

Expression of HtrA1 in Chondrocytes

Matrix Degradation, Inhibition of Matrix Synthesis, and the Expression of a Hypertrophic Phenotype.

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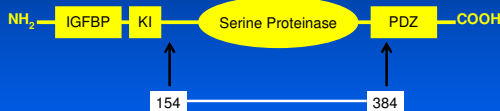
HtrA1: Introduction

- Evolutionarily conserved, secreted serine proteinase
 - high homology to a bacterial enzyme: *HtrA*
- Temperature Requirement
- Three other family members: HtrA2, HtrA3 & HtrA4
- Disease-associated roles in:
 - Macular degeneration
 - Tumor growth
 - Osteoarthritis



HtrA1: Domain Structure

HtrA1: *alias* PRSS11



- 154 -384 construct: catalytically active + retains antagonism of signaling via TGF β family members
 - C. Oka et al Development, 2004



HtrA1 and Osteoarthritis

- Direct proteolytic activity vs. several matrix proteins
 - Z. Yang et al., ORS, 2006; A. Tsuchiya et al., Bone, 2005.
- Activity enhanced by PDZ-binding peptides.
 - Murwantoko et al., Biochem J., 2004.
- Upregulated expression in OA vs. normal cartilage
 - S.-I. Hu et al., JBC 1998
- Generation of catabolically active protein fragments
 - S. Grau et al, JBC, 2005.
- Antagonism of TGF β family signaling pathways.
 - C. Oka et al, Development, 2004.
- Induced chondrocyte phenotypic changes.
 - S. Grau et al., JBC, 2005; Z. Yang et al., ORS, 2006.



Objectives

- Understand potential of HtrA1 to contribute to chondrocyte phenotype & to depletion of extracellular GAG
 - direct addition of recombinant HtrA1
 - overexpression of hHtrA1
 - siRNA knockdown of HtrA1 in human OA chondrocytes
- Determine HtrA1 substrates & cleavage sites in cartilage proteins
 - identify small peptide substrates

Normal bovine chondrocytes



Methods

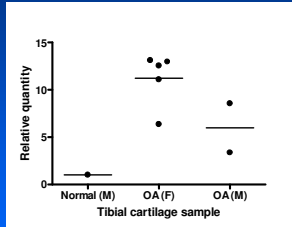
- Normal bovine cartilage
- Human cartilage
 - OA: Total joint replacement
 - Normal: Organ donor
- HtrA1 overexpression via electroporation: Amaxa
- Transfection of siRNA's using Dharmatect 4
- GAG synthesis/content via S³⁵ incorporation
 - chondrocyte monolayers plated on collagen films in Cytostar-T plates
 - quantitation via scintillation counting
- Gene expression via Taqman
- LC/MS analysis of peptides

Sequential collagenase/trypsin
→ monolayer cultures



Disease-Associated Expression of HtrA1 mRNA

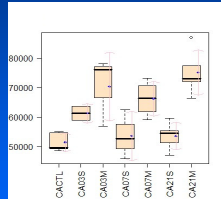
Human Cartilage



Upregulated HtrA1 expression in human OA cartilage consistent with previous reports

Rat cartilage: Meniscal Tear (MCT)

HTRA1 Affymetrix Array

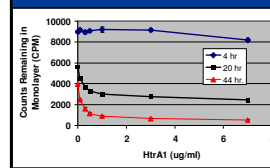


3, 7 and 21 days post-surgery
CTL = no surgery
S = sham MCT surgery
M = MCT surgery

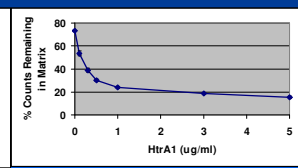
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HtrA1 Degradation of Prelabeled Chondrocyte Extracellular Matrix

Total CPM in Cell Matrix



Percent CPM in Cell Matrix @ 44 h

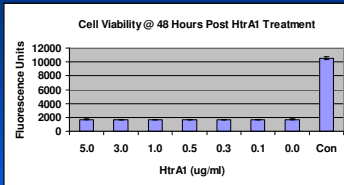


- Bovine chondrocyte monolayers
- Cytostar T Plates + collagen film
- 3 days in 10% Serum + S³⁵ Sulfate
- S³⁵ Sulfate removed, cells washed
- Recombinant HtrA1 added in 10% serum

hr 4 20 44
Count media 44
Matrix Scintillation Count

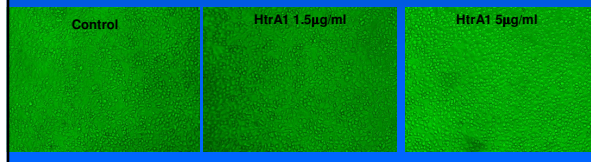
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HtrA1: Cell Viability

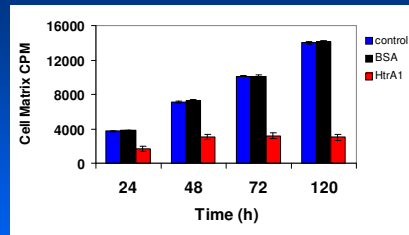


Relative LDH Release
• CytoTox-ONE™

Cell Morphology: 96 hours



HtrA1 Treatment Bovine Chondrocytes → Suppression of GAG Synthesis

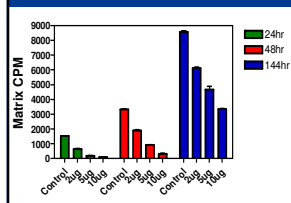


Hours 0 24 48 72 120
S³⁵ + HtrA1 or BSA @ 5µg/ml Remove HtrA1, wash + fresh S³⁵
Scintillation Count

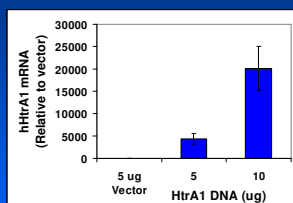
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Expression of hHtrA1 in Chondrocytes → Suppression of Matrix GAG Accumulation

Matrix GAG



Relative Expression hHtrA1 mRNA

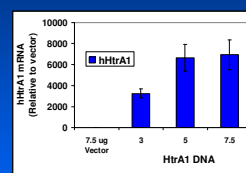


Hours: -24 0 24 48 144
Electroporate HtrA1 (2, 5, 10 µg) Vector control (5 µg) 10% Serum + S³⁵ Sulfate
Scintillation Count hHtrA1 mRNA

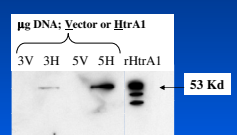
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Expression of hHtrA1 in Bovine Chondrocytes: 96 Hrs Post-Electroporation

hHtrA1: mRNA



hHtrA1: Protein



Hours: -24 0 96
Electroporate HtrA1 DNA (3, 5, 7.5 µg) Vector DNA (3, 5, 7.5 µg) 10% Serum
mRNA: TaqMan Protein: Western

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