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Regulation of ERAP1 and ERAP2 genes and their disfunction in human cancer

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ABSTRACT

The endoplasmic reticulum (ER) aminopeptidases ERAP1 and ERAP2 are two multifunctional enzymes playing an important role in the biological processes requiring trimming of substrates, including the generation of major histocompatibility complex (MHC) class I binding peptides. In the absence of ERAP enzymes, the cells exhibit a different pool of peptides on their surface which can promote both NK and $CD8^+$ T cell-mediated immune responses. The expression of ERAP1 and ERAP2 is frequently altered in tumors, as compared to their normal counterparts, but how this affects tumor growth and anti-tumor immune responses has been little investigated. This review will provide an overview of current knowledge on transcriptional and post-transcriptional regulations of ERAP enzymes, and will discuss the contribution of recent studies to our understanding of ERAP1 and ERAP2 role in cancer immunity.

1. ERAP biological functions

The endoplasmic reticulum (ER) aminopeptidases ERAP1 and ERAP2 are two homologous enzymes belonging to the oxytocinase subfamily of M1 metalloproteases with which they share two domains essential for their enzymatic activity, $HEXXH(X)_{18}E$ Zn-binding and GAMEN substrate recognition sequences [1]. Studies on the evolution have suggested that *ERAP1* and *ERAP2* arose by gene duplication [2]. In human, *ERAP1* and *ERAP2* genes share 49% sequence identity and are located on chromosome 5q15 in a 167 Kb segment in the opposite direction, likely sharing regulatory elements. Of note, *ERAP2* gene is absent in rodent genome although it was present in the primate-rodent common ancestor [2].

ERAP1 and ERAP2 enzymes play an important role in the biological processes requiring trimming of amino acid residues at the N-terminus of polypeptides. They have complementary functions by selecting substrates according to N-terminus and internal sequences [3]. ERAP1 preferentially cleaves hydrophobic amino acid residues, except proline, and peptides with a hydrophobic C-terminus, whereas ERAP2 preferentially hydrolyzes the basic residues Arg and Lys. Several evidences suggest a concerted action of the two enzymes: a) perfect co-localization in the ER, b) co-immunoprecipitation of ERAP1 with ERAP2, and vice versa, c) co-elution in chromatographic microsome fractionation, and d) existence in multimeric complexes [4–6]. In a recent study, the use of engineered ERAP1 and ERAP2 complexes showed that the physical interaction between the two enzymes results in a change of their enzymatic properties and an increase efficiency in processing various antigenic precursors compared with the enzyme mix [6,7]. The authors also demonstrated that upon dimerization with ERAP2, ERAP1 adopts a conformation that increases its affinity for peptides both with hydrophilic and hydrophobic N and C termini [7].

In the ER, ERAP1 and ERAP2 play a key role in generating optimal length of peptides for MHC class I binding [6,8–10]. The majority of antigenic peptides is generated by the proteasome and have the C-terminus suitable to act as anchor for MHC class I binding [11]. These peptides are transported into the ER by the transporter associated with antigen presentation (TAP), where amino acid residues that flank the N-terminus of antigenic precursors are trimmed by ERAP enzymes. Antigenic peptides bind MHC class I molecules to form peptide-MHC (pMHC) class I complexes that are then transported on the cell surface to be recognized by effector T and NK cells. Although both enzymes take part in this process, the role of ERAP2 in antigen processing and immunity has been studied less extensively than ERAP1. This probability is due to the fact that being absent in rodents [2] and in a quarter of the human population (see below, [2]), ERAP2 has always been considered an accessory molecule of ERAP1. Recent findings

Abbreviations: ERAP, endoplasmic reticulum aminopeptidase; MHC, major histocompatibility complex, ER, endoplasmic reticulum; SNP, single nucleotide polymorphism; HCMV, human cytomegalovirus; TCGA, The Cancer Genome Atlas

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highlighting an emerging role of ERAP2 in immunity are discussed in detail in another review in this issue by Lopez de Castro and Stratikos [12].

