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Evidence for CTHRC1 Cleavage and Role in Promoting Metabolic Efficiency: Potential Implications for Endurance Athletic Performance

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CTHRC1 is cleaved to a more active form that promotes metabolic efficiency

Evidence for CTHRC1 Cleavage and Its Role in Promoting Metabolic Efficiency: Potential Implications for Endurance Athletic Performance

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Introduction

- CTHRC1 is a secreted protein transiently expressed by some activated fibroblasts during wound and tissue repair.
- Circulating CTHRC1 is expressed in bone with effects on body composition and metabolism.
- CTHRC1 has been studied as a marker for some pathological conditions such as rheumatoid arthritis and certain cancers.
- CTHRC1 contains a signal peptide for secretion via the ER-Golgi pathway, followed by 16 amino acids that make up a putative propeptide.
- We investigated propeptide cleavage and the potential significance of cleavage to *in vivo* CTHRC1 levels.
- We also studied the effect of CTHRC1 on energy expenditure and cellular metabolism.

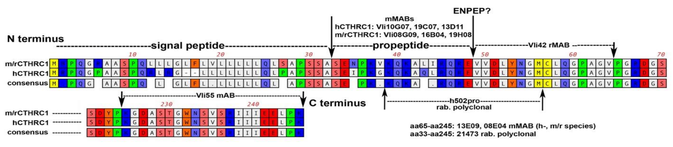

Figure 1. Schematic of the epitopes within the CTHRC1 protein sequence recognized by our antibodies.

Methods

1. To measure uncleaved versus truncated CTHRC1, we developed epitope specific ELISAs able to detect CTHRC1 in the pg/ml range in conditioned media (CM) or plasma.
2. CTHRC1 cleavage was confirmed by Western blot using epitope specific antibodies.
3. Factors that affect cleavage were analyzed with *in vitro* experiments.
4. CTHRC1 levels were measured in healthy volunteers and ultramarathon runners participating in the 2009 TransEurope FootRace with ELISA.
5. We measured body composition and metabolic rate of wildtype and Cthrc1 knockout mice using NMR and metabolic cages.
6. Using Seahorse analysis we measured the effects of CTHRC1 on cellular metabolism in endothelial cells.

Results

- The N-terminal 16 amino acids of CTHRC1 are cleaved by cells suggesting it is a propeptide. Cleavage by transiently transfected cells is promoted by serum in the media and also by cell lysate (Fig. 2, data not shown).
- A variety of cell types cleave exogenous CTHRC1, and under some conditions cleavage is inhibited by protease inhibitors (Fig. 3, data not shown).
- Healthy human subjects have both forms of CTHRC1 (all but one subject had detectable total CTHRC1 ranging from 88pg/ml to >400ng/ml) (Fig. 4).
- Most endurance athletes had detectable and high levels of full length CTHRC1 (Fig. 5).
- Cthrc1 null mice have less lean tissue and more fat than wildtype mice, are less active and have a higher basal metabolic rate at rest (Table 1).
- Endothelial cells transduced with Cthrc1 have increased mitochondrial respiratory capacity and coupling efficiency (Table 2).
- We also have preliminary data showing that the truncated form of CTHRC1 is more active than the full length form at regulating cellular metabolism (data not shown), providing additional evidence that the N-terminus encodes a propeptide.

Discussion

CTHRC1 is a secreted protein that is cleaved in both *in vitro* and *in vivo* conditions, and cleavage may be due to protease activity. We hypothesize that cell injury or cell death is associated with the release of a protease that could cleave CTHRC1, allowing for the generation of active CTHRC1 at sites of tissue injury, including myocardial infarction.

Circulating CTHRC1 could be cleaved by cells at the target tissue to generate the active form.

Our data demonstrate that levels of CTHRC1 in circulation vary widely among healthy human subjects with the highest levels found in endurance athletes, questioning published reports associating CTHRC1 levels with pathology.

Our findings suggest that CTHRC1 functions as a hormonal regulator of mitochondrial function with implications for cell survival after tissue injury and repair, as well as for endurance athletic performance.

Graphs and Figures

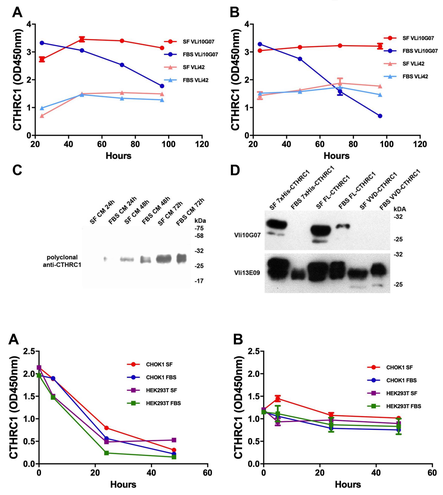

Figure 2. CTHRC1 in CM from CHOK1 or HEK293T cells transfected with human full length CTHRC1. Levels of full length CTHRC1 decline in samples containing serum (FBS) in CHOK1 cells (**A**) and HEK293T cells (**B**), while total levels of CTHRC1 (determined with Vli42 antibody) remain steady. In the absence of serum, levels of full length CTHRC1 remain stable. (**C**) CTHRC1 in CM in the presence (FBS) and absence (SF) of serum over time are shown by immunoblotting with a polyclonal antibody recognizing both forms of CTHRC1. In the presence of serum both cleaved and full length CTHRC1 are apparent within 48 hours. (**D**) HEK293T cells were transfected with different constructs of CTHRC1 and CM were analyzed by immunoblotting 72h later. Samples were probed with monoclonal antibodies to the full length form of CTHRC1 (Vli10G07) and monoclonal antibodies to all forms of CTHRC1 (Vli13E09).

Fig 3. Cells cleave exogenous CTHRC1. (**A**) Levels of full length CTHRC1 in CM measured with 10G07 antibody decline rapidly. (**B**) Total CTHRC1 levels in CM measured with VLi42 antibody remain steady. Cleavage is independent of serum in media.

	Wildtype Mice	*Cthrc1* Null Mice	p value
Body Weight (g)	28.80 ± 0.51	28.80 ± 1.03	1.0
Lean Body Mass (g)	22.91±0.49	19.81±0.48	0.0007
Body Fat (g)	3.92±0.21	7.57±0.74	0.0003
Running Wheel Speed (average, m/s)	0.263 ± 0.014	0.201 ± 0.015	0.033
Running Distance (m/24h)	6164±370	3346±607	0.0013
Still per 24h (%)	56.28±1.66	65.82±1.86	0.0021
EE at rest/g lean mass	0.019±0.001 (kcal/h/g)	0.023±0.001 (kcal/h/g)	0.014

Table 1. Basal metabolic rate as determined by energy expenditure (EE) at rest is increased in *Cthrc1* null mice. NMR was used to determine lean and fat mass in *Cthrc1* null and wildtype mice on the C57BL/6J background.

in pmol/min/µg	Control (ß-gal)	Cthrc1	p value
Basal Respiration	18.54 ± 1.305	18.40 ± 1.435	0.9433, n.s.
Maximal Respiration	48.94 ± 3.529	77.96 ± 6.262	0.0004
ATP Production	13.18 ± 0.8670	14.59 ± 1.099	0.3194, n.s.
Reserve Respiratory