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Targeting HLA-B27 Misfolding as a Potential Therapeutic Treatment of Ankylosying Spondylitis

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Abstract

Human Leucocyte Antigen (HLA)-B27 associated inflammatory arthritic diseases, referred to as the spondyloarthropathies (SpAs) remain enigmatic. Genome wide associations scans (GWAS) have revealed other strong genetic associations with the SpAs but have yet to provide a telling insight into the cause of disease and the role of the strongest genetic marker HLA-B27. Recent observations suggest that misfolding of HLA-B27 may be a contributory factor to the development of inflammatory arthritis. Targeting aberrant conformations of HLA-B27 and/or the pathways triggered by misfolding HLA-B27 molecules may provide a window for a different form of therapeutic approach for this group of inflammatory arthritic diseases. Here I will focus on the evidence supporting a role for HLA-B27 misfolding in disease pathology and our recent studies, which have provided a rationale for why targeting unusual intracellular HLA-B27 conformations may be a potential future therapeutic approach for treating the SpAs.

Keywords: HLA-B27; Unfolded protein response; Autophagy; Spondyloarthropathy

Abbreviations: HLA: Human Leucocyte Antigen; GWAS: Genome Wide Associations Scans; SpAs: Spondyloarthropathies; ER: Endoplasmic Reticulum; β2m: Beta-2 Microglobulin; HC: Heavy Chain; ERAD: ER Associated Degradation; UPR: Unfolded Protein Response; AS: Ankylosying Spondylitis; XBP-1: X Box Binding Protein-1; p: Position; D: Aspartic Acid; H: Histidine; Y: Tyrosine; S: Serine; V: Valine; E: Glutamic Acid; A: Alanine; G: Glycine; TNFα: Tumour Necrosis Factor Alpha; C: Carboxy

Introduction

HLA-B27 is a Major Histocompatibility Complex (MHC) class I molecule whose predominant function is the presentation of short 8-10 amino acid long peptides to cytotoxic CD8+T cells. HLA-B27 exhibits a strong association with a group of inflammatory arthritic diseases referred to as the spondyloarthropathies (SpAs) [1]. The SpAs are characterized by inflammation at the entheses, followed by skeletal fusion, which predominantly occurs at the sacroiliac joint and along the vertebral column. In addition, the SpAs are characterized by inflammation of other organ systems such as the gastro-intestinal tract, which is perhaps the next most common inflammatory lesion, manifesting as either a 'Crohns' or 'colitis' like disease [2]. It remains unknown why these specific sites are targets for disease development but it has been proposed that exposure to mechanical stress could be a contributory factor [3].

MHC class I heavy chains (HCs) assemble within the endoplasmic reticulum (ER) via transient associations with ER resident chaperones. MHC class I HCs form non-covalent interactions with the light chain beta-2 microglobulin (β2m) and associate with peptides of 8-10 amino acids long, before transiting through the secretory pathway to the plasma membrane [4]. MHC class I molecules, which can misfold at any point along the assembly process, are expelled from the ER and targeted for degradation by the ubiquitin-proteasome pathway [5]. The pathway for degrading ER associated proteins is known as ER associated degradation (ERAD). However, different proteins may employ different components of the ERAD pathway [6].

Conclusion

HLA-B27 misfolding and the spondyloarthropathies

HLA-B27 exhibits unusual characteristics that set it apart from most other classical MHC class I molecules [7]. The HLA-B27 HC exhibits an enhanced propensity to misfold [8] and form unusual conformations such as disulfide-bonded HC homo-dimers [9,10]. HLA-B27 expressing transgenic rats developed a spontaneous AS like disease that was dependent on high transgene copy number [11,12]. Disease incidence correlated with the expression of HLA-B27 HC-dimers [13].