Studies in mice lacking ERAP1 have revealed the key role of ER peptide trimming in MHC class I antigen presentation [8–10,13]. Although the loss of ERAP1 results in a modest reduction of MHC class I surface expression, as compared to those deriving from the inhibition of the other antigen processing components (i.e., TAP, LMP2, LMP7 and β2m) [14–19], immunization of ERAP1-deficient mice with splenocytes from wild-type mice, and vice versa, immunization of wild-type mice with splenocytes from ERAP1-deficient mice, resulted in potent $CD8^+$ T cell responses [15]. These findings suggest that the lack of ERAP1 alters the normal repertoire of peptides bound to MHC class I and, consequently, the $CD8^+$ T cells responses in mice. The repertoire of peptides bound to MHC class I molecules can also affect NK cell-mediated immune responses. We demonstrated that loss of ERAP1 affects the susceptibility of both murine and human tumor cells to recognition and killing by NK cells [20,21]. The murine T-cell lymphoma RMA was rejected in the syngeneic mice following the inhibition of ERAP1 mainly through a tumor-specific NK cell response due to impaired pMHC class I engagement of Ly49C/I NK cell-inhibitory receptors [20]. Similarly, inhibition of ERAP1 yielded the human DAOY medulloblastoma cell line more susceptible to NK cell-mediated killing due to a poor recognition of pMHC class I complexes by KIRs and CD94-NKG2A NK cell-inhibitory receptors, thus suggesting that ERAP1 inhibition may be exploited for boosting immune responses to established tumors [21]. Importantly, inhibition of ERAP1 enhanced NK cell-mediated killing of lymphoblastoid cell lines (LCL) in both alloreactive and nonalloreactive settings, regardless of the presence of KIR-KIR ligand matching [21]. In another study, the inhibition of ERAP1 in the murine colorectal carcinoma CT26 elicited specific CTL responses against a cryptic tumor antigen that was normally destroyed by ERAP1, resulting in tumor growth arrest and enhanced survival [22]. These studies clearly demonstrated that ERAP1 expression can be critical for immune evasion of tumors. A further recent evidence of the role of ERAP1 in T-cell mediated tumor rejection is provided by the Kloetzel group [23]. The authors show that ERAP1 controls the efficacy of adoptive T-cell transfer in a mouse model of genetically depleted ERAP1 by operating both on the direct antigen presentation in tumor cells and on the antigen cross-presentation in the adoptive T-cell transfer recipient's cells [23]. The authors show that ERAP1 is required for the proliferation of $CD8^+$ T cell after adoptive T-cell transfer and its lack in transplanted recipients results in a failure of adoptive T-cell therapy [23].

ERAP1 is also known to modulate the development of the innate immune system and its responses during the initial stages of pathogen recognition [24]. ERAP1-deficient mice display exaggerated innate immune responses during pathogen recognition characterized by increased activation of mature NK and NKT cells and production of pro-inflammatory cytokines, such as IFNγ [24]. Furthermore, ERAP1 regulates the production of IL-12 by $CD11c^+$ DCs during pathogen recognition that correlated with an increased phagocytic activity of splenic DCs and macrophages [24]. More recently, the same authors [25] found that ERAP1-deficient mice have a reduced number of a subset of regulatory T cells, "Tr1-like" cells, and tolerogenic dendritic cells, which are important for Tr1 cell differentiation [25]. ERAP1 is also involved in other biological processes, including the regulation of innate and inflammatory immune responses by promoting the shedding of cytokine receptors on the cell surface, such as TNF receptor 1 (TNFR1), IL-6 receptor α (IL-6R α), type II IL-1 decoy receptor (IL-1RII) and IL-1 β receptor (IL-1 β R1) [26–30], post-natal angiogenesis by regulating the proliferation and migration of endothelial cells [31], and in the nitric oxide synthesis [32]. ERAP1 and ERAP2 enzymes are also known to regulate the metabolism of several peptide hormones involved in the regulation of blood pressure [31].

According to these multiple functions, ERAP enzymes are known by alternative names, such as adipocyte-derived leucine aminopeptidase (A-LAP), puromycin insensitive leucine-specific aminopeptidase (PILS-AP) and aminopeptidase regulating type I TNF receptor (TNFR1) shedding (ARTS-1) for ERAP1, and leukocyte-derived arginine aminopeptidase (L-RAP) for ERAP2. The two enzymes hereafter will be referred to ERAP1 and ERAP2. In this review we will provide an overview of current knowledge on transcriptional and post-transcriptional regulations of ERAP enzymes and we will discuss the contribution of recent studies to our understanding of their role in cancer immunity.

