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Review Article

The Role of Endoplasmic Reticulum Aminopeptidase 1 Biology in Immune Evasion by Tumours

Abstract

Expression of MHC I at the cell surface is essential for presenting peptides to circulating cytotoxic T cells. Interference with a number of components of the antigen processing machinery is an immune evasion mechanism that has been highlighted in a number of malignancies. Endoplasmic reticulum aminopeptidase 1 (ERAP1), a key component of the antigen processing pathway, undertakes the final N-terminal processing of peptide epitopes for MHC I presentation. ERAP1 acts to regulate and define the repertoire of peptides at the cell surface; changes in ERAP1 activity have significant consequences on CD8+ T cell and NK cell responses. Single nucleotide polymorphisms (SNPs) in ERAP1 have been strongly associated with a number of autoimmune conditions, viral infections and cancer. The presence of SNPs in ERAP1 significantly alter the ability to generate stable peptide epitopes for presentation, thereby altering the peptidome and subsequent immune response. Interestingly, ERAP1 variants have been significantly associated with prognosis and survival in virally induced cancer. Here we discuss the emerging role of ERAP1 activity in malignancy, and the contribution of ERAP1 SNP variants in the generation or destruction of epitopes in tumour cells. This potential immune evasion strategy employed by a number of tumour cells may therefore predispose individuals to cancer based on their ability to generate the required epitopes and form adequate immune response.

Immune System and Cancer

Cancer immune editing is the protective process by which the host immune system is able to control/prevent tumour growth and shape the immunogenicity of tumours. Recently, the hallmarks of cancer proposed by Hanahan and Weinberg have been modified to recognize the emerging role of the immune system and immunological evasion as an enabling characteristic of cancer [1]. The dynamic process of immune editing is defined into three categories 'Elimination, Equilibrium and Escape' [2-4]. The elimination phase represents the original concept of immune surveillance, with innate and adaptive immune responses largely involving natural killer (NK) cells, macrophages, IFN-γ secretion and cytotoxic T cells to recognize and eliminate transformed cells. The equilibrium phase involves selective pressure from lymphocytes and IFN-γ to maintain the balance between the host immune system and tumour cells. This is the longest of the three phases, with potential to result in a novel population of tumour clones with the acquisition of mutations and a reduced immunogenicity. The final stage is immune escape, allowing the growth of tumour cells in an immune competent environment, with resistance to detection and elimination. This results in tumour growth and the formation of solid malignancies [2-4]. In these final stages, the tumour cells undergo a number of immune evasion strategies, often comprising multiple strategic mechanisms within an individual cell. One such strategy is the loss of antigen expression at the cell surface and loss of components of the major histocompatibility complex class I (MHC I) antigen processing pathway. In particular a reduction in MHC I expression itself has been demonstrated in a wide variety of tumours.

Additionally, loss of expression of other components of the antigen processing machinery (APM), TAP1, LMP2 and LMP7 play a major role in immune evasion [5-8]. Here we discuss the role of the endoplasmic reticulum aminopeptidase 1, ERAP1, a key enzyme in the generation of optimal peptide ligands for MHC I expression, in immune evasion and tumour growth.

Antigen processing

Endogenously derived peptides are processed by the MHC I antigen processing pathway in antigen presenting cells, including tumour cells. MHC I present a diverse array of optimal peptide ligands at the cell surface for immunesurveillance by circulating $CD8^+$ cytotoxic T lymphocytes (CTL). Initially the proteasome, a multi subunit complex in the cytosol, is responsible for degradation of proteins targeted for degradation through the ubiquitin-proteasome system to generate smaller length peptide fragments [9]. However, like other components of the APM, IFN-γ stimulation up regulates the proteasome and induces a change in the catalytic subunits of the proteasome core: up regulating LMP2, LMP7 and LMP10 forming the immunoproteasome. This complex generates distinct antigenic epitopes with a higher affinity to MHC I with the correct hydrophobic C-terminal region of the peptides for stable binding. Once degraded by the proteasome, the peptides are transported into the endoplasmic reticulum (ER) by the transporter associated with antigen processing (TAP). TAP is comprised of two non-covalently associated subunits, TAP1 and TAP2, preferentially transporting peptides longer then a 12 amino acids into the ER in an ATP dependent manner [10]. In the ER, immature MHC I heavy chain (HC) associates with β2-microglobulin (β_2m), with the folding process aided by the association with chaperones calnexin, calreticulin, BiP and ERp57. The recruitment of TAP and the peptide editor tapasin form the