It was proposed that the slow rate of maturation of HLA-B27 was related to HC-homodimer formation within the ER [10]. HLA-B27 maturation (as determined by measuring the rate of acquisition of endoglycosidase H resistance, which monitors post-translational oligosaccharide processing during transit from the ER through the Golgi apparatus) occurs at a slower rate when compared to most other MHC class I HCs [10]. Another key observation in the animal model was the development of a transgenic rat with 'improved' HLA-B27 folding by the introduction of high copies of human β2m. Such transgenic animals exhibited skeletal disease, more akin to the human AS. However, the gut inflammation, which was the more prevalent manifestation of disease in the original transgenic models, was no longer evident. These observations suggested HLA-B27 associated SpA pathologies as being comprised of distinct diseases with HLA-B27 misfolding associated more with gut pathologies [14]. It was therefore proposed that HLA-B27 misfolding within the ER led to disease development through the activation of the Unfolded Protein Response (UPR) [15]. The UPR is a cellular response to protein misfolding within the ER [16], which can trigger the release of inflammatory mediators [17]. HLA-B27 expression in rat derived macrophages correlated with the activation of a key transcription factor of the UPR, the X box binding protein-1 (XBP-1) [18]. XBP-1 exists as an mRNA in the cytosol and following UPR activation, a 26 nucleotide intronic sequence is spliced from the mRNA by the ER resident IRE1 kinase.

The spliced XBP-1 (XBP-1s) is a potent transcription factor that targets folding chaperones and ERAD machinery components [19] and has been implicated in having a role in inflammatory bowel disease [20]. Controversy exists whether the UPR can be detected in AS patients [21,22]. However, UPR activation is temporal and the timing of activation can determine cellular fate [23]. Within apparent homogenous cell populations, the UPR can be heterogeneous in the timing of its induction. It was observed that cells could either induce XBP-1s early or late on in response to UPR inducing agents. It was proposed that early onset of XBP-1s activation would lead to a faster accumulation of downstream transcriptional targets many of which are ERAD components [23]. Interestingly our recent observations demonstrated an up-regulation of the ERAD component, the E3 ubiquitin ligase HRD1 in a population of AS HLA-B27+patients. The detection of elevated levels of HRD1 suggested that UPR induction can leave a 'footprint' [24] and is detectable depending on the selected target protein.

Subsequently, a link has been demonstrated between the UPR and autophagy [25], which is a process employed by cells to eliminate and recycle intracellular components. Autophagy has been demonstrated to induce pro-inflammatory cytokine production, especially IL-23 which is elevated in SpA patients [26-28]. Therefore, there appears to be a link between HLA-B27, induction of pathways related to misfolding protein clearance and pro-inflammatory cytokine production.

However, a conundrum does exist with the hypothesis that misfolding HLA-B27 participates in inflammatory arthritic disease. The UPR and autophagy can be viewed as defense mechanisms employed by the cell to protect against the accumulation of misfolded or dysfunctional proteins. If misfolded or unusual conformations of HLA-B27 do indeed induce the UPR and/or autophagy pathways, why does a disease state follow? It is possible that misfolded or dimeric forms of HLA-B27 may present a particular problem to the cell in undergoing disposal under normal physiological conditions (Antoniou unpublished).

We have demonstrated that HLA-B27 HC-dimers are unusually long lived [24] (Panayoitou and Antoniou, unpublished). The apparent long half life of HLA-B27 HC-dimers could well be due to the unusual conformations adopted within the ER, making disposal problematic. Alternatively, the nature of the misfolding event may not or only partially trigger pathways leading to ERAD, resulting in inefficient disposal. Overall the effect could be chronic triggering of the UPR and/or autophagy resulting in the deregulated production of inflammatory cytokines such as TNFα, IL-17 and IL-23.

The misfolding hypothesis should provide an explanation for the lack of association of various HLA-B27 subtypes. HLA-B2*27:05 and 04 are two of the most common subtypes associated with AS. Two HLA-B27 subtypes which exhibit no association with disease are HLA-B*27:09 and 06 which express limited differences when compared to HLA-B*27:05 and 04. Polymorphisms between these subtypes are predominantly found within the F pocket of the antigen binding groove at positions (p) 114 and 116. The p114-116 residues determine the C-terminal sequence of associated peptides and heavy chain folding characteristics. HLA-B*27:09 exhibits an aspartic acid (D) to histidine (H) change at p116. HLA-B*27:06 expresses five differences compared to HLA-B*27:05 i.e. D77S, V152E, H114D, D116Y and A211G. The residues expressed by HLA-B*27:06 at p77 and p152 are shared with HLA-B*27:04 however 04 shares the same F pocket residues with HLA-B*27:05. Compared to HLA-B*27:04 and 05, the 06 subtype differs at p114 and p116.

