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Original Research

The prognostic benefit of tumour-infiltrating Natural Killer cells in endometrial cancer is dependent on concurrent overexpression of Human Leucocyte Antigen-E in the tumour microenvironment

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Abstract ***Background:*** Human Leucocyte Antigen- E (HLA-E) has been reported as both a positive and negative prognostic marker in cancer. This apparent discrepancy may be due to opposing actions of HLA-E on tumour-infiltrating immune cells. Therefore, we evaluated HLA-E expression and survival in relation to the presence of intratumoural natural killer (NK) cells and cytotoxic T cells (CTLs).

Methods: Tissue microarrays (TMAs) of endometrial tumours were used for immunohistochemical staining of parameters of interest. The combined impact of clinical, pathological and immune parameters on survival was analysed using log rank testing and Cox regression analyses.

Results: Upregulation of HLA-E was associated with an improved disease-free and disease-specific survival in univariate analysis (HR 0.58 95% CI 0.37–0.89; HR 0.42 95% CI 0.25–0.73, respectively). In multivariate analysis, the presence of NK cells predicts survival with a hazard ratio (HR) 0.28 (95% confidence interval (CI) 0.09–0.91) when HLA-E expression is upregulated; but it is associated with a worse prognosis when HLA-E expression is normal (HR 13.43, 95% CI 1.70–106.14). By contrast, the prognostic benefit of T cells was not modulated by HLA-E expression.

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Conclusions: Taken together, we demonstrate that the prognostic benefit of NK cells, but not T-cells, is influenced by HLA-E expression in endometrial cancer (EC) and propose a model to explain our observations.

1. Introduction

Endometrial cancer (EC) is the most common gynaecological cancer in the western world, and EC incidence has increased by more than 40% in the last two decades [10,23]. There is a need for novel treatment options as prognosis has not improved over the years. Such innovative treatment options may come in the form of immunotherapy, exemplified by recent reports on the efficacy of checkpoint inhibitors in EC patients [19]. Besides treatment options, cancer immunology could contribute to improved selection of patients at risk of recurrence that would benefit from adjuvant treatment. CD8 positive, cytotoxic T cells (CTLs) are a strong prognostic biomarker for survival in EC [7,11,36]. In addition, other immune cell populations such as innate Natural Killer (NK) cells may also be of importance [26,35,37,38].

Recognition of tumour cells by CTLs and NK cells occurs through binding to Human Leucocyte Antigen (HLA) molecules on the cell surface [15,25]. For CTLs, specificity is determined via antigen-derived peptides presented in the context of classical HLA-Ia molecules. For NK cells, recognition occurs through interaction of Killer Cell Ig-like receptors with classical HLA-I molecules. In addition, NK cell activity is modulated by an interaction between HLA-E and NKG2x/CD94 receptors such as NKG2A and NKG2C. HLA-E is expressed in most tissues and inhibits NK cell activity by binding to the NKG2A/CD94 receptor complex [5,13,30,31,38,39]. Alternatively, HLA-E activates NK cells by binding to the NKG2C/CD94 receptor complex [30,34]. In addition, upregulated HLA-E expression in ovarian cancer neutralised the positive relation between CTL and survival [11]. Similarly, CTL only had a positive effect on survival in HLA-E negative tumours in non-small cell lung carcinoma [31].

Previously, our group reported on the association between downregulated expression of HLA-Ia, unfavourable clinicopathological variables and worse disease-specific survival in EC [3,6]. It is unclear whether HLA-E expression on tumour cells is of prognostic importance in EC. A relation between HLA-E expression and survival has been shown in other cancers types such as colorectal cancer and breast cancer. Both loss of expression as well as upregulated expression of HLA-E turned out to be related to improved survival [1,2,8,11,20,27,29,31,40,41]. Zeestraten *et al.* linked loss of expression for HLA-E to improved prognosis in colorectal cancer [40]. On the other hand, Benevolo *et al.* found upregulated expression of HLA-E to be related to improved survival [2].

We hypothesised that HLA-E expression affects survival in EC, by interacting with NK cells and/or CTLs. We therefore analysed the expression of HLA-E in a well-described cohort of 355 EC patients and evaluated the interaction of HLA-E with NK cells and CTLs on disease-free and overall survival (OS) in EC.

2. Materials and methods

2.1. Patients

In total, 355 consecutive patients treated for EC at the University Medical Center Groningen between 1984 and 2004 were enrolled as described previously [3,7]. Patients who had received radiotherapy prior to surgery or treatment for another neoplasm were excluded. Treatment and adjuvant treatment was in conformance with national and international guidelines [23]; [24]. Tissue was classified according to International Federation of Gynaecology and Obstetrics (FIGO) criteria by an experienced gynaecological pathologist and reclassified to conform to the updated/revised FIGO 2009 classification where necessary (HH). Follow-up visits included history taking and a physical examination and were performed for a period of 5 years. Follow-up was completed until December 2014 and entered into a password-protected database. Study-specific patient numbers were used, untraceable to patient identities. According to Dutch legislation, no approval by an Institutional Review Board is necessary. This cohort is also described in previous investigations on cancer immunology in EC including the role of CTLs, and the data from such previous investigations on cancer immunology in EC were also used for this study [7,36].

2.2. Immunohistochemistry (IHC)

TMAs were constructed as described previously [3,7]. Three 0.6 mm core biopsies were taken from representative areas of tumour centre and transferred to a blank paraffin block. Recipient blocks were placed in an incubator at 37° Celsius for 15 min to facilitate adhesion. For staining, sections of 4 μm were cut and applied to aminopropyltriethoxysilane (APES)-coated slides; the