Tumour and placenta establishment: the importance of antigen processing and presentation.

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*Abstract*

Classical and non-classical MHC class I (MHC I) molecules displayed at the cell surface are essential for the induction of innate and adaptive immune responses. Classical MHC I present endogenously derived peptides to CD8+ T cells for immunosurveillance of infected or malignant cells. By contrast, non-classical MHC I, in particular HLA-G, also display peptides, but primarily act as immunomodulatory ligands for the innate immune response and are an important component for extravillous trophoblast invasion to form the placenta in pregnancy. Endoplasmic Reticulum AminoPeptidase 1 (ERAP1), which trims peptides in the ER to generate ligands for MHC I loading, is a key regulator of the peptide repertoire and has a significant impact on the formation of stable MHC I at the cell surface. ERAP1 also plays a role in angiogenesis, cell cycle progression and migration, events that are shared between tumour cells and placenta formation. Here we discuss the similarities between tumour and extravillous trophoblast cells in their immune modulatory, invasion, migration and proliferation properties in the context of ERAP1 and its role in establishment of solid tumours and placenta formation.

*Cellular immune recognition: Major histocompatibility complex*

Major Histocompatibility Complex class I (MHC I) molecules are a fundamental component of pathogenic recognition by immune cells, mediating both adaptive and innate immune responses. Classical MHC I, HLA-A, -B and -C, are highly polymorphic and have evolved to present a diverse array of intracellular peptides of specific length, 8-12 amino acids, to CD8+ T cells [1, 2]. This immunosurveillance mechanism is a major component of the adaptive immune response and provides antigen-specific memory to the host [3]. Innate immune response pathways may also be activated in response to alterations in MHC I expression levels; killer immunoglobulin-like receptors (KIR) and leukocyte immunoglobulin-like receptors (LILR) expressed on Natural Killer (NK) cells bind to MHC I and induce either inhibitory or activating signals. Interestingly, in situations where MHC I is downregulated (in malignant or virally infected cells), inhibitory KIR fail to engage with their ligand, resulting in a lack of inhibitory signaling cascade which would, under normal circumstances, abrogate cytolytic activity of NK cells. This results in NK-mediated cellular destruction and is an important component of self-tolerance [4, 5] By contrast, the cell surface presentation of non-classical MHC I, HLA-E and -G, is peptide dependent, but as they contain few polymorphisms, the diversity of peptides presented by these molecules is reduced [6, 7]. Similarly to classical MHC I, HLA-E is IFN-**** inducible, and presents peptides mainly derived from classical HLA leader sequences [8]. However, HLA-G, which is expressed on trophoblasts, presents endogenously derived cytosolic peptides, but lacks the IFN-**** inducible response and is up-regulated in an indirect manner through IFN-**** inducible transcription factors [7, 8]. These findings suggest that whilst non-classical MHC I have the capacity to present peptides to immune cells, this may not be their primary function and instead these non-classical stable peptide-MHC I (pMHC I) complexes serve as ligands for immune tolerance [9]. The role of HLA-G in immunomodulation is further reinforced by the association of increased HLA-G expression and prolonged allograft survival *in vivo* [10, 11].

A key strategy in the immune-evasion of tumour cells and viruses is down-regulation of cell surface MHC I through modulating the expression of key components of the antigen processing and presentation (APP) pathway such as the proteasome, MHC I, endoplasmic reticulum aminopeptidase 1 (ERAP1) and components of the peptide loading complex (PLC): TAP, tapasin, ERp57 and calreticulin [12] (Figure 1). During the development of the placenta, cell surface expression of classical MHC is limited to HLA-C only; however high levels of non-classical HLA-G and HLA-E is expressed. How this is modulated by components of the APP is currently unknown.

*Antigen processing*

The major antigen processing event, resulting in optimal length and highly stable pMHC I complexes, is undertaken within the ER by ERAP1 and ERAP2 [13]. ERAP1 shares 49% and 40% homology with ERAP2 (also known as leukocyte-derived arginine aminopeptidase, L-RAP) and insulin regulated aminopeptidase (IRAP, also known as placental-leucine aminopeptidase, P-LAP) [14]. These aminopeptidases belong to the oxytocinase family of M1 metalloproteases, classified by the presence of specific Zn binding motif HEXXH(X)18E and active site GAMEN motifs [14, 15]. Additionally, ERAP1 is also known as puromycin insensitive leucyl-specific aminopeptidase (PILSAP)/ adipocyte-derived leucine aminopeptidase (A-LAP) and aminopeptidase regulator of TNF receptor superfamily 1 (TNFRSF1) shedding (ARTS-1) based on the alternative cellular functions of ERAP1 [16]. Both ERAP1 and ERAP2 play major roles in antigen processing, however, ERAP1 has been shown to have additional functions in other cellular processes such as cell migration, cell differentiation and angiogenesis. Therefore, we will only discuss the role of ERAP1 in tumour growth and placental formation in this review.

