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Effects of polymorphic variation on the mechanism of Endoplasmic Reticulum Aminopeptidase 1

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ABSTRACT

Endoplasmic Reticulum Aminopeptidase 1 (ERAP1) generates antigenic peptides for loading onto Major Histocompatibility Class I molecules (MHCI) and can regulate adaptive immune responses. During the last few years, many genetic studies have revealed strong associations between coding Single Nucleotide Polymorphisms (SNPs) in ERAP1 and common human diseases ranging from viral infections to cancer and autoimmunity. Functional studies have established that these SNPs affect enzyme activity resulting to changes in antigenic peptide processing, presentation by MHCI and cellular cytotoxic responses. These disease-associated polymorphisms are, however, located away from the enzyme's active site and are interspersed to different structural domains. As a result, the mechanism by which these SNPs can affect function remains largely elusive. ERAP1 utilizes a complex catalytic mechanism that involves a large conformational change between inactive and active forms and has the unique property to trim larger peptides more efficiently than smaller ones. We analyzed two of the most consistently discovered disease-associated polymorphisms, namely K528R and Q730E, for their effect on the ability of the enzyme to select substrates based on length and to undergo conformational changes. By utilizing enzymatic and computational analysis we propose that disease-associated SNPs can affect ERAP1 function by influencing: (i) substrate length selection and (ii) the conformational distribution of the protein ensemble. Our results provide novel insight on the mechanisms by which polymorphic variation distal from the active site of ERAP1 can translate to changes in function and contribute to immune system variability in humans.

1. Introduction

Cytotoxic T-lymphocyte (CTL) responses towards infected or aberrant cells are central to the ability of the adaptive immune response to fight pathogens. CTLs recognize infected cells by means of specific antigenic peptides bound onto specialized receptors of the Major Histocompatibility class I (MHCI) on the surface of all somatic cells. These antigenic peptides are derived from the proteolytic digestion of intracellular proteins and represent a sample of the protein content of the cell (Lazaro et al., 2015). Recognition of antigenic peptides that do not belong to the normal protein content of the cell indicates infection or malignant transformation, eliciting specific cytotoxic responses that eradicate the cell (Weimershaus et al., 2013).

During the last decade the trimming of N-terminal extended precursor peptides by ER-resident aminopeptidase ERAP1 has been recognized to be key for the generation of antigenic peptides (Weimershaus et al., 2013; Evnouchidou et al., 2009). ERAP1 is necessary for the generation of many antigenic epitopes but can also over-trim others, leading to their destruction (Blanchard and Shastri, 2008). It is a 110 kDa zinc-aminopeptidase that belongs to the M1 family of metallopeptidases, but has several unique properties that fit well with its biological role. First, it can efficiently process many different peptide substrates, consistent with the vast variety of peptide sequences that it may encounter in the ER. Secondly, it prefers to trim larger peptides over smaller ones, resulting to the accumulation of products of 8-9 amino acids long, a length consistent with the binding preferences of MHCI (Chang et al., 2005). Lastly, ERAP1 activity can be dependent on the whole peptide sequence, a property that can affect the pool of antigenic peptides available for MHCI presentation (Evnouchidou et al., 2008).

Crystallographic and biochemical analysis of ERAP1 has revealed that during its catalytic cycle the enzyme can undergo a signif-

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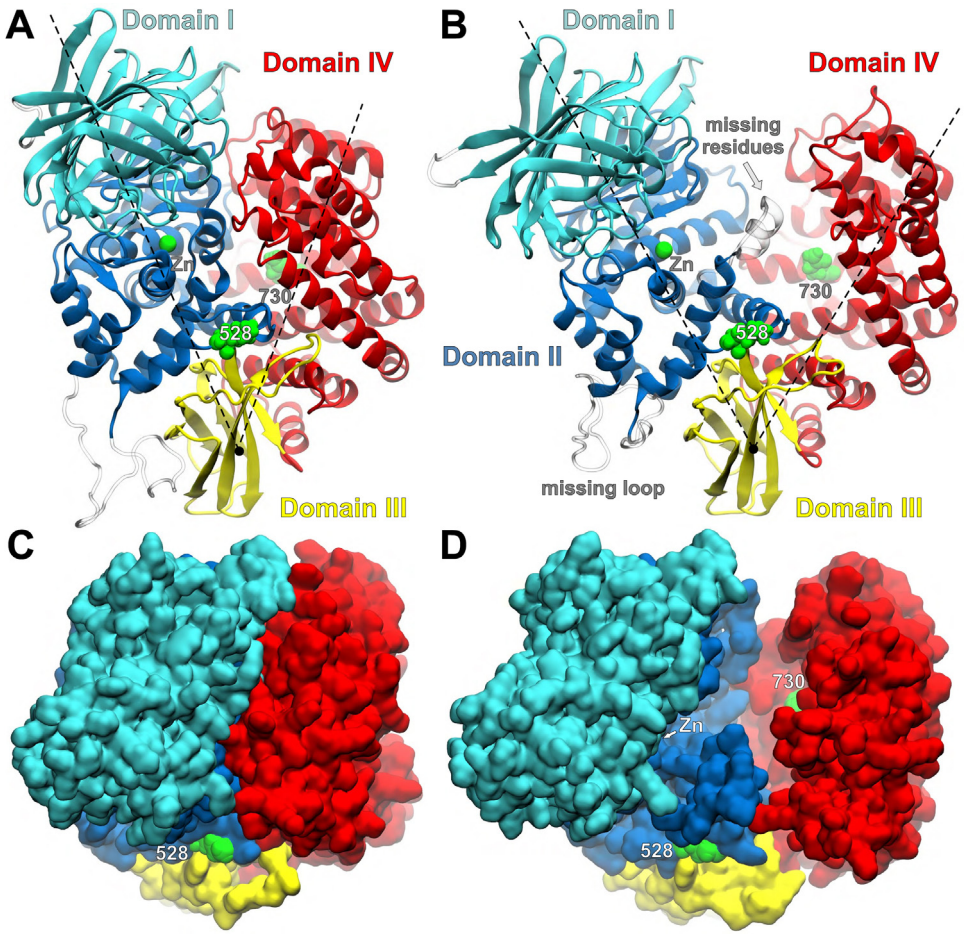