peptide loading complex (PLC), optimizing the binding of high affinity peptides onto MHC I. During this process, peptides in the ER often require further processing at the N-terminal region to generate the highest affinity peptides of optimal length (8-10 amino acids) for stable MHC I association. This processing event is undertaken by ER resident aminopeptidases, ERAP1 and ERAP2, and is fundamental for the generation of optimal epitopes (11-14). Finally, once high affinity peptides are bound, stable hetero-trimeric complexes of peptide-MHC-β_2m (pMHC I) dissociate from the PLC and transit via the golgi apparatus to the cell surface. The correct function of the antigen processing pathway, including expression of the APM, is essential for the formation of stable pMHC I at the cell surface. Often, in cancerous cells, a number of the APM are down regulated, which results in a dysfunctional pathway and a reduction of MHC I at the cell surface. This appears to be an emerging key feature of a number of tumour cells and a clear strategy for tumour immune evasion.

Endoplasmic reticulum aminopeptidase 1 in antigen generation

ERAP1 and the mouse homologue, Erap1, play a major role in antigen processing, responsible for the final N-terminal trimming of peptides [12,15]. This process generates a pool of high affinity epitopes within the ER for stable MHC I loading. In addition to ERAP1, a second aminopeptidase, ERAP2, exists in humans and shares 49% homology [16]. Like other APM, ERAP1 expression is up regulated in response to IFN-γ stimulation [12]. Interestingly, the tissue distribution of ERAP1 correlates with that of MHC I expression, however ERAP2 does not share the same expression profile, suggesting that ERAP1 has a more dominant role in MHC I antigen generation [12,17,18]. While the majority of MHC I antigens are generated through this endogenous processing pathway, professional antigen presenting cells, such as macrophages and dendritic cells are able to load MHC I with internalized antigens through cross-presentation. Exogenous antigens are internalized via endocytosis or phagocytosis where they are either transported into the cytosol and undergo the more conventional presentation pathway via the ER, or they are subject to processing and MHC I loading within endosomal compartments. The role of ERAP1 in generating optimal antigens for cross-presentation is largely unknown, although a reduction of ERAP1 expression was reported to reduce the efficiency of cross-presentation in murine DCs [19]. This suggests that ERAP1 may be involved in this process, which would be limited to its localization within the ER. Interestingly, insulin regulated aminopeptidase (IRAP), from the same M1 metalloprotease family as ERAP1 and ERAP2, is an endosomal resident aminopeptidase shown to have a major role in generating antigens for cross-presentation in these compartments [20]. This highlights the distinct, but important roles different M1 metalloproteases have in cross-presentation.

The substrate specificities of ERAP are unique. Whilst ERAP1 has been proposed to prefer hydrophobic and aromatic amino acids and has a distinct trimming hierarchy towards amino acids with a length preference of 9-13 amino acids, ERAP2 is thought to preferentially trim basic positively charged amino acids with no length requirements [17,21,22]. However, when tested in cell lines, a distinct hierarchy of amino acid trimming based on their properties is not apparent. This disparity is likely due to the nature of the assay used (cell based transfection of minigenes versus recombinant protein) [21,22]. Another unique characteristic of ERAP1 is the inability to process X-pro-X bonds [21]. The inability of TAP to transport peptides with a proline at position 2, together with the presence of a number of MHC I peptides containing this motif requires these peptides to enter the ER as N-terminally extended versions. Therefore ERAP1 is essential for generating these epitopes in the ER from their precursors [10,23]. Although ERAP1 normally acts individually within the ER, it is able to form heterodimers with ERAP2, potentially to increase the trimming efficiency and broaden the substrate specificity through the complementary specificity of ERAP1 and 2 [11,17]. This heterodimer complex is able to generate epitopes that are sub-optimally generated by ERAP1 alone *in vitro* but has not yet been demonstrated *in vivo* [17]. Nonetheless, only a small proportion of ERAP1 form these complexes, correlating with the discrepancy in tissue distribution of the two enzymes and the dominant role of ERAP1 in antigen processing.