2. ERAP transcript variants

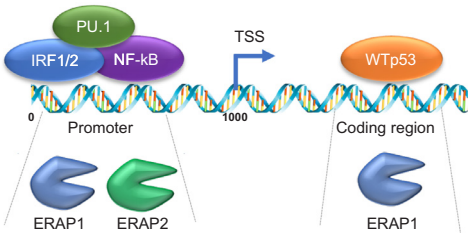
ERAP1 and *ERAP2* genes are ubiquitously expressed in all human tissues. According to Human Protein Atlas (www.proteinatlas.org) [33], which integrates RNA and protein expression of 33 different tissues, ERAP1 is mainly expressed in the fat, duodenum and small intestine, whereas ERAP2 is more abundant in the lymph nodes, spleen and appendix.

Several transcript variants encoding different ERAP1 and ERAP2 protein isoforms have been identified (https://www.ncbi.nlm.nih.gov). Human *ERAP1* produces at least four transcripts encoding two major protein isoforms, isoform a (ERAP1a) and isoform b (ERAP1b). The two isoforms share the same amino acid sequences except few amino acids at the C-terminus and different 3′ untranslated regions (UTRs). A recent study demonstrated that the two isoforms (reported by the authors as ERAP1_19E and ERAP1_20E for the different terminal exons) undergo different post-transcriptional regulation mechanisms through their 3′ UTRs [34]. The 19E isoform is induced by IFNγ and underwent suppressive translational control. Conversely, the 20E isoform is expressed more abundantly and it is not responsive to IFNγ stimulation [34]. Kim and colleagues also found that the 20E isoform is the most expressed in human cells [35]. The two isoforms 19E and 20E appear to have different functions resembling a regulatory gene and a housekeeping gene, respectively [34]. *ERAP2* exists in at least three transcript variants which result from an alternative splicing event in correspondence to the single nucleotide polymorphism (SNP) rs2248374 within the 5′ splice site of exon 10 [2]. Two transcripts encode the full-length protein, while the third one is degraded by nonsense-mediated decay [2]. Since the variant rs2248374 is maintained at a frequency of 50%, three quarters of individuals (with AA and AG genotypes) express a functional ERAP2, whereas the remaining individuals (with GG genotype) fail to express ERAP2. LCLs from individuals with GG phenotype have reduced levels of MHC class I surface expression, suggesting that the lack of ERAP2 may result in a pool of antigenic peptides with low affinity to MHC class I molecules [2].

3. Regulation of ERAP1 and ERAP2 expression

The expression of *ERAP1* and *ERAP2* genes is regulated at genetic, transcriptional and post-transcriptional levels. *ERAP* genes are highly polymorphic. Different genetic variants associated to several immune mediated diseases and virally induced cancer, have been found to affect gene expression at both the RNA and protein levels [36–38]. Hanson and colleagues demonstrate that disease risk-associated SNPs in *ERAP1* and *ERAP2* affect the expression, and that those in *ERAP1* are associated with altered splicing of the transcript [38]. Thus, in addition to the functional effects of coding variants in *ERAP1*, the disease-associated SNPs in both *ERAP* genes influence disease risk acting on transcription and splice variation. More recently, Paladini and colleagues identified an allelic variant (rs75862629) mapping in the intergenic region between *ERAP1* and *ERAP2* that affect their expression [39]. The authors associated the presence of the G variant of rs75862629 with low levels of ERAP2 mRNA and high levels of ERAP1 mRNA in human LCLs, thus suggesting a concerted inverse regulation between the two enzymes [39].

Similar to MHC class I and the antigen processing components, the expression of *ERAP* genes is regulated by interferons (IFNs)



	ERAP1 promoter			ERAP2 promoter		
Transcription factor	Position	DNA strand	Consensus sequence	Position	DNA strand	Consensus sequence
IRF1	22-46	(-)	ccatggagtaaagtGAAAgcaagta	n.p.		
IRF2	996-1020	(-)	ccaggaccgaaagtGAAAgtggagc	963-987	(+)	ttagaacagaaagtGAAAgcaaaag
PU.1	n.p.			42-62	(+)	aaaaaaaaAGAAaagaaaagg
	n.p.			983-1003	(-)	actgaaggGGAAtttacttttt
	n.p.			1549-1569	(-)	agggaggtGGAAgttgcagtg
	n.p.			421-441	(+)	agcagagaGGAAgttgaagcc
NF-kB (p65)	336-350	(-)	ccaggactTTCCtgc	n.p.		
p53*	19030-19052	(+)	TGTCATGTGTcagGCACATGTTA	n.p.		

