Unveiling Vimentin-Collagen Hybrid Peptides: Potential Neo-Antigens produced by Cathepsin K Linked to Autoimmunity in Rheumatoid Arthritis

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Introduction

Around 0.5-1% of the world population suffers from rheumatoid arthritis (RA). Post-translationally modified forms of peptides (such as cirtrullinated) from self-proteins such as vimentin (VIM) and type II collagen (TIIC) have been implicated in the induction of RA. Nothing is known whether modified peptides consisting of hybrids between self-proteins (hybrid neo-antigens) can contribute to the pathogenesis of RA. Here, we demonstrate that cathepsin K, a key protease involved in cartilage and bone degradation in RA is capable to catalyze transpeptidase reactions leading to hybrid peptides. This study investigates the potential of human cathepsin K (hCatK) to fuse self-peptides from VIM and TIIC to form hybrid potential neo-antigens. Fusion products were identified by LC-MS/MS and labeled transpeptidase donor peptides based on a antigenic vimentin peptide sequence revealed wide-spread formation of TIIC/vimentin hybrid peptides.

Induction of rheumatoid arthritis & hypothesized mechanisms behind peptide fusion by cysteine proteases

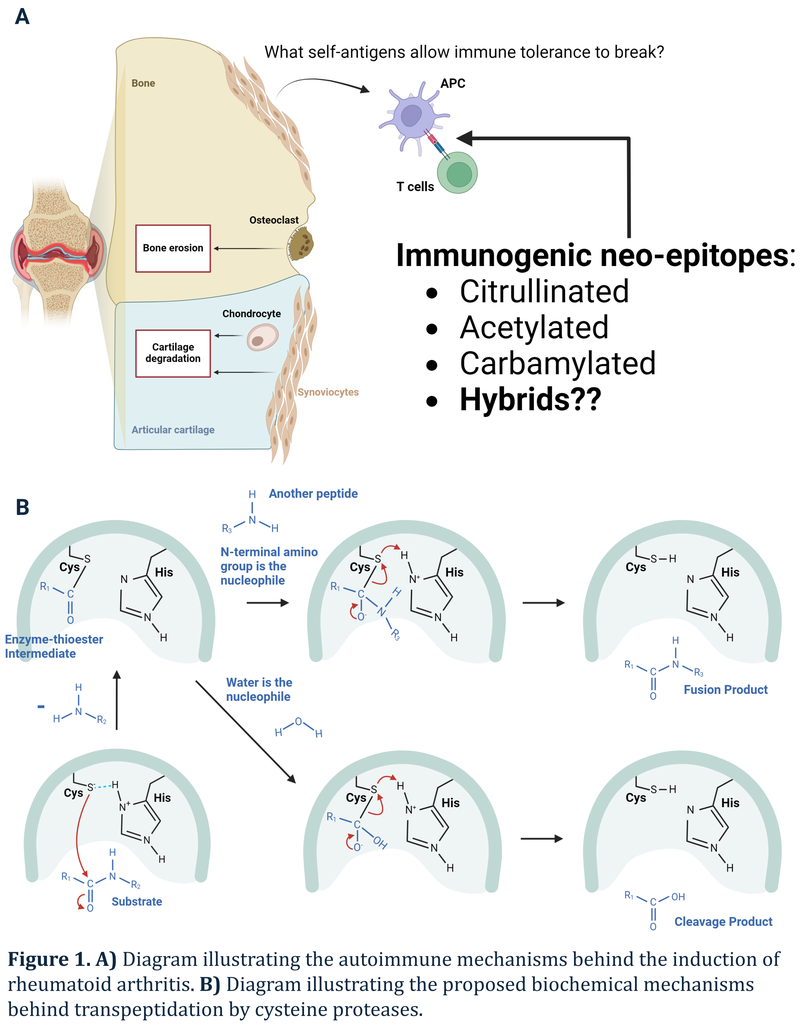


Figure 1. A) Diagram illustrating the autoimmune mechanisms behind the induction of rheumatoid arthritis. **B)** Diagram illustrating the proposed biochemical mechanisms behind transpeptidation by cysteine proteases.