The regulation of the peptide repertoire at the cell surface is a key feature of ERAP1, and disruption of processing activity has significant consequences on the number and sequence of peptides presented. Immunization of wild type (WT) mice with Erap1 deficient cells elicited a robust $CD8^+$ T cell response in response to the altered peptide repertoire presented in ERAP1 deficient cells compared with ERAP1 competent cells [24,25]. Interestingly, loss of ERAP1 results in fewer peptides presented at the cell surface, but those that are presented are often N-terminally extended, resulting in structurally distinct MHC I [24]. Further investigation into the requirement of ERAP1 revealed that peptides fall into three categories; independent, dependent and sensitive [24]. Those that are ERAP1 independent are presented regardless of ERAP1 activity and are likely to be optimal length when entering the ER. ERAP1 dependent peptides are only displayed at the cell surface in the presence of ERAP1 presumably as they are N-terminally extended and require processing before being high affinity for binding. The ERAP1 sensitive peptides are destroyed by normal ERAP1 activity and as a result are only presented when ERAP1 activity is absent. Therefore ERAP1 has the ability to alter the specificity and magnitude of T cell responses through the peptide repertoire presented to CTL. The direct effect of peptide processing by ERAP1 on MHC I expression was demonstrated by the generation of Erap1 deficient mice, which exhibit a significant reduction in MHC I at the cell surface [19,26,27]. H2-L^d was the most noticeable reduction in expression (70%) with other MHC I alleles having 20-30% reduction when Erap1 activity was absent [26]. The effect of reduced ERAP1 expression is also seen in human cells, with approximately 20% reduction in MHC I expression [28]. However, the exact magnitude of reduction in MHC I may be dependent on the variant of ERAP1 expressed within the cells. The effect of reduced ERAP2 expression has not been extensively studied, however a partial suppression of MHC I is obtained when ERAP2 expression is reduced [17]. Nonetheless, due to the disparity in tissue distribution with MHC I, altering ERAP2 expression may not be expected to have such a significant effect as ERAP1. Interestingly, the effect on MHC I expression with reduced ERAP1 activity is not as pronounced as seen with the loss of other APM components, such as TAP or tapasin [26].

Currently, the mechanism of ERAP1 activity is unknown, however the recent crystal structures have revealed a 4 domain protein which may adopt 'open' and 'closed' conformations in order to undertake peptide binding and substrate cleavage, respectively [29,30]. The movement of domains III and IV would allow for critical alterations in the structure of the active site of domain II, containing Zn binding motif and GAMEN motif, to become enzymatically active [30]. Although no crystal structures with peptides of physiological length are available, studies have proposed the molecular ruler hypothesis for ERAP1 trimming mechanism [29-31]. This proposed mechanism suggests ERAP1 itself acts as the molecular template, implied both by the conformational changes undertaken by ERAP1 to become enzymatically active, and the ceasing of ERAP1 activity when peptides <8 [13,31]. Conversely, a second hypothesis proposes that the presence of MHC I is essential in generating peptides. In the presence of the correct MHC I, ERAP1 generates final length peptide, whereas in the absence, these epitopes are destroyed and an increase in N-terminally extended epitopes is observed [32]. This was further reinforced when peptide tethered to MHC I in a single-chain trimer complex was shown to be trimmed to the correct length [22]. These studies demonstrate that dysregulation of ERAP1 peptide processing function have significant consequences on the $CD8^+$ T cell response.

Effect of polymorphisms on ERAP function

ERAP1 and 2 are naturally polymorphic molecules. There is increasing evidence of polymorphic ERAP variants having predisposition to a number of diseases, primarily autoimmune conditions and virally induced cancer [33-38], Interestingly, the association with ERAP and autoimmune diseases also confers strong genetic associations with distinct HLA-I alleles [35,36,39]. Therefore it is likely that the presence of SNPs have a significant impact on immune regulation through altered antigen processing of epitopes required for stable presentation on particular MHC I alleles.

The most extensively studied link is that between ERAP1 SNPs and the chronic autoimmune inflammatory condition ankylosing spondylitis (AS: $p=1 \times 10^{-26}$), where five ERAP1 SNPs were originally identified through genome-wide association studies (GWAS; rs27044, rs30187, rs2287987, rs10050860, rs17482078) [33,40]. AS has a significant association with HLA-B27 suggesting that ERAP1 might affect the generation of HLA-B27 specific epitopes. Other SNPs have since been associated with as well as the original SNPs being associated with increased risk of other diseases; Behçets disease, psoriasis, type 1 diabetes and multiple sclerosis (Table 1), all having a strong HLA components [40]. These genetic associations have led to a number of extensive studies to elucidate the effect that these SNPs have on function in an attempt to shed light on mechanism of disease pathogenesis.

