iv) Early detection of recurrence: After a complete remission, a rise in CA125 precedes tumor recurrence in 75% of all patients, with lead times up to more than 1 year; (v) Diagnosis and differential diagnosis when used alone or in combination with other markers [6].

CA125 as a biomarker for early detection of ovarian cancer

Early detection is an attractive approach to reducing mortality from OC but the translational research community faces many challenges. Screening for OC using tools with high sensitivity is potentially cost-effective [93] but because OC is so rare, very high specificity is needed to achieve an acceptable positive predictive value (PPV). Overall, incidence rates in the post-menopausal population are about 45 per 100,000 in the U.S. Thus to achieve a PPV of 10% a screening test with 80% sensitivity would need to have specificity of 99.6%.

Despite these challenges, CA125 is used clinically in the U.S. Practice guidelines [94] recommend ovarian cancer screening starting at age 35, or 5–10 years earlier than the earliest age of ovarian cancer diagnosis in the family, for HR women who have not elected prophylactic surgery. Transvaginal sonography (TVS) and the serum marker CA125 are often used every 6–12 months. CA125 is elevated in the serum of most women with OC, but pre-operative serum levels of CA125 are below the conventional cutoff of 35 U/ml in roughly 50% of clinically detected stage I cases [95] and in the majority of women with occult cancers identified at prophylactic surgery [96]. The ability of TVS to identify OC while it is still curable is similarly debatable, as a substantial proportion of women diagnosed with advanced stage, serous OC have normal appearing ovaries by TVS as little as 3–12 months prior to clinical diagnosis [97]. In addition, despite frequent screening in the HR population, when TVS detects ovarian malignancy the disease is often advanced [98].

Reports suggest that sensitivity for early stage disease is limited in the HR population even when both CA125 and TVS are used together in a multimodal strategy. Hogg reviewed findings

from 12 studies using CA125 and TVS to screen over 6,000 HR women [99]. Excluding borderline and germ cell tumors there were 38 ovarian cancers identified, only 9 of which were stage I; 15 cancers diagnosed within a year of a screen were missed by both CA125 and TVS. Similarly, Stirling identified 2 Stage I invasive cancers among 12 ovarian cancers detected in a cohort of 1,100 HR women participating in a screening program [100]. To improve sensitivity while maintaining good specificity, the Risk of Ovarian Cancer Algorithm (ROCA) was developed for use in a multimodal screening strategy [101]. The ROCA uses a change point model to interpret longitudinal CA125 values in the context of other variables including age and menopausal status, in order to stratify women based on their risk of having OC at the time of the screen. Women are asked to return for repeat CA125 testing and/or TVS screening if their CA125 levels are abnormal.

Two prospective screening trials targeting HR women are currently underway. Neither includes a control arm as it is considered unethical to randomize HR women to a non-screening arm. Both use the ROCA to estimate an individual's risk of having OC based on serial CA125. The U.K. Familial Cancer Screening Study is screening over 1500 HR women using annual CA125 and TVS testing. In the U.S., the Cancer Genetics Network Risk of Ovarian Cancer (CGN/ROCA) study is screening over 2,200 HR women at multiple centers. CA125 is measured quarterly to stratify women using ROCA. Women at usual risk return to routine screening, intermediate risk women are referred for TVS, and elevated risk women are referred for TVS and subspecialty consultation. TVS is performed annually at some centers.

Recent research has sought to improve on existing screening methods by adding known or novel markers to a panel that includes CA125. A number of novel candidate markers have emerged including CA 72-4 and M-CSF [102], HE4 [103], Mesothelin [72], the kallikreins 6, 10 and 11 [104], and B7-H4 [105]; some can be detected by immunohistochemistry in OC tissue that does not express CA125 [106]. HE4 is less likely than CA125 to be elevated in women with benign tumors [103], and a panel combining HE4 with CA125 (both on a bead-based platform) performs better than either marker used alone [88].

Longitudinal algorithms have also been proposed to improve performance of CA125 as an early detection marker. The lead time gained by screening is a function of the characteristics of the screening tests used, screening frequency, and the decision rule(s) used to select women for definitive diagnostic procedures. A decision rule that incorporates marker history can potentially improve lead time for markers that are relatively stable over time within women (lower variability within than between women), because smaller changes in these markers' levels are needed to distinguish signal from noise. ROCA uses a change point model to interpret longitudinal CA125 values in the context of other variables including age and menopausal status, in order to stratify women based on their risk for OC at the time of the screen. In a prospective screening trial of 6,682 average-risk women the specificity and PPV for ROCA at the prevalence screen were 99.8% and 19% respectively [107], a significant improvement in screening performance compared to a single threshold rule such as above 30 U/ml. A parametric empirical Bayes (PEB) approach has also been proposed [108,109] to tailor the screening decision rule to the individual woman. It provides a positive result at lower levels of CA125 by accounting for marker history within each woman [108] without any sacrifice in specificity [109]. Cut-off levels assigned by the PEB are lower for most women than a single threshold rule with comparable specificity [109], yielding longer lead times for screen-detected cancers. The PEB approach can be easily generalized to a panel that includes novel markers such as HE4.