Fig. 1. The transcription factors on the ERAP1 and ERAP2 promoter genes. Binding site and consensus sequences of IRF1/2, PU.1, NF-kB (p65) and p53 transcription factors in the promoter regions of *ERAP* genes (from The Eukariotic Promoter Database, https://epd.vital-it.ch/) were identified by Genomatix software (https://www.genomatix.de/). The promoter regions from −1,000 to +20,000 bp relative to transcription start site (TSS) were analyzed. Promoter ERAP_1: Chromosome [NC_000005.10]; Position [96807945]. Pomoter ERAP_2: Chromosome [NC_000005.10]; Position [96876500]. * Wang et al., 2013. n.p. = not present.

[8,10,40,41] and Tumor Necrosis Factor-α (TNF α) [42]. ERAP1 is also induced by lipopolysaccharide (LPS) in macrophages [43]. Bioinformatic analyses of *ERAP1* and *ERAP2* gene promoters revealed the presence of multiple potential transcription factor binding motifs, such as those for IRF1/2, p53, PU.1 and NF-kB transcription factors (Fig. 1). Several authors have shown that both IRF1 and IRF2 bind to the promoters of *ERAP1* by regulating the constitutive and the IFNγ-induced expression [41,44]. IRF1 and IRF2 also bind the *ERAP2* promoter, but while IRF2 regulates the constitutive expression, IRF1 is the main regulator of IFNγ-induced ERAP2 expression [41,45]. Tanioka et al. found that also PU.1, a member of the E26 transformation-specific family of transcription factors, is involved in the regulation of ERAP2 expression [45]. The co-expression of IRF1 and PU.1 in HEK293 cells resulted in the maximum expression of *ERAP2* gene, that instead was suppressed by IRF2 [45]. The expression of *ERAP1* and *ERAP2* genes is also regulated by the transcription factor NF-kB [42]. Both the transcripts and the proteins of ERAP1 and ERAP2 were reduced following the inhibition of expression and nuclear translocation of p65, a subunity of NF-kB, in neuroblastoma cell lines. Consistently, the exogenous expression of p65 NF-kB in p65-low neuroblastoma cell lines (SH-SY5Y, IMR32, LA-N-5, SK-N-BE(2)c) increased ERAP1 and ERAP2 protein levels over 10 times [42]. Chromatin immunoprecipitation assay revealed a direct binding of p65 NF-kB protein to responsive elements located upstream of the transcription start site of *ERAP1* and *ERAP2* genes [42]. More recently, we demonstrated that IRF1 and p65 NF-kB synergistically further enhance the expression of ERAP proteins in the most aggressive neuroblastoma cell lines [41]. Of note, the co-expression of IRF1 and p65 NF-kB determined an increase in the surface expression of MHC class I molecules and all components of antigen processing machinery, thus rendering neuroblastoma cells susceptible to recognition by antigen-specific cytotoxic $CD8^+$ T cells [41]. *ERAP1* has been proved to be also directly regulated by p53, a tumor suppressor and transcriptional regulator known to protect cells from malignant transformation [46]. Gene expression analysis in a $p53^{-/-}$ colon carcinoma cell line (HCT116) revealed an increased expression of ERAP1, and to less extend of ERAP2, in the cells transfected with wild-type p53, but not with different p53 mutants (R175H, C176F, G245D, R273C, R280T, R282W). Experiments in $p53^{+/+}$ HCT116 cells with or without treatment with the p53 activator nutlin-3a also revealed that ERAP1 mRNA was upregulated by p53 [46]. Chromatin immunoprecipitation analysis demonstrated that p53 upregulates ERAP1 expression through the binding to a responsive element located in an intron of the *ERAP1* gene [46]. Niizeki et al. observed that the expression of ERAP1 increased during *in vitro* differentiation of mouse embryonic stem (ES) cells into endothelial cells (EC) and was regulated, at least in part, by the transcription factor PEBP2 [47]. Interestingly, similar to IRFs and NF-kB [41,42,44], p53 is also directly or indirectly involved in the regulation of MHC class I molecules and/or components of the antigen processing pathway [46,48,49]. IRFs and p53 transcription factors are known to act as tumor suppressors whose functional loss, in combination with other genetic alterations, may significantly increase risk of malignancy [49,50]. The relationship between these transcription factors, inflammation and cancer has been widely reviewed [49–51].