Individually, SNPs alter the enzymatic function of ERAP1, albeit to different extents compared with WT. The first SNP identified with an association with disease risk was K528R (rs30187: WT amino acid, position, mutant amino acid) which linked with an increased predisposition with essential hypertension [41]. This SNP has the most frequent association with disease, presumably as it lies at a critical position at the junction of domains II and III with the potential to affect the conformational change from open to closed structure of ERAP1 [30]. This is also the most extensively studied individual polymorphic variant, and not surprisingly, results in a reduction of enzymatic activity (40%), a reduction in the ability to generate final length epitopes and an overall reduction in MHC I presentation [22,39,41,42]. Other SNPs also alter the activity; Q730E (rs27044) and R725Q (rs17482078) are inefficient at generating epitopes and are proposed to reside within a 'regulatory domain' region in domain IV binding the C-terminal region of the peptide [30,39,42]. M349V (rs2287987) alters the substrate specificity and is the closest SNP to the active site region in domain II [22,30]. Interestingly, D575N (rs10050860) has little impact on the activity of ERAP1 in comparison to WT, positioned in domain III [41]. Combinations of polymorphisms confer disease 'susceptible' and 'protective' haplotypes based on frequency in cases versus controls, as demonstrated in AS with the protective haplotype (R528/E730) having better overall trimming function than the susceptible haplotype [43,44]. Genetic studies also revealed amino acid positions 528 and 575 in tight linkage disequilibrium, altering the hierarchy of function depending on the amino acids present [45]. The minor allele at both positions (K528/N575) has the greatest activity, whilst the major alleles (R528/D575) have the poorest activity. In the context of AS, the presence of these SNPs altered the generation of high affinity ligands for HLA-B27 [45]. These findings were also consistent with the N575 individually having little effect, but acts to rescue the trimming phenotype when in combination with other SNPs (V349/Q725) [22].

As ERAP1 expression affects the peptide repertoire, in turn affecting the stability and expression of MHC I at the cell surface,

Table 1: Allelic variation of ERAP1 and ERAP2 and specific disease linkage.

SNP	Gene	Amino acid / position	Disease linkage (HLA I association with disease)
rs3734016	ERAP1	E56K	HPV cervical carcinoma
rs26653	ERAP1	R127P	HPV cervical carcinoma / Psoriasis (Cw*0602)
rs2287987	ERAP1	M349V	AS (B*27)
rs30187	ERAP1	K528R	AS (B*27) / Psoriasis (Cw*0602) / Type 1 diabetes (A*02, A*24) / Multiple Sclerosis
rs10050860	ERAP1	D575N	AS (B*27) / Behçet's disease (B*51)
rs17482078	ERAP1	R725Q	AS (B*27) / Behçet's disease (B*51)
rs27044	ERAP1	Q730E	HPV cervical carcinoma / AS (B*27)
rs2549782	ERAP2	K392N	AS (B*27) / preeclampsia / resistance to HIV infection

studies have begun to look at the direct effect of polymorphic ERAP1 variants on MHC I expression, in particular HLA-B27. ERAP1 SNPs directly alter the HLA-B27 peptidome producing longer length epitopes, in turn affecting the stability of HLA-B27 at the cell surface [43,46]. The effect of combined SNPs is more pronounced when 5 AS associated SNPs were present in ERAP1, reducing the ability to generate optimal epitopes for HLA-B27 [44]. When assessing both chromosomal copies, those with overall dysfunctional trimming activity were unable to restore both H2-K^b and HLA-B27 levels].