CA125 as biomarker for risk of ovarian cancer

CA125 is the only serum marker that has been evaluated in preclinical serum markers, allowing it to be classified as a risk marker as well as a diagnostic and potentially an early detection

marker. The literature is consistent in its evidence that CA125 is a predictive marker that becomes increasingly powerful with proximity to diagnosis. Two decades ago, CA125 levels were evaluated in the JANUS serum bank for 105 women who subsequently developed ovarian cancer and 323 matched controls [110]. Median CA125 levels were 18.0 over 5 years prior to diagnosis in women with OC, but only 10.9 in healthy women. Within 18 months of diagnosis, median CA125 was 27.2. A case-control study using the same data demonstrated that CA125 is >30 U/ml in 50% and 24% respectively of patients up to 18 and 60 months before diagnosis [110].

More recently and with longer follow up, preclinical CA125 levels were estimated for 668 ovarian cancer patients (478 invasive and 190 borderline) and 1989 matched controls, using the JANUS databank and a nested case-control design. Among the 478 ovarian cancer patients, over the entire period 15% had elevated CA125 at the time of serum sampling, while 6% of the controls were positive. A statistically significantly higher-risk of OC was observed in women with elevated CA125 (OR = 3.1). Importantly, restricting the analysis to cases with serum sampling within 2 years of diagnosis and matched controls gave a higher OR of 13.0 [111]. In the same study, the OR for a BRCA1 mutation was estimated to be 29. The ability of CA125 to identify women destined to be diagnosed with OC within the next 2 years is an important finding that has not been adequately explored for its translational potential. Evidence from the JANUS databank is supported by a UK cohort study of 49 incident cancers observed in 22,000 post-menopausal women age ≥ 45 observed for 6.8 years. Elevated CA125 (>30 U/ml) was a powerful predictor of subsequent disease (RR=35.9, 95% CI 18.3–70.4 within 1 year and RR=14.3, 95% CI 8.5–24.3 within 5 years) [112].

These data suggest that CA125 could serve as a *predictive marker* for OC, and generate the hypothesis that CA125 signals precursor lesions such as adnexal dysplasia. Support for this hypothesis comes from a study of serum CA125 level for the prediction of adnexal dysplasia and cancer in women at hereditary HR [113]. CA125 was obtained from 424 women at hereditary HR of ovarian/tubal cancer attending the VU University Medical Center (Amsterdam, the Netherlands) between 1993 and 2005. Serum samples obtained at the second-to-last (n = 64) and last (n = 98) visit before surgery were tested in women who underwent adnexal surgery for diagnostic (n = 9) or prophylactic (n = 89) reasons. Serum samples obtained from 370 age-matched healthy women were used as controls. Both the absolute value ($P < .0001$) and the serial change ($P < .0001$) of CA125 were predictive for OC (n = 8). For adnexal dysplasia (n = 23), the absolute value of CA125 ($P = .003$) was predictive, but the serial change in CA125 was not ($P = .32$). The odds ratio for adnexal dysplasia versus nondysplasia in the highest tertile (CA125 levels > 14 U/mL) compared with the lowest tertile (CA125 < 10 U/mL) was 6 (95% CI, 1.32 to 36.66). The authors conclude that in HR patients both the absolute value of serum CA125 and the change in serial CA125 are predictors for cancer, and that the absolute value of CA125 is predictive for adnexal dysplasia. They recommend that CA125 values be taken into account in the decision to elect prophylactic surgery.

CA125 AS A THERAPEUTIC TARGET

Because OC occurs in the peritoneal cavity, the regional administration of therapy is possible and theoretically attractive. Biologic therapies for EOC are being developed and ongoing clinical trials are investigating the use of CA125 as a target for immunotherapy.

Oregovomab (OvaRex monoclonal antibody [MAb]-B43.13; Unither Pharmaceuticals, Wellesley Hills, MA) is an immunotherapeutic agent for investigational use in the immunotherapy of patients with ovarian adenocarcinomas expressing CA-125. The active component of oregovomab is the modified murine MAb-B43.13, an IgG_{1k} subclass immunoglobulin. MAb-B43.13 binds with high affinity ($1.16 \times 10^{10}/M$) to CA-125[114].