The expression of *ERAP* genes is also regulated at the post-transcriptional level. Two microRNAs from human cytomegalovirus (HCMV), miR-US4 and miR-UL112-5p, have been demonstrated to bind the 3′ UTR of ERAP1 by reducing expression at both the RNA and protein levels [35,52]. Of note, the rs17481334 G variant, naturally occurring in the ERAP1 3′ UTR, contrary to A variant, preserves ERAP1 from HCMV miR-UL112-5p-mediated degradation, contributing to establish a mechanism of HCMV-mediated immune evasion [52].

4. ERAP expression in cancer

Defects in the expression and function of *ERAP* genes have been detected in various solid and hematological tumors, including melanoma, leukemia-lymphomas and carcinomas of breast, colon, lung, skin, chorion, cervix, prostate, kidney and bladder (reviewed in [53]). A comparison in the distribution of ERAP proteins between neoplastic and normal counterparts from the same tissue showed changes that may have happened during malignant transformation [54]. These changes include: a) low expression of ERAP1 and ERAP2 proteins as the most frequent phenotype observed in tumors, regardless of tumor histotype; b) down-regulation of one or both enzymes as the most frequent condition in breast, ovary and lung carcinomas that derive from tissues co-expressing both enzymes; c) up-regulation of both ERAP proteins in

colon and thyroid carcinomas that derive from tissues lacking detectable levels of both; and d) ERAP1/ERAP2 imbalance in essentially all the tested tumor histotypes. Most important, low expression of either ERAP1 or ERAP2 resulted in low levels of functional trimming activities [54]. A discordant expression of ERAP1 and ERAP2 was also detected in renal cell carcinoma lesions as compared to the normal kidney epithelium [55]. No association was detected between the expression of ERAP1 and ERAP2 and the clinical features [55]. In cervical carcinoma, ERAP1 was expressed at high levels in 85% of the samples and at low levels in remaining samples [56]. Low ERAP1 expression was associated with worse overall survival and presence of metastases in cervical carcinoma patients [56]. In another study, ERAP1 expression was partially or totally lost in cervical intraepithelial neoplasia and cervical squamous cell carcinoma as compared to normal epithelium of uterine cervix [57]. Steinbach et al. demonstrated that ERAP1 is overexpressed in HPV16-positive cervical carcinoma cell lines and cervical cancer samples as compared to HPV16-negative samples [58]. However, no role of ERAP1 in the generation of specific HPV antigenic peptides has been identified so far. In endometrial carcinoma, ERAP1 was expressed in 64% of the cases analyzed and found correlated with CA-125 levels, thus suggesting a role in tumor development and differentiation [59–61]. In esophageal carcinoma lesions, the expression of ERAP1 was lost or reduced in 20 and 28% of patients, respectively, and significantly associated with the depth of tumor invasion [62]. Low expression of ERAP1 has been associated with poor clinical outcome of patients affected by triple-negative breast carcinoma [63]. A discordant expression of ERAP1 and ERAP2 was also detected in tumor cell lines, including a set of cell lines derived from glioblastoma multiforme lesions [64–66]. Melanoma cell lines displayed considerable diversity in their *ERAP* gene promoter activities, thus suggesting that the presence of genetic, transcriptional, and post-transcriptional control mechanisms are involved in the regulation of ERAP expression [64].

The role of coding SNPs in ERAP enzymes in tumor immunity and cancer predisposition has been widely reviewed [53,67]. Several functional *ERAP1* variants were correlated with increased metastases and decreased overall survival of cervical [68–71] and non-small-cell lung carcinoma patients [72]. Yao et al. [72] evaluated the association of four *ERAP1* SNPs from two genetically distant populations, Chinese Han and Polish Caucasian. A significant association with all SNPs was detected in Chinese but not in Poles patients [72].

