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Detection of glyco-mucin profiles improves specificity of MUC16 and MUC1 biomarkers in ovarian serous tumours

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ABSTRACT

The CA125 assay detects circulating MUC16 and is one of the most widely used cancer biomarkers for the follow-up of ovarian cancer. We previously demonstrated that detection of aberrant cancer-associated glycoforms of MUC16 as well as MUC1 in circulation could improve the yield of these serum assays. Our aim was to refine ovarian cancer biomarkers by detection of aberrant glycoforms (Tn, STn, and T) of MUC16 and MUC1 in ovarian cancer tissue using Proximity Ligation Assays (PLA).

We studied two series of serous ovarian tumours, a pilot series of 66 ovarian tumours (27 cystadenomas, 16 borderline tumours and 23 adenocarcinomas) from Centro Hospitalar S. João, Porto and a validation series of 89 ovarian tumours (17 cystadenomas, 25 borderline tumours and 47 adenocarcinomas) from the Portuguese Institute of Oncology Francisco Gentil, Lisbon. PLA reactions for MUC16/Tn, MUC16/STn, MUC1/Tn and MUC1/STn were negative in benign lesions but often positive in borderline and malignant lesions, in both series. An even better yield was obtained based on positivity for any of the four glyco-mucin profiles, further increasing sensitivity to 72% and 83% in the two series, respectively, with 100% specificity. The strategy is designated glyco-mucin profiling and provides strong support for development of PLA-based serum assays for early diagnosis.

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1. Introduction

Worldwide, ovarian cancer is the 7th cancer with highest incidence and the 8th cause of cancer death in women (Ferlay et al., 2012). The large majority of ovarian cancers are epithelial in origin and, within those, serous carcinomas constitute nearly 70% (Seidman et al., 2004) and represent the most relevant contributors to ovarian cancer mortality (Prat, 2012). Despite the downward trends in survival (Barnholtz-Sloan et al., 2003; De Angelis et al., 2014) and in mortality rates (Hirabayashi and Marugame, 2009) observed in the last decades in many settings, there is a large heterogeneity in the across countries and an ample margin for improving diagnosis at treatable stages. This may be accomplished through the development of new strategies or combination of existing methods, capable to overcome limitations of those currently available.

The CA125 assay is the most successful cancer serum biomarker and it detects the large mucin MUC16 (Bast et al., 1981), which is heavily decorated with N and O-glycans (Hattrup and Gendler, 2008). The CA125 assay is approved for monitoring ovarian cancer patients after treatment to predict recurrence but not for diagnostic purposes due to low specificity (Goonewardene et al., 2007). More recently, attempts were made to combine several tumor markers to increase sensitivity and specificity of cancer detection (Kondalsamy-Chennakesavan et al., 2013), but CA125 is still the preferred biomarker (Cramer et al., 2011; Mai et al., 2011; Zhu et al., 2011). Several other membrane-bound mucins, including MUC1 and MUC4, are overexpressed in ovarian carcinomas, although these are more widely expressed in other organs (Singh et al., 2008; Skates et al., 2004). Increased serum levels of MUC16 in non-malignant gynecological conditions and diseases, especially in those that produce ascites, are serious limitations to the use of serum CA125 assay as a diagnostic tool. The CA125 assay is based on detection of MUC16 with monoclonal antibodies such as OC125 (Bast et al., 1981) and M11 (Nustad et al., 1996) that react with similar epitopes in the tandem repeat SEA region of protein (Bressan et al., 2013; Marcos-Silva et al., 2014). We recently confirmed that these antibodies bind the protein backbone, albeit a particular conformational epitope, without substantial influence by glycosylation (Marcos-Silva et al., 2014).

Mucins are characterized by dense decoration of O-glycans and these O-glycans themselves may serve as biomarkers. Thus, perhaps the most common phenotypic character of carcinoma cells is expression of truncated immature O-glycans due to a variety of mechanisms leading to incomplete O-glycosylation (Gill et al., 2011). Several studies showed expression of T (Galβ1-3GalNAc-α1-O-Ser/Thr), Tn (GalNAcα1-O-Ser/Thr) and STn (Neu5Acα2-6GalNAcα1-O-Ser/Thr) in carcinoma cells in effusions (Davidson et al., 2000) and in tissue sections, where both Tn and STn correlated with higher histological grade and poorer survival (Ghazizadeh et al., 1997). In ovarian carcinomas STn antigen was detected on MUC16 using a sandwich ELISA on peritoneal fluid of ovarian cancer patients and co-localizes with MUC16 on cancer tissues (Akita et al., 2012). Furthermore, we recently demonstrated that circulating MUC16 in ovarian cancer patients carry the STn glycosylation, and that selective detection of the STn MUC16 glycoform using an antibody capture array assay improved specificity of MUC16 compared to the CA125 assay (Chen et al., 2013). This suggests that detection of specific glycoforms of circulating mucins may represent a strategy towards improved biomarker assays. However, we identified sensitivity of the array assay as an obstacle in that the array assay performed equal or poorer than the routine CA125 assay in terms of overall sensitivity. This limits its use for early diagnosis regardless of the improved specificity.

Here, we have further studied the expression of glycoforms of mucins in ovarian cancer and developed highly sensitive mucin glycoform specific Proximity Ligation Assays (PLA). As a proof of concept we demonstrate that selective detection of Tn and STn glycoforms of MUC16 as well as MUC1 provide high specificity and sensitivity in tissue screening of ovarian serous neoplasia. We have previously demonstrated that PLA (Soderberg et al., 2006) can successfully be applied in tissue sections for detection of glycoforms of mucins (Conze et al., 2010), and we identified Tn/STn glycoforms of MUC1 in a large percentage of mucinous ovarian carcinomas (Pinto et al., 2012). We studied a total of 155 serous ovarian tumours (44 cystadenomas, 41 borderline tumours and 70 adenocarcinomas), encompassing a test and a validation series, using PLA probes for MUC1, MUC16, Tn, STn, and T antigens. Our results show that identification of MUC1 and MUC16 glycoforms with the Tn and STn glycans constitute a very promising biomarker signature to distinguish malignant/borderline serous ovarian tumours from benign lesions. The strategy is designated glyco-mucin profiling and the results provide strong support for further development of PLA-based serum assays with the potential for early diagnosis.

2. Materials and methods

2.1. *Patient selection and tissue microarray construction*

Pilot study: A series of 66 serous ovarian tumours, diagnosed between 2000 and 2012, was selected from the Pathology Department of Centro Hospitalar S. João, Porto, Portugal (CHSJ Porto series). The series was extracted from all ovarian lesions surgically removed in the same period ($n = 1492$, including occasional findings in hysterectomy specimens, metastasis, etc) and the 66 cases were selected on the basis of the quality/representativity of the histological material, clinical information and staging. Formalin-fixed paraffin-embedded histological sections were reviewed and the diagnosis confirmed. Patients' ages ranged from 25 to 88 years. Tumours were characterized for histologic type and stage (FIGO classification) (Supplementary Table S1).

From the 66 cases, 44 were arrayed in six Tissue Microarray (TMA) blocks with at least two tissue cores (1.5 mm in diameter) from each tumour sample. TMAs were built after careful review of hematoxilin & eosin stained sections by an experienced pathologist (LD) with selection of representative tumour areas. TMA blocks were designed and constructed according to rules previously described (Avninder et al., 2008; Simon et al., 2004). The remaining 22 cases were whole tissue