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Down-regulation of proteasomal subunit MB1 is an independent predictor of improved survival in ovarian cancer

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ABSTRACT

Objective. To investigate the expression and to determine the prognostic impact of components of the antigen processing and presentation pathway (APPP) in ovarian cancer.

Methods. Expression of MB1, LMP7, TAP1, TAP2, ERp57, ERAP1, β_2-microglobulin and the α-chains, HLA-B/C and HLA-A, of the MHC class I molecules was evaluated on tissue microarrays containing primary tumor samples from 232 FIGO stages I–IV ovarian cancer patients. Expression levels were correlated to clinicopathological data and disease specific (DSS) survival.

Results. Patients with expression of all components of the MHC class I complex, i.e. HLA-A^+–β_2-m^+ and HLA-B/C^+–β_2-m^+ patients, more often had expression of LMP7, a component of the immunoproteasome than patients with other phenotypes ($p<0.001$). These patients were also more prone to loss of MB1, part of the constitutive multicatalytic proteasome ($p<0.05$). Nuclear MB1 expression was an independent predictor of worse DSS (HR 1.94, 95% CI 1.16–3.26, $p=0.012$). The HLA-B/C^+–β_2-m^+ phenotype was an independent predictor of a better prognosis (HR 0.63, 95% CI 0.40–0.99, $p=0.047$). Median DSS was longer for patients with normal nuclear expression of LMP7 (57.4 vs. 31.0 months, $p=0.029$).

Conclusions. The prognostic influence of the proteasomal subunit MB1 and the MHC class I complex in ovarian cancer provides a rationale for targeting these specific APPP components in ovarian cancer.

Introduction

Ovarian cancer is the second most common gynecological cancer and the leading cause of death from gynecological malignancies in the Western world [1]. The exploration of immunotherapy as a new treatment modality for this disease relies on evidence of improved clinical outcome when intra-tumoral T-lymphocytes are present in ovarian cancer patients, which is most likely a reflection of an anti-tumor immune response [2]. Immunotherapy can potentially overcome the poor survival rate seen with standard treatment, i.e. surgical debulking and adjuvant platinum based chemotherapy [3,4], by inducing or augmenting anti-tumor immune responses. Different strategies have been employed, including adoptive transfer of anti-tumor T cells or natural killer (NK) cells, as well as active immunization with tumor antigens, such as p53. Contrary to some promising murine studies [5–7], immunotherapeutic trials in human subjects have only reached minimal clinical benefit [8,9]. This may partly be due to the development and exploitation of immune escape mechanism by the tumor. Down-regulation or absence of components of the antigen processing and presentation pathway (APPP) is believed to be such a mechanism as presentation of antigens either processed within the MHC class I or the MHC class II pathway is a prerequisite for recognition by $CD8^+$ cytotoxic and $CD4^+$ helper T-lymphocytes, respectively [10,11].

The mainstay of the anti-tumor immune response is via MHC class I. In the MHC class I pathway, cytoplasmic and nuclear proteins are degraded by the interferon-γ inducible subunits LMP7, LMP2 and LMP10 (components of the immunoproteasome), which can replace subunits MB1(X), delta(Y) and zeta(Z) of the multicatalytic constitutive proteasome [10–14]. Immunoproteasomes are generally thought to be more efficient at the production of antigenic peptides than the constitutive proteasome [13] and they tend to produce more and on average slightly longer peptides [15]. Immunoproteasomes are preferentially located near the endoplasmic reticulum (ER), whereas the constitutive proteasome can be found throughout the cytoplasm [16]. Proteasomes may also be present in the nucleus, especially in case of cell stress [17]. Next, peptides of a suitable length are transported from the cytoplasm into the endoplasmic reticulum by

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the transporter associated with antigen processing (TAP), consisting of subunits TAP1 and TAP2 [10,11,13,14,18]. Once in the ER, aminopeptidases such as endoplasmic reticulum aminopeptidase 1 (ERAP1) can further trim peptides to a correct size for adequate MHC class I binding. Chaperones (e.g. ERp57) facilitate loading of peptides into the MHC class I molecule [10,11,19–21]. Finally, a fully assembled heterotrimeric MHC class I/peptide complex (α-chain, β_2-microglobulin and a bound antigenic peptide) is transported to the cell surface, where it may be recognized by $CD8^+$ cytotoxic T-lymphocytes [11,13,14,20]. A schematic overview of this pathway is given in Fig. 1.

Although defects and down-regulation in all of the aforementioned components have been identified in several types of cancer, most APPP defects have some, little or no significant relation with clinical parameters and disease outcome [11,22–32]. In ovarian cancer, a study of five APPP components recently showed that the number of down-regulated components is an independent prognostic factor [33]. We investigated the expression and clinical relevance of these and essential additional components of the APPP in paraffin embedded tissues obtained from a large series of well-documented ovarian cancer patients.