5. Expression and mutation analysis of ERAP genes according to cancer genomic data set

The heterogeneous expression of ERAP1 and ERAP2 in tumors detected at the protein level matches with that observed at the mRNA level. Publicly available data from The Cancer Genome Atlas (TCGA) allow the identification of genetic alterations occurring in 9,125 tumors (www.cbioportal.org). According to TCGA, ERAP1 is detected in all tumor specimens although at variable levels (from 500 to 3491 units), resulting more expressed in acute myeloid leukemia (AML), stomach adenocarcinoma (STAD) and kidney renal clear cell carcinoma (KIRC), and less expressed in testicular germ cell carcinoma (TGCT), uveal melanoma (UVM) and adrenocortical carcinoma (ACC) (Fig. 2A). ERAP2 is detected in all type of tumors, but not in all individuals, as those with rs2248374-GG genotype fail to express the enzyme (Fig. 2A). As shown in a representative type of cancer, the expression of ERAP1 is not coordinated with that of ERAP2 (Fig. 2B and C). *ERAP1* and *ERAP2* genes are rarely mutated in cancer (Fig. 3). Compared to *TP53*, which is highly mutated in different cancer types (up to 75% of head and neck carcinomas), genetic alterations in the *ERAP1* and *ERAP2* genes occur in a small fraction of tumors. An overview of *ERAP1* and *ERAP2* genetic alterations including deep deletions, amplifications and mutations is shown in Fig. 3. Deep deletions are detected in 5% of prostate adenocarcinoma (PRAD) and even less in STAD, AML, and mesothelioma (MESO), whereas amplifications are more frequent in KIRC and ACC

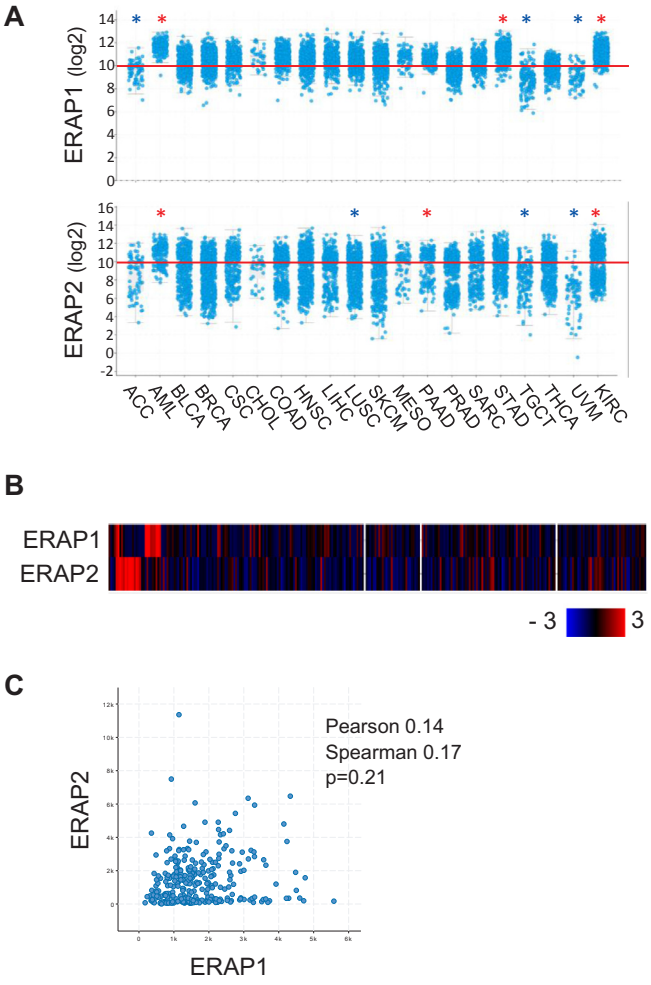


Fig. 2. Expression of ERAP1 and ERAP2 across TCGA tumor tissues. A, Data from the cBioPortal website (www.cbioportal.org) showing mRNA expression of ERAP1 and ERAP2, evaluated by RNAseq, in 9,125 cancer samples of various origin. Red bars were added to compare ERAP1 and ERAP2 expression levels. Red and blue asterisks indicate the higher and the lower ERAP1 and ERAP2 expressing tumor types, respectively. B, Heat map showing the mRNA expression of ERAP1 and ERAP2 in 297 cervical squamous cell carcinoma patients. C, Scatter plot showing the correlation between the mRNA expression of ERAP1 and ERAP2 in 297 cervical squamous cell carcinoma patients. Pearson and Spearman correlation coefficients and the *p* value are reported. ACC: Adrenocortical carcinoma; AML: Acute Myeloid Leukemia; BLCA: Bladder Urothelial Carcinoma; BRCA: Breast invasive carcinoma; CSC: Cervical squamous cell carcinoma; CHOL: Cholangiocarcinoma; COAD: Colon adenocarcinoma; HNSC: Head and Neck squamous cell carcinoma; LIHC: Liver hepatocellular carcinoma; LUSC: Lung squamous cell carcinoma; SKCM: Skin Cutaneous Melanoma; MESO: Mesothelioma; PAAD: Pancreatic adenocarcinoma; PRAD: Prostate adenocarcinoma; SARC: Sarcoma; STAD: Stomach adenocarcinoma; TGCT: Testicular Germ Cell Tumors; THCA: Thyroid carcinoma; UVM: Uveal Melanoma; KIRC: Kidney renal clear cell carcinoma.