Fig. 1. Crystallographic structures of ERAP1 in the closed (A, PDB: 2YD0) and open states (B, PDB: 3MDJ), illustrating the domain organization of the enzyme, the catalytic Zn and the polymorphic residues at positions 528 and 730. The missing residues (417–433) in the open state, and the missing loop (486–514) in both X-ray structures were modeled and are shown in gray. The interdomain angle theta is defined using the centers of mass of domains I and II (excluding the missing loop), domain III and domain IV, and is 53° in the closed and 67° in the open state. (C and D) Surface representation of the two ERAP1 states from the top of the substrate binding site indicating the position of the two polymorphic residues.

icant conformational change that involves reorganization of key structural domains of the enzyme from an open to a closed state (reviewed in (Stratikos and Stern, 2013)). An extended internal cavity hypothesized to accommodate the peptide-substrate is only exposed to the external solvent in the open conformation (Fig. 1). Therefore, the open state has been suggested to facilitate initial substrate capture and the closed state to enhance catalysis by organizing catalytic residues and specificity pockets. Larger peptides may facilitate this transition through a mechanism of self-activation involving a still unmapped regulatory site in the enzyme (Nguyen et al., 2011; Gandhi et al., 2011; Kochan et al., 2011). Overall, it appears that ERAP1 has evolved to specialize for generating antigenic peptides for MHCI.

Several population genetic studies have associated several coding single nucleotide polymorphisms (SNPs) in ERAP1 with predisposition to major human diseases ranging from viral infections to cancer and autoimmunity (reviewed in (Fruci et al., 2014; Alvarez-Navarro and Lopez de Castro, 2014)). These associations have been repeatedly confirmed especially for autoimmune diseases often in the context of specific MHCI alleles, suggesting that the disease-link lies within the role of ERAP1 in generating antigenic peptides (Alvarez-Navarro and Lopez de Castro, 2014; The Australo-Anglo-American Spondyloarthritis et al., 2011). Several studies have also demonstrated that these disease-associated variants affect ERAP1 enzymatic activity and selectivity, cellular antigen presentation and concomitant cytotoxic responses (Evnouchidou et al., 2011; Chen et al., 2014; Reeves et al., 2013; Garcia-Medel et al., 2012; Sanz-Bravo et al., 2015; Alvarez-Navarro et al., 2015; Martin-Esteban et al., 2014). Interestingly, in several of those studies, changes in ERAP1 activity due to polymorphic variation at positions 528 and 730 have been correlated with the production of different lengths of antigenic peptides although the effects of peptide sequence were not investigated in those studies (Garcia-Medel et al., 2012; Sanz-Bravo et al., 2015; Alvarez-Navarro et al., 2015). Specific ERAP1 haplotypes have been proposed to constitute a range of different activities that correlate to disease predisposition (Reeves et al., 2013; Seregin et al., 2013). This association is reminiscent of the natural variability in MHCI molecules: polymorphisms in the MHC locus define thousands of MHCI alleles within the population and affect antigenic peptide binding and presentation as well as resulting adaptive immune responses to pathogens. Most polymorphisms in MHCI cluster within and around the peptide binding pocket, directly affecting peptide binding or interactions with the T-cell receptor (Reche and Reinherz, 2003). In contrast, most disease-associated polymorphisms in ERAP1 are located distal to the active site and are scattered to different structural domains (Nguyen et al., 2011). As a result, little insight exists on how a diverse set of natural polymorphisms dispersed over the struc-

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