Identified substrate-donor cleavage sites and fusion products catalyzed by cathepsin K

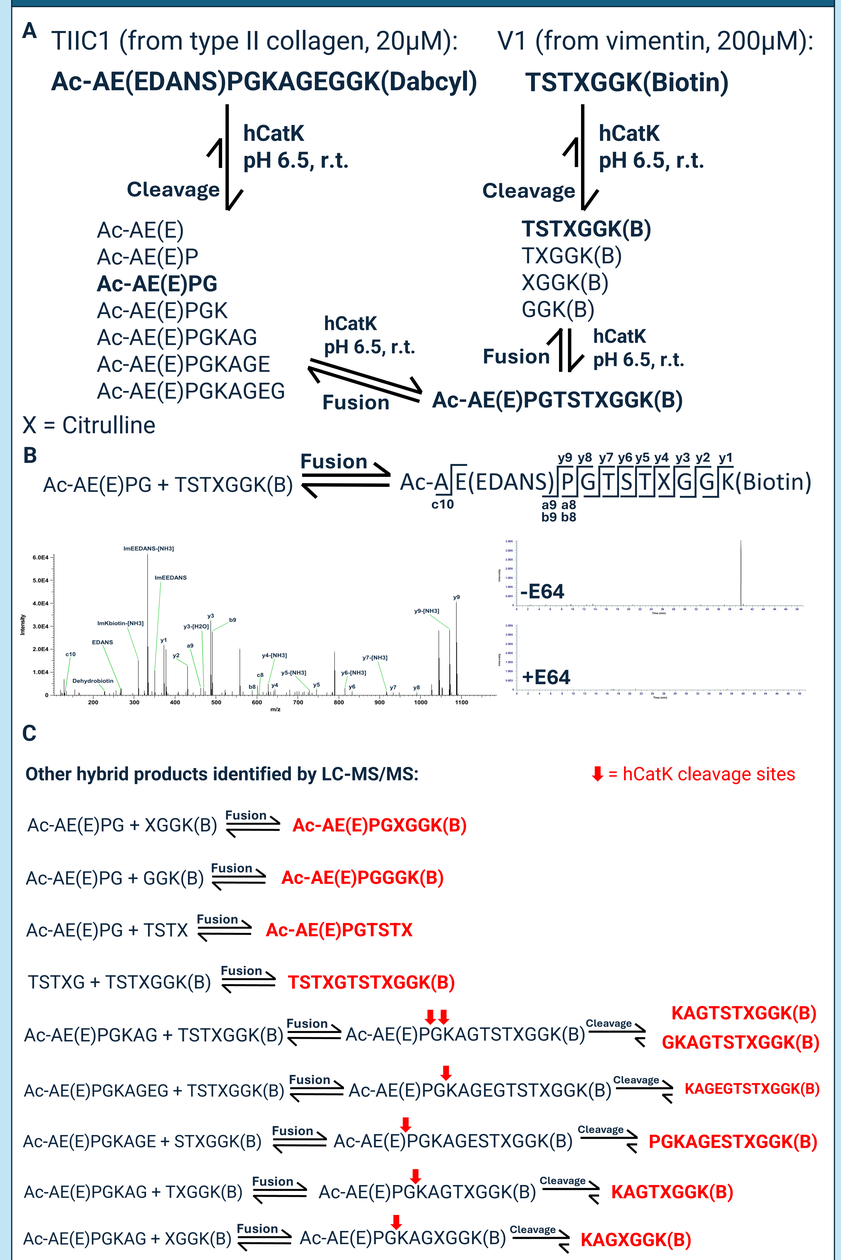


Figure 2. Identification of hybrid peptides between type II collagen and vimentin small peptides using LC-MS/MS. A) Schematic illustration of cleavage, then fusion between small peptides. **B)** LC-MS spectra and LC-MS/MS fragmentation pattern of one hybrid peptide identified following co-incubation of TIIC1 (20µM) and V1 (200µM) with hCatK (500nM) for 5 hours. **C)** List of all hybrid peptides identified by LC-MS/MS along with the hypothesized mechanism behind their formation. None of these hybrid products were identified in control samples containing hCatK inactivated with E64.