More recently SNPs in ERAP1 were shown to form naturally occurring distinct combinations, forming 9 allotypes (individual chromosomal ERAP1 containing multiple SNPs) within healthy individuals in the population [22]. This not only further confirmed the polymorphic nature of ERAP1, but also highlighted the importance in determining the whole ERAP1 coding sequence. The cumulative effect of these SNPs were revealed to significantly alter the enzymatic activity and substrate specificity, classifying ERAP1 into three functional categories; hypoactive, hyperactive and efficient in the generation of antigens [22]. Further to this, the overall trimming capacity of both chromosomal copies of ERAP1 within an individual plays a fundamental role in the repertoire of peptides presented at the cell surface [28]. Interestingly, in most instances there are two heterozygous copies of ERAP1 within an individual, often having different trimming functions when assessed. Strikingly, ERAP1 all type combinations were able to define individuals with AS from controls based both on sequence and trimming function [28]. Therefore, the contribution of both chromosomal copies must be considered when analyzing the ERAP1 trimming function in relation to disease.

These studies suggest the emerging importance of SNPs in the generation of optimal peptide antigens in disease pathogenesis, and the requirement of ERAP1 as a peptide editor and regulator of the repertoire presented at the cell surface. As yet, there are no specific antigenic epitopes for AS or many other autoimmune conditions with ERAP1 association. However, the alterations in the generated HLA-B27 peptidome by ERAP1 SNPs are hypothesized to play an important role in the AS disease pathogenesis through either direct recognition or through the generation of aberrant forms of HLA-B27. The effect of ERAP1 SNPs on the global peptide repertoire is therefore likely to be important in pathogenesis of other HLA related diseases. Conversely, there are many known HPV-16 and -18 immunogenic epitopes in HPV driven carcinoma, another ERAP1 associated disease. Therefore, it would be interesting to determine the role ERAP1 plays on the generation of these specific epitopes for stable presentation.

ERAP1 in cancer: expression

It is well documented that one strategy employed by host tumour cells to escape immune detection is to alter essential components of the antigen processing pathway. Significantly, in many tumours a down regulation of MHC I is reported [5,7,8,47,48]. Since ERAP1 is essential in shaping the peptide repertoire and significantly affects MHC I, several studies have begun to investigate ERAP1 expression in tumours [8,49-52]. The impact of ERAP1 on immune response in cancer through peptide editing function and generation of tumour antigens was demonstrated in the murine CT26 colorectal carcinoma model [53]. Normal expression of Erap1 in CT26 destroys the immunogenic tumour antigen GSW11; CTL targeted to this antigen is required for tumour protection. Inhibition of Erap1 function resulted in a ~75-fold increase in presentation of GSW11, enabling the generation of a strong anti-tumour response and ultimately tumour rejection, revealing levels of Erap1 expression directly correlate with levels of GSW11 presentation and anti-GSW11 CTL stimulation [53]. Changes in ERAP1 expression have been observed in human tumours [50-52,54,55]. Interestingly an increase in ERAP1 expression in colorectal adenoma carcinoma compared with normal gut tissue was observed [50]. It is tempting to suggest that this increase is due to an effective immune evasion strategy which leads to the destruction of immunogenic tumour epitopes reducing anti-tumour $CD8^+$ CTL responses.

Reduced Erap1 expression also altered innate immune responses in murine T cell lymphoma and in medulloblastoma [56,57]. The resultant unstable MHC I complexes in cells with reduced ERAP1/Erap1 expression failed to engage with inhibitory receptors on NK cells, resulting in NK cell activation and killing leading to tumour rejection. The increase in tumour infiltrating NK cells followed by CD8+ cells suggests rejection of murine lymphoma occurs through an initial innate NK cell response but complete control of tumour rejection relies on the collaboration of innate and adaptive responses [56,57].

A number of studies have revealed that viral infection can interfere with components of the APM, in particular TAP, LMP2, LMP7 and MHC I expression [58]. Deficiency of ERAP1 activity impairs the $CD8^+$ T cell response to pathogens *toxoplasma gondii*, lymphocytic choriomeningitis virus (LCMV) and cytomegalovirus (CMV), thus becoming susceptible to infection most likely due to the lack of presentation of pathogenic antigens [14,59,60]. The molecular mechanisms that underlie the reduction in MHC I and various components of the APM in tumours are largely unexplained. ERAP1 has been significantly associated with an increased risk of developing of HPV-driven cervical carcinoma where partial loss of expression was significantly associated with reduced overall survival [8]. Although as yet there is no evidence for a direct effect of HPV on ERAP1 expression, viral microRNAs from CMV directly reduce ERAP1 expression by binding to 3'UTR region, reducing transcription [59]. The direct impact of this was a reduction in CTL response to CMV viral proteins, highlighting ERAP1 as a susceptible target for viral interference and a mechanism of immune evasion by the virus itself. In a recent study to try to elucidate the mechanism of ERAP1 down regulation in a subset of cervical carcinoma, *Mehta et al* revealed loss of expression occurred at the pre-transcriptional level, with a loss of heterozygosity and genetic variation in SNPs [49]. Epigenetic modifications, including hypermethylation, are tumour events documented in oesophageal squamous cell carcinoma (SCC) and melanoma to reduce MHC I expression [61,62]. However, when assessed in Uighur women with cervical carcinoma, DNA methylation did not play a role in the down regulation of ERAP1 expression [6]. Interestingly, in melanoma cell lines there is some evidence of disparity between mRNA and protein expression, suggesting that there may be both transcriptional and post-transcriptional modifications that alter ERAP1 protein expression [51].