ERAP1 plays a fundamental role in the trimming of N-terminal extensions of peptides to generate the stable antigens for MHC I binding. The tissue distribution of ERAP1 mimics MHC I expression, suggesting a dominant role of ERAP1 in the generation of these MHC I-specific epitopes [13, 17]. The quality and quantity of peptides displayed at the cell surface are tightly regulated by ERAP1 activity and loss of expression significantly alters the repertoire of peptides, resulting in i) a reduction in total number of peptides and ii) increased length of peptides presented by MHC I [18]. ERAP1 preferentially cleaves hydrophobic aromatic amino acids over polar charged residues and displays a distinct hierarchy of substrate specificity [19]. The importance of ERAP1 in peptide generation and stable MHC I presentation is further reinforced by the observed reduction in both classical and non-classical MHC I expression in the absence of ERAP1 [20]. Interestingly, however, this reduction in pMHC I is not as profound as that demonstrated by the loss of other components of the APP pathway, such as the immunoproteasome subunits LMP2/7 and the TAP transporter responsible for the supply of peptides into the ER (figure 1). This supports the observation that a significant subset of MHC I peptides are ERAP1 independent [20].

The precise mechanism of ERAP1 trimming activity remains unclear and is a focus of several studies. Evidence supporting a ‘molecular ruler mechanism’ suggests ERAP1 acts independently with an internal molecular template for peptide length. Enzymatic cleavage efficacy of ERAP1 significantly reduces when peptides reach the critical 8/9amino acid in length. The crystal structure of ERAP1 suggests a C-terminal binding pocket adjacent to the N-terminal active site binding region when adopting the ‘active-closed’ conformation supporting the requirement for substrates with a free C-terminus [21, 22]. By contrast, evidence from cellular based experiments indicates that the correct MHC I must be present for the generation of epitopes; the absence of the correct MHC I results in destruction of the peptide [23]. In addition, we and others have demonstrated ERAP1 trimming activity of an N-terminally extended peptide tethered to MHC I in a single-chain trimer complex can occur [19, 24].

*ERAP1 in HPV induced cervical carcinoma*

ERAP1 polymorphism and expression level has been associated with progression and overall survival of human papillomavirus (HPV) driven cervical carcinoma, as well as alterations in expression levels in other cancers from a variety of primary tumour sites: colorectal, ovary, breast, lung and thyroid carcinomas [25-27]. However, there appears to be little correlation of ERAP1 expression between tumours of similar histological origin [27]. Interestingly, ERAP1 is highly polymorphic forming discrete allotypes formed from multiple SNP combinations in the same gene [19]. Moreover, these allotypes fall into functionally distinct categories: efficient, hypoactive and hyperactive and as such, significantly alter the peptide repertoire displayed at the cell surface. Since ERAP1 is polymorphic, both the increase and decrease in ERAP1 expression observed may prove invaluable for evasion of the host responses to tumour antigen, depending on i) the host ERAP1 allotypes expressed and their subsequent function and ii) the required generation of, or lack of, specific tumour associated antigens. In cervical carcinoma, the minor allele present at the ERAP1-127 and -730 loci in combination with SNPs in TAP and LMP7 increase the risk of cervical carcinoma three-fold [28]. Additionally, the minor allele homozygote phenotype at ERAP-56 and -127 conferred a worse overall 10 year survival [29]. Although the mechanism underpinning these correlations between ERAP1 SNPs, as well as genetic alterations of other APP components (TAP/LMP7), with cervical carcinoma is currently unknown, numerous studies have demonstrated the significant effect of ERAP1 SNPs on the ability to process peptides and the effect on the cell surface MHC I presentation [19, 30-32]. Therefore, SNPs in ERAP1 are likely to alter the regulation of peptides, specifically HPV-derived peptides, presented in cervical carcinoma patients. When determining the effect of loss of ERAP1 expression, a partial loss is associated with reduced MHC I cell surface presentation and a worse overall survival [33]. This reduction in both ERAP1 mRNA and protein expression is, at least in part, a result of loss of heterozygosity, and approximately 50% of patients displaying a loss of ERAP1 expression have this characteristic [34]. Intriguingly, the patterns of genetic association of ERAP1 and other APP machinery components with cervical carcinoma risk differ depending on the background genetic composition and may even depend on the HPV type distribution when assessed in Indonesian and Dutch populations [35].