pH-dependent fusion of type II collagen fragments with a biotin-labeled vimentin peptide by cathepsin K

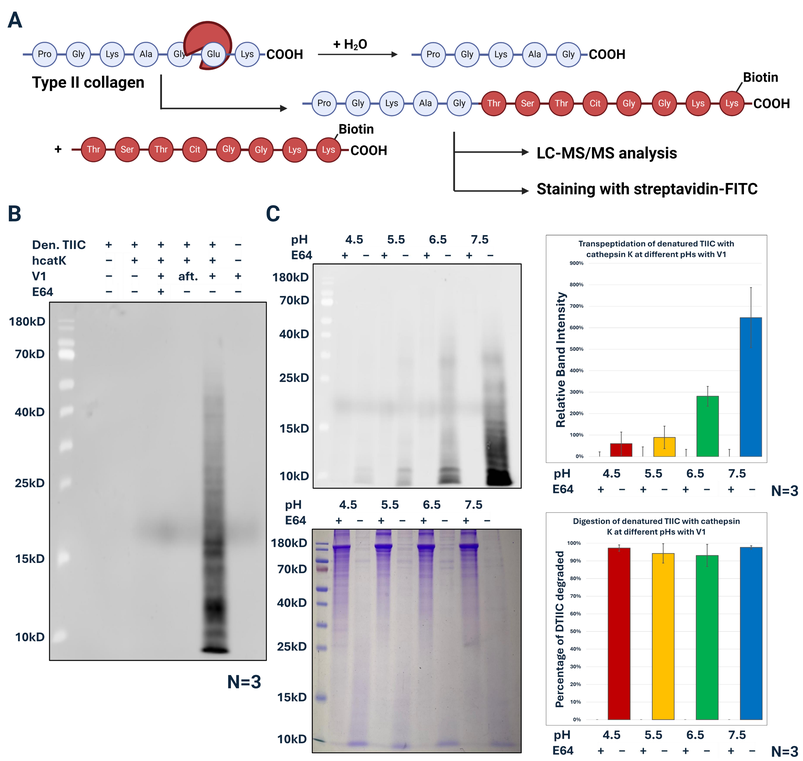
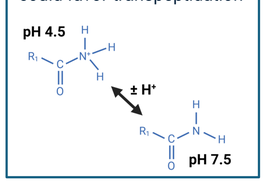


Figure 3. Transpeptidation between denatured type II collagen fragments and the biotinylated V1 peptide. Type II collagen was denatured (DTIIC) by incubation at 70°C for 20 min. **A)** Schematic illustrating fusion between type II collagen fragments and the V1 peptide through transpeptidation. **B)** DTIIC was co-incubated with 5nM of hCatK for 8h at 28°C. High-molecular-weight bands can be observed only when the biotinylated V1 peptide is co-incubated with an active form of hCatK, along with DTIIC. **C)** The co-incubation between DTIIC and V1 in the presence of 50nM of hCatK was performed in four different pH conditions ranging from pH 4.5 to 7.5. The strength of the bands corresponding to potential fusion products between DTIIC fragments and V1 correlates with increasing pH. However, the digestion pattern observed in the same conditions is similar throughout.

pH-dependent effect: Deprotonation of the nucleophilic amino group could favor transpeptidation



Summary

LC-MS/MS shows that hCatK can catalyze fusion between small RA-related peptides

Biotin assays show pH-dependency behind the fusion between full-length TIIC fragments and a citrullinated vimentin peptide

Acknowledgements

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