(Fig. 3A). Of note, these genetic alterations affect both genes. Thirty-eight missence and 9 truncating mutation are associated with ERAP1 gene (Fig. 3B). Similarly, 42 missense and 13 truncating mutations are associated with *ERAP2* gene (Fig. 3B). Interestingly, although overall mutations in *ERAP1* and *ERAP2* genes account in a small fraction of tumors (0.6% and 0.8%, respectively), some amino acid residues (Y145, A637, S868 for *ERAP1* and G340, R225, W496 for *ERAP2*) are found mutated in more than one tumor (Fig. 3B). These mutations are distributed along the entire gene. Their potential function in affecting tumorigenesis need further investigation. Genome-wide sequencing

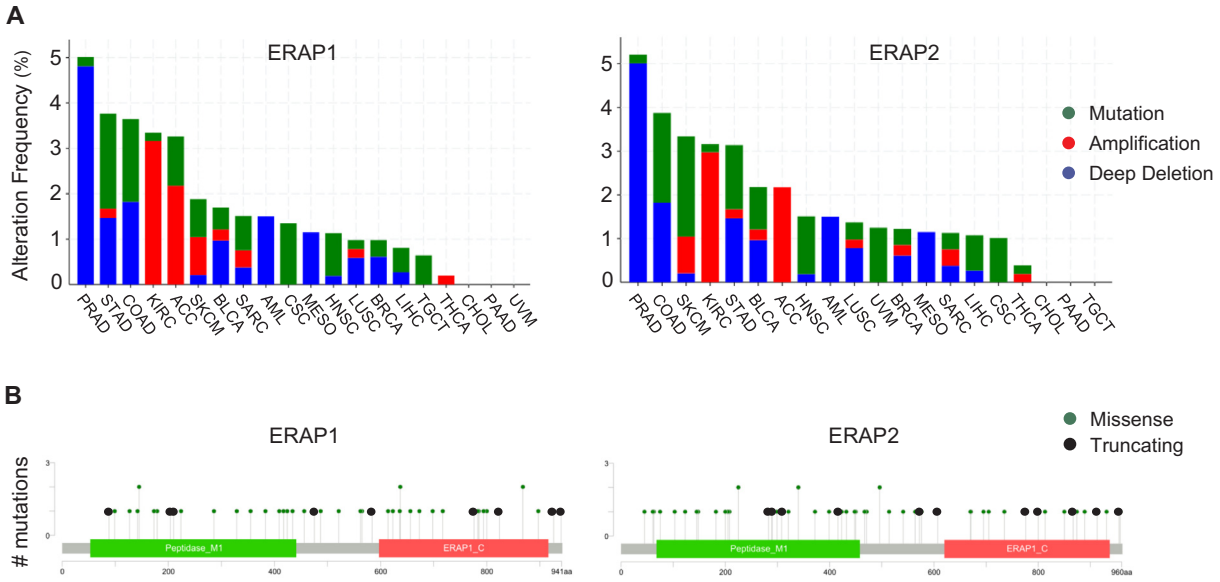


Fig. 3. Alterations frequency of *ERAP1* and *ERAP2* genes across TCGA tumor tissues. A, Alterations of *ERAP1* and *ERAP2* genes were visualized by cBioportal for Cancer Genomics. Mutation, amplification and deletion are shown in different colors. The most frequent alterations of *ERAP1* and *ERAP2* genes are deletions in pancreatic adenocarcinomas. B, Graphical summary of *ERAP1* and *ERAP2* missense and truncating mutations mapped to protein domains. Totally 47 and 55 mutation sites were detected for *ERAP1* and *ERAP2* genes, respectively. Peptidase_M1: Peptidase family M1 domain (53-441 amino acid for ERAP1 and 68-458 amino acid for ERAP2). ERAP1_C: ERAP1-like C-terminal domain (597-916 amino acid for ERAP1 and 620-937 amino acid for ERAP2). ACC: Adrenocortical carcinoma; AML: Acute Myeloid Leukemia; BLCA: Bladder Urothelial Carcinoma; BRCA: Breast invasive carcinoma; CSC: Cervical squamous cell carcinoma; CHOL: Cholangiocarcinoma; COAD: Colon adenocarcinoma; HNSC: Head and Neck squamous cell carcinoma; LIHC: Liver hepatocellular carcinoma; LUSC: Lung squamous cell carcinoma; SKCM: Skin Cutaneous Melanoma; MESO: Mesothelioma; PAAD: Pancreatic adenocarcinoma; PRAD: Prostate adenocarcinoma; SARC: Sarcoma; STAD: Stomach adenocarcinoma; TGCT: Testicular Germ Cell Tumors; THCA: Thyroid carcinoma; UVM: Uveal Melanoma; KIRC: Kidney renal clear cell carcinoma.