Materials and methods

Patients

Patients were identified from a database containing clinicopathological and follow-up data of all epithelial ovarian cancer treated with primary debulking surgery according to standard treatment protocols by gynecological oncologists of the University Medical Center Groningen (Groningen, The Netherlands) between May 1985 and April 2003. Patients were selected if sufficient paraffin embedded tissue was available. All patients were staged according to the FIGO classification, and resected tumors were graded and classified by a gynecological pathologist based on the World Health Organization criteria. Adjuvant chemotherapy was given if indicated. Follow-up of all patients was performed regularly up to 10 years with gradually increasing intervals.

Institutional review board approval

All clinicopathologic and follow-up data of patients referred to the Department of Gynecologic Oncology of the UMCG are prospectively collected and stored in a computerized database. For the present study, all relevant data were retrieved from our computerized database and transferred into a separate password-protected anonymous database. Patient identity was protected by study-specific unique patient codes. Patients' true identity was only known to two dedicated data managers, who have daily responsibility for the larger database. In case of uncertainties with respect to clinicopathologic and follow-up data, the larger databases could only be checked through the data managers. Based on this information, according to Dutch law no further approval from our Institutional Review Board was needed.

Tissue samples and construction of tissue microarrays

Paraffin-embedded tissue blocks and corresponding hematoxylin and eosin stained slides constructed from tumor tissue obtained at

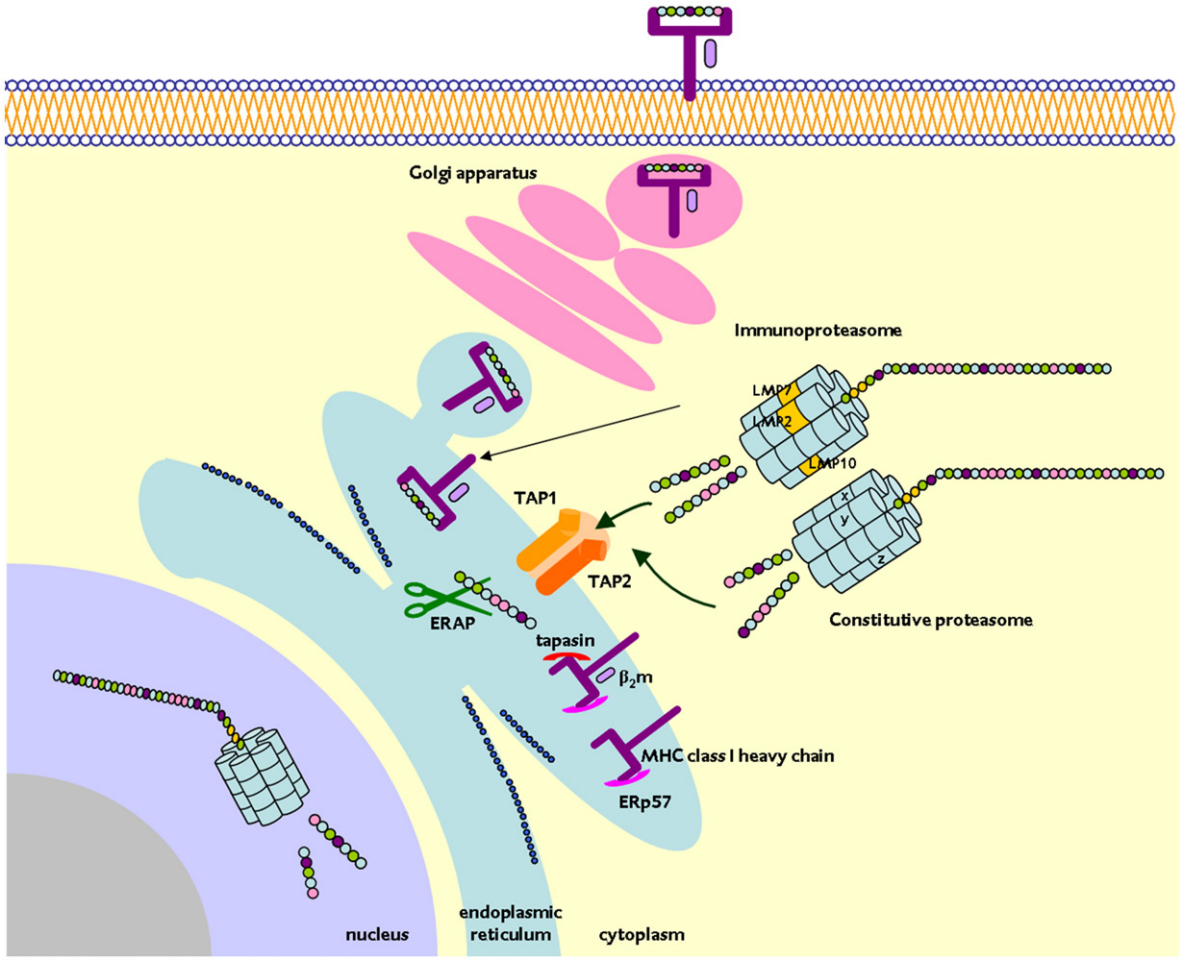

Fig. 1. Schematic overview of MCH class I dependent antigen processing and presentation pathway.