*Similarities between MHC I properties of invading tumour cells and trophoblasts*

The establishment of solid tumours is a complex multistep process involving interplay between different cell types and a surrounding tumour microenvironment to promote survival. Interestingly, there are a number of shared immune evasion strategies adopted by tumour cells and cells involved in the establishment of pregnancy, another multistep process that results in implantation and growth of a semi-allogeneic foetus. Immunomodulatory mechanisms have evolved at the foetal-maternal interface to allow invasion and growth of extravillous trophoblasts (EVT), cells differentiated from the original cytotrophoblast with an invasive phenotype comparable to cancer cells, into maternal decidua [36]. Similarly to establishment of tumour, where the surrounding microenvironment is manipulated to sustain growth, the surrounding decidua of the endometrium secrete a number of proteins, such as leukemia inhibitory factor (LIF), to promote invasion and growth of the trophoblast cells and is essential for placenta formation [36].

The lack of classical MHC I expression is a common feature of tumour cells and has been extensively documented in a variety of malignancies to ‘hide’ from host immune attack through alterations in expression of APP components [12]. Interestingly, EVTs do not express classical HLA-A and -B but do express HLA-C and HLA-G, and yet are not attacked by the host immunity [37]. This difference may highlight the roles of HLA-C and HLA-G as inhibitory ligands for KIR in the trophoblast, specifically KIR4DL2, receptors, which enable the expression of MHC I but prevent detection by the immune response [8, 38]. Whilst the expression of HLA-G was initially believed to be restricted to trophoblasts, regulating the NK cell component of the foetal-maternal interface during pregnancy, expression has now been confirmed on a number of cell types, such as macrophages and dendritic cells to function as an immunomodulatory molecule, as well as pancreatic cells, adult thymic stem cells and tumour cells [8, 39-41]. One proposed mechanism for this immunomodulation is as a result of HLA-G being less polymorphic which may allow maternal and paternal HLA-G-peptide complexes (pHLA-G) to adopt similar conformations reducing the likelihood of an alloreactive maternal CTL response to the foetus. HLA-G present nonameric peptides derived from the cytosol of the cell, with a specific binding motif (XI/LPXXXXXL) and are likely to require ERAP1 to generate these stable epitopes [7, 8]. Interestingly, presentation of peptide antigens may not be the primary function of HLA-G expression as stable complexes interact with inhibitory receptors at the cell surface that are not peptide-specific [42]. However, these interactions do require the generation of optimal epitopes to allow stable expression of the complex at the cell surface and engagement with inhibitory receptors. ERAP1 is expressed in the ER of EVTs and choriocarcinoma cells and is up-regulated in the presence of LIF, correlating with an increase in HLA-G expression. By contrast, ERAP1 has little effect on HLA-G expression in the absence of LIF [43]. Interestingly, cell surface MHC I expression has been linked with prognosis and outcome in a number of cancers, with a lower expression correlating with a worse survival [44]. ERAP1 expression mimics that of MHC I expression in a variety of tumour types, and alterations in ERAP1 expression in tumour cell lines resulted in abnormal cell surface MHC I expression [26, 27]. In addition, the tumour suppressor p53 alters cell surface MHC I levels through transcriptional regulation of ERAP1 expression in human colon carcinoma cell lines, suggesting that ERAP1 modulation of MHC I expression may be significant in the magnitude of immune response and overall survival in cancer [45]. In the context of HLA-G, these findings suggest ERAP1 may have a significant function in the generation of peptides for stable HLA-G complexes presented at the cell surface during EVT invasion and implantation. This is a significant finding, as loss of ERAP1 expression or altered function by the presence of allotypic variation may impact the immune recognition of EVTs and in turn affect the outcome of the pregnancy.

*The role of ERAP1 in cell proliferation, migration and angiogenesis*

There are a significant number of studies suggesting additional cellular functions of ERAP1; angiogenesis and neovessel formation, cell migration, and bioactive hormone cleavage as part of the renin-angiotensin system (figure 2) [46-49]. These are all distinct mechanisms that are orchestrated to support the growth of solid tumours and foetus implantation and allowing immunologic tolerance to support survival.