studies have revealed thousands of genes mutated in a small fraction of tumors (passenger) yet may still be important for cancer initiation and progression. In general, passenger mutations are located on functional features or conserved residues which are shared by other genes with a common functional role (i.e., oncodomain hotspots) [73] in processes known to influence cancer initiation or progression such as ubiquitination, proteolysis, metabolic proteins and genes involved with actin binding and the cytoskeleton [74–84].

6. Potential role of ERAP in cancer immunotherapy

Several studies have highlighted that ERAP1 and ERAP2 may be important targets for boosting T cell- and NK cell-mediated immune responses to established cancer [20–22]. These results are also supported by a recent work [23], which demonstrated that ERAP1-dependent cross-presentation of cell-associated antigens has an important role in the efficacy of adoptive T cell transfer. Of note, this study show that NK cells of ERAP1$^{-/-}$ Rag$^{-/-}$ recipients mice tolerate ERAP1-deficient TAg^{+} hepatocellular carcinoma (HCC) cells but mediate killing of ERAP1-expressing TAg^{+} HCC. These data support previous evidences [20,21] that the cross-reactivity between peptides and NK cell-inhibitory receptors could favor activating signals and consequently NK cell-mediated anti-tumor killing. By contrast, ERAP1$^{+/+}$ Rag$^{-/-}$ mice recipients showed a different phenotype since they tolerated at the same manner both ERAP1-expressing and ERAP-deficient TAg^{+} HCC cells [23]. These data further suggest that the modulation of ERAP1 could represent a benefit for NK cell-based immunotherapy only in specific conditions such as the expression of activating ligands on tumor cells and that of inhibitory receptors on NK cells. More recently, two studies have shown that expression of ERAP enzymes render tumor cells more sensitive to immunotherapy based on the neutralization of immune checkpoint inhibitors [85,86]. In the first study, low levels of ERAP2 were associated with improved response to anti-PD-L1 in patients with the luminal subtype of bladder cancer [85]. Consistent with these results, an *in vivo* loss-of-function CRISP genetic screen demonstrated that depletion of ERAP1 in a mouse transplantable tumor model increased the efficacy of anti-PD-1 immunotherapy [86]. The presentation of cancer-specific antigens as well as successful infiltration of T cells are essential for effective antitumor immune responses [87]. We have shown that the inhibition of ERAP1 leads to greater recruitment of intratumoral T and NK cells and a substantial change of the repertoire of peptides presented on the cell surface to immune effector cells [20]. Given the central role of ERAP enzymes in manipulating immune response, they are emerging as attractive targets for cancer immunotherapy. In this context, there is a strong interest in the development of new pharmacological inhibitors of ERAP enzymes. A detailed description of the currently available ERAP inhibitors is reported in another review in this issue by Hanson and colleagues [88].

7. Conclusions

The expression of ERAP1 and ERAP2 is frequently altered in tumors as compared to their normal counterparts, with low expression associated with poor prognosis. However, what is the contribution of these enzymes to tumor growth and activation of anti-tumor immune responses is not well understood. So far, few studies demonstrate the direct involvement of ERAP enzymes in tumor growth and in the generation of specific tumor epitopes. The reduced expression of ERAP1 in ERAP1-expressing tumors, such as lymphoma, colorectal carcinoma and melanoma, resulted in an increase of both NK and CD8^{+} T cell-mediated antitumor immune responses [20,89,90]. Although ERAP1-deficient mice do not develop tumors spontaneously [8,14–16,18], the use of ERAP1-deficient tumor models obtained from the crossing of ERAP1-deficient mice with mouse tumor models or by their treatment with chemical agents will be useful to completely dissect the role of ERAP enzymes in tumor development and cancer immunity.

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