HLA-B*27:09 and 06 subtypes do exhibit biochemical differences, which include differences in stability/flexibility [29,30], cysteine residue exposure [8] and use of the MHC class I accessory molecule tapasin [31]. We demonstrated that the F pocket residues could influence HC-dimer formation and the rate of maturation. The most pronounced effects were observed with the HLA-B*27:06 F pocket residues, which reduced HC-dimer formation and led to a more rapid maturation rate [32]. Thus, disease and non-disease associated HLA-B27 subtypes do exhibit differences in their ability to fold and misfold.

Exploiting the UPR and autophagy induced ERAD as a therapeutic tool

We wanted to determine whether misfolded HC-dimeric HLA-B27 conformers were indeed targets for UPR induced ERAD [24,32]. To this end, we inhibited expression of XBP-1 and

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some of its key ERAD target genes. We determined that EDEM1, derlin 1, derlin 2 and calnexin did participate in HLA-B27 HC-dimer degradation [24,32]. The most pronounced effect on dimer degradation followed the inhibition of HRD1 [24]. Thus a UPR mediated ERAD pathway can target aberrant HLA-B27 HC-dimers for clearance by the cell.

As the UPR and autophagy pathways can be viewed as intracellular defense mechanisms against the accumulation of dysfunctional proteins, the question arose if inflammatory arthritic disease can be instigated by HLA-B27 misfolding, could the UPR/autophagy pathways be exploited for therapeutic intervention? Our proof of principle study subjected cell lines expressing HLA-B27 HC-dimers to UPR inducing pharmacological agents such as tunicamycin (glycosylation inhibitor), thapsigargin (ER calcium gradient inhibitor) and MG132 (proteasome inhibitor). These pharmacological agents exhibit a biphasic mode of action i.e. initial perturbation of protein folding leading to the accumulation of misfolding proteins, followed by triggering of the UPR and elimination of misfolded proteins. Our studies demonstrated that HLA-B27 HC-dimers could be eliminated following incubation with UPR inducing pharmacological agents and the degradation pathway was dependent on an intact ERAD pathway [24].

Our observations would indicate that under the correct conditions, HLA-B27 HC-dimers can be eliminated. Therefore under 'normal' conditions the UPR induced ERAD pathway or indeed autophagy may not be fully engaged to effectively remove aberrant protein conformations. Our unpublished observations also suggest that autophagy can target HLA-B27 HC-dimers (Panayoitou and Antoniou unpublished). Therefore can the UPR and/or autophagy be exploited as therapeutic treatment for the SpAs? Such an intervention strategy would either aim to trigger the UPR to fully induce ERAD pathways or specifically target ERAD components that enhance the removal of misfolded HLA-B27.

Alternatively, strategies can be sought to reduce the burden of misfolded conformers on the ER. Our studies have demonstrated that activating or 'pushing' the UPR to fully activate the ERAD process, can target the unusual conformations adopted by HLA-B27. In addition pharmacological induction of the UPR under our experimental conditions maintained monomer expression levels [24]. This raised the distinct possibility that the UPR can be exploited and targeted to rid cells of HLA-B27 conformations thought to be responsible for disease as a potential therapeutic avenue. Such an approach of inducing physiological degradation pathways is not unheard of and has been used successfully under experimental conditions. For instance the autophagy pathway has been pharmacologically enhanced to remove mutated α1-antitrypisin aggregates responsible for causing liver fibrosis in α1-antitrypsin deficiency [33]. Therefore, exploiting degradation pathways does seem a plausible approach in targeting misfolding HLA-B27.

The cause of SpAs and the role of HLA-B27 in disease development remain unknown. Recent observations suggest that misfolding of HLA-B27 may indeed be a contributory factor to at least some of the pathologies associated with the SpAs. Recent studies have provided the proof of principle that the UPR and possibly autophagy could be exploited as novel therapeutic intervention strategies targeting unusual HLA-B27 protein conformations.

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