ERAP1 expression is up-regulated in embryonic stem cells during differentiation into endothelial cells (EC [47]). Interestingly, ERAP1 expression is required for cellular migration and proliferation of ECs through activation of integrins essential for cell adhesion: a vascular endothelial growth factor (VEGF) dependent process [46]. Additional studies have highlighted a role for ERAP1 in activation of RhoA, a member of the Rho family of GTPases that regulate the reorganisation of actin cytoskeleton [46, 48]. Further investigation of ERAP1 in VEGF stimulated EC proliferation revealed phosphatidylinositol-dependent kinase 1 (PDK1) as a substrate for ERAP1 trimming activity, processing 9 amino acids from the N-terminus. This trimming event allows the formation of ERAP1-PDK1-S6K complex, phosphorylating and activating S6K, allowing G1/S phase cell cycle progression in a VEGF dependent manner (figure 2) [49]. Interestingly, an ERAP1 active site mutant lacking enzymatic activity has a dominant-negative effect, forming a complex with PDK1 but since it does not trim the required N-terminal amino acids, no association or activation of S6K occurs. This dominant negative effect resulted in a reduction in angiogenesis and was also shown to halt tumour growth, providing significant evidence for ERAP1 in cell cycle regulation in cancer [49].

Sufficient nutrient and oxygen supply is vital for survival of foetus and tumour cells. Establishment of the necessary vasculature through angiogenesis is accomplished by invasion and subsequent proliferation of tumour or trophoblast cells within the surrounding tissue. Although trophoblasts invade and remodel local maternal spiral arteries, a number of signaling proteins, such as VEGF, alongside a high proliferative capacity, a property of both cell types, are reportedly involved in angiogenesis in tumour and EVT cells. Interestingly, ERAP1 is sensitive to VEGF stimulation, increasing in expression when stimulated with VEGF in EC, and correlates with increased angiogenesis, implicating ERAP1 in tumour neovessel formation and embryonic vascular development [47]. The degradation of bioactive hormones angiotensin II (AngII) and kallidin to AngIII/IV and bradykinin, respectively, suggests a role for ERAP1 in the renin-angiotensin system (RAS) which is responsible for blood pressure regulation and angiogenesis (figure 2) [50]. The local RAS system present at both the site of tumour and within the placenta aids the formation of neo-vessels during angiogenesis and the foetal-placental vasculature as well as invasion of both trophoblasts and tumour cells [51, 52]. Peak expression of ERAP1 in luteal cells during pregnancy supports the role of ERAP1 in facilitation of AngII conversion under VEGF stimulation [53]. Conversely, overexpression of ERAP1 in endometrial carcinoma suppresses angiogenesis by reducing AngII mediated VEGF secretion and cellular migration, suggesting ERAP1 regulates renin-angiotensin system in cancer [54]. Significant alterations in placental RAS regulation may result in preeclampsia, a pregnancy specific condition characterised by hypertension and proteinuria, as well as contributory factors such as first pregnancy, change in partners and components of the compliment system (MBL, C3 and C5) [52]. There is currently no information regarding the involvement of ERAP1 in other pregnancy pathologies, either through antigen processing or alternative cellular functions. Given the association of ERAP1 (both expression and SNP) with HPV+ cervical carcinoma, it would be interesting to investigate whether there is any association with the development of cervical carcinoma and poor pregnancy outcome in these patients in the context of ERAP1.

Taken collectively in the context of ERAP1, these data suggest a significant role for ERAP1 in angiogenesis, cell proliferation and migration: characteristics that are essential in maintaining the growth of solid tumours and establishment of a viable pregnancy.

*Conclusions*

ERAP1 has been extensively studied in antigen processing and cancer, however less information is available for its role in embryo implantation and pregnancy.

Nevertheless, we discuss here the similarities between the events requiring ERAP1 activity in tumour cells and their potential importance in EVT invasion. It is evident that ERAP1 has significance in antigen processing and may be required to generate stable HLA-G ligands for EVTs to evade maternal immune responses. In contrast, the role of ERAP1 in cellular processes such as migration, proliferation and angiogenesis seems vital for invasion events. The similarities between tumour invasion and embryo implantation suggest a common pattern for which the involvement of ERAP1, in both an antigen processing capacity and regulation of cellular processes, may be important. Since ERAP1 has recently been shown to be highly polymorphic in individuals, further investigation is required to elucidate the whether these variants have an impact in both tumour formation and pregnancy.

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