The levels of expression of both ERAP1 and ERAP2 have been investigated in leukemia, melanoma and carcinoma cell lines [50-52,55]. Complete loss of ERAP1 and ERAP2 expression is only documented in one melanoma cell line, where both proteins were detectable in all others, however to a highly variable degree. This is consistent with only partial loss of ERAP1 expression seen in cervical carcinoma patients [8]. Additionally, ERAP1 expression was found in all normal cell lines, however ERAP2 was highly variable [52]. This is consistent with the lack of ERAP2 protein expression in approximately 25% individuals in the populations [63]. There appeared to be very little correlation between levels of ERAP1 and ERAP2 expression in the same cell line and are likely to be independent in their expression. Added to this, ERAP1 was shown to be more closely coordinated with MHC I expression than ERAP2, consistent with the normal tissue distribution patterns of ERAP1 mimicking MHC I expression and being more dominant in antigen processing [50-52]. When assessed for function, the majority of cell lines appeared to be enzymatically active, with the activity correlating with expression. Interestingly, a subset of cell lines displayed high levels of expression but the proteins appeared to be functionally impaired [52]. Recently, ERAP1-349 variant has been identified in melanoma cell lines, however there is no evidence to date that this alters protein expression rather than function [51]. However, these studies did not determine the presence of SNPs within ERAP1 and ERAP2 molecules when assessing altered trimming function, and considering they are highly polymorphic molecules, the presence of certain SNP combinations may account for the reported reduction in enzymatic activity even when protein is expressed.

MHC I expression was notably increased when ERAP1 was silenced in melanoma cell lines implying that in melanoma, ERAP1 has little impact on expression of MHC I, however this does not rule out alterations in the repertoire of peptides presented due to the presence of SNPs [51]. Conversely, reconstitution of ERAP1 and ERAP2 altered MHC I expression, suggesting that both ERAP1 and ERAP2 affect MHC I expression but to a different extent in tumours of different origins [52]. Despite the lack of a link between ERAP1 or ERAP2 expression in particular cancers it would be important to identify the polymorphic variants that are present within each tumour to correlate changes in expression to ERAP1 function and the potential effect on antigen generation and presentation.

ERAP1 in cancer: SNPs and virally driven cancer

Cancers that arise from human papillomavirus (HPV) infection account for approximately 5% of cancers worldwide. To date there are over 15 oncogenic strains of HPV, but HPV-16 and -18 are the highest risk strains associated with the majority of anogenital cancers and an increasing fraction of head and neck cancer [64]. HPVs are small non-enveloped DNA viruses that infect the squamous cell epithelium and result in malignant transformation as a result of viral persistence and immune evasion. HPV-E6 and E7 oncoproteins are mainly responsible for the maintenance of transformed cells as a result of their effect on cell cycle regulation. E6 and E7 oncoproteins inhibit p53 and pRb tumour suppressor genes respectively. The inhibition and loss of negative control of pRb by E7 results in an increase in p16 expression and is often used as a surrogate marker for HPV expression.