Oregovomab behaves as an active immunotherapeutic agent through a unique mechanism. The antibody forms immune complexes with serum soluble CA125 and the CA-125/B43.13 complex binds to antigen-presenting cells such as macrophages and dendritic cells. The binding of the antibody/antigen complex is more efficient than either antibody or tumor antigen alone. The components of the complex are cross-presented in the context of both class II and I major histocompatibility complex which triggers induction of CA125-specific immune responses, including anti-CA125 antibodies against various epitopes and CA125-specific B and T cell responses [115,116], but also CD4 and CD8 T-cell responses specific for B43.13. Administration of a technetium-99m-labeled anti-CA-125 monoclonal antibody (B43.13) after immunoscintigraphy was shown to increase the survival time of EOC patients [117,118]. Clinical studies demonstrated that oregovomab is well tolerated. In addition, it induced immune responses to CA125 maintained during concomitant chemotherapy [119] that were correlated with a significant survival benefit [120–122].

Another type of biologic therapy based on anti-idiotypic vaccine has been more recently undertaken using **abagovomab** (formerly ACA125) in patients with epithelial ovarian, fallopian tube or primary peritoneal cancer. This antibody functionally mimics the CA125 antigen and induces humoral and cellular CA125-specific immunity [123,124]. A phase I study demonstrated that this approach too is well tolerated by patients [125]. A study in a mouse model system suggests that the co-injection or the fusion of IL6 to the anti-idiotypic antibody could improve vaccination efficacy [126].

Targeted therapies with anti-CA125 antibodies conjugated to cytotoxic drugs are currently under study in animal models. A recent publication compares the toxicity and the efficacy of two antibodies, one directed against a unique epitope in the extracytoplasmic domain of CA125 and the other directed against CA125 repeat sequences and reports superior efficacy *in vitro* and *in vivo* without compromising safety of targeting the repeat CA125 domains [127]. Finally, efforts have been undertaken to develop anti-CA125 antibodies specific for the cell-associated form of the antigen, which is of particular interest for targeted therapy [128].

CONCLUSIONS

CA125 is a biomarker that has potential utility across the spectrum: risk, early detection, diagnosis, prognosis, monitoring and therapy.

CA125 structure has challenged biochemists for 2 decades but has recently been described. Yet, the complexity of its post-translational modifications, and in particular of its glycosylations, necessitates more effort to explore possible links between specific glycosylation variants and physiological or disease states.

CA125 function is barely understood but fascinating. Some recent evidence points toward a potential immunosuppressive role of this complex glycoprotein.

Finally, CA125 represents an attractive therapeutic target and numerous groups have been developing various approaches, including antibodies against unique or repeat domains of CA125, anti-idiotypic antibodies, antibodies specific for the membrane-bound form of CA125, and antibodies naked or coupled to cytotoxic drugs or fused with cytokines such as IL6. All these approaches present great potential and should be aggressively pursued, particularly considering the grim prognosis of OC.

FUTURE PERSPECTIVE

Tumors actively release or induce the secondary release of a wide range of soluble factors that contribute to peripheral tolerance and tumor escape [129,130]. To do so, they often utilize pre-

existing mechanisms of immunotolerance, such as the release of cytokines (IL10, IL-6, IL-8, TGF and VEGF), gangliosides, prostaglandins and matrix metalloproteases that can influence both the activity and maturation of immune cells or influence the degradation of the extracellular matrix and the regulation of angiogenesis. Recent data published by various groups suggest that CA125 contributes to the prevention of the immunological rejection of the fetus through its interaction with NK cells, and to the immune-evasive traits of ovarian epithelial cancer cells. Thus, understanding the mechanisms that trigger the release of CA125 from the cell surface, such as phosphorylation events or binding to soluble receptors such as mesothelin or anti-CA125 autoantibodies might improve immunotherapeutic responses. Alternatively, blocking the binding of CA125 to its receptors through competition such as antibodies, biobodies [131] or small molecules, or alteration of CA125 glycosylation might improve the evolution and the prognosis of OC. Finally, understanding the immunosuppressive strategies developed by CA125 could be of utility to design novel therapies for autoimmune and inflammatory disorders.

EXECUTIVE SUMMARY

The need for ovarian cancer (OC) early detection

- Ovarian cancer is often diagnosed at late stage when the disease is rarely cured.
- CA125, one of the earliest identified biomarkers for cancer, remains the most useful ovarian cancer serum marker despite its limited sensitivity in early stage disease and its inadequate specificity for malignancy.

Structure and function of CA125

- Mucin-type *O*-linked glycoprotein of high molecular mass with various subspecies and unusual features (branched core 1 antennae in the core type 2 *O*-glycans) and robust *N*-glycosylations.
- The MUC16 gene was identified in 2001.
- Novel evidence suggests a role in immunological tolerance.

CA125 as a biomarker for ovarian cancer

- Quantification of soluble CA125 is possible using available antibodies and kits
- CA125 is not sensitive or specific enough to be used alone as an early detection marker; in combination with other markers it may be useful for risk assessment and/or early detection.

CA125 as a therapeutic target

- Oregovomab has been tested in human trials suggesting that it is well tolerated and potentially efficacious
- Abagovomab has been shown in Phase I trial to be safe in humans
- Targeted antibodies and other approaches, including preventing binding of CA125 to mesothelin, are under investigation.

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