Polymorphic ERAP1 variants are associated with an increased risk in the development of cervical carcinoma, the third most common cancer affecting women worldwide. In a cohort of Dutch women, *Mehta et al* identified the presence of minor allele at position ERAP1-127 and ERAP1-730 in combination with the major allele at TAP2-651 and LMP7-145 loci SNPs gives rise to a threefold increased risk of development of cervical carcinoma and is an independent predictor of survival [38]. Further investigation revealed the homozygous haplotype ERAP1-56 major with 127-minor allele to be significantly associated with reduced overall survival [38]. Interestingly, in a subset of Indonesian cases, where HPV is endemic, the ERAP1-575 minor allele has been implicated to increase the risk of cervical carcinoma development in combination with the major allele at TAP2-379 and -651. Like the cohort of Dutch cases, this study confirmed SNPs in ERAP1 to have an increased association, however the patterns of association appear to differ between different populations [65]. This may be as a result of altered distribution of HPV oncogenic subtypes and genetic composition of different populations. Recently, ERAP1-349 has been reported in melanoma cells lines. Upon genotyping analysis, this mutation was not found in any cervical specimens regardless of ERAP1 expression and may therefore prove unique to distinct types of cancer [38,51,65]. This SNP is close to the active site region and has been previously demonstrated to impact upon the antigen processing function of ERAP1, altering both the function and the substrate specificity of ERAP1 [22].

SNPs within ERAP1 significantly alter the trimming activity and ability to generate optimal antigens for peptide expression, thereby altering the repertoire of peptides presented. Although there is no direct evidence yet of the effect of these SNPs in ERAP1 on the presentation of E6 and E7 epitopes at the cell surface, it is likely that ERAP1 is dysfunctional in the generation of specific immunogenic HPV epitopes, reducing the T cell response to cells infected with HPV. This suggests ERAP1 has a significant impact on immunological control of tumour expansion.

Reduction in MHC I expression is observed in a significant proportion of HPV- driven cancers (40% cervical carcinoma cases and 50% of squamous cell carcinoma of the head and neck (SCCHN)). HPV-positive SCCHN cases have significantly increased over the last 40 years [66] with reduced MHC I expression correlating with a more aggressive phenotype and worse overall outcome [5,48]. Recently, levels of TIL have been shown to be a predictor of prognosis in SCCHN and correlate with levels of MHC I expression: high TIL correlates with high MHC I [67]. Interestingly, HPV-positive SCCHN (accounting for 40-80% incidence depending on anatomical site) has an overall better long term survival and outcome when compared to stage-matched HPV-negative SCCHN. One potential explanation for this is the protective effect of TILs through an adaptive immune response targeted towards HPV specific epitopes, demonstrated by the presence of HPV-16 specific T cells at the tumour site [68,69]. In addition, within the subset of HPV-positive tumours, outcome and survival stratifies based on their TIL status, with TIL^{high} patients having approximately 94% 3 year survival rate compared to TIL^{low} patients (approximately 37% 3 year survival rate) with similar survival rates to those in HPV-negative patients. This highlights TIL status as a potential tool for prognosis and identifying those at high risk [67].

The correlation between TIL status and SCCHN prognosis indicates that the ability to generate and present HPV-tumour antigens to elicit T cell responses are fundamental to rejecting the tumour. It will be important to determine how ERAP1 polymorphisms and expression correlate to TIL level and the generation of HPV-specific eptiopes.

Conclusions

Here we have discussed the emerging evidence for a role of ERAP in cancer pathogenesis. Successful antigen presentation by MHC I is key for immune regulation, with disruption of components in this pathway proving unfavourable to host immune control of tumour growth. Specifically, the significant role of ERAP in generation of peptide antigens is vital in the modulation of NK and CTL responses. It is well established that polymorphisms within ERAP1 significantly alter activity, in turn affecting the quality and quantity of the peptidome. Therefore altered ERAP1 activity, through the expression of particular allotypes that alter function (either increase or decrease activity) in combination with particular MHC I alleles may prove detrimental in mounting an efficient immune response to malignant cells. Reducing ERAP1 expression has been shown to have a positive effect on both innate and adaptive immune responses [53,56] resulting in tumour regression and providing a target for therapy. Recently, novel ERAP1 inhibitors have been generated which, upon reduced ERAP1 activity, were able to generate robust T cell response to cancer cells [70], further suggesting that regulating ERAP1 activity would provide a novel target for cancer therapy. Additionally, ERAP1 may be used as a prognostic marker and independent indicator of overall survival, as demonstrated in cervical carcinoma [38]. In light of these recent findings, it is of vital importance to increase our knowledge of genetic variation in ERAP in relation to cancer through GWAS and functional studies. This may result in a novel target for categorizing high and low risk cancer predisposing based on ERAP1 gene sequence and function, proving invaluable in future diagnosis and immunotherapeutic treatment of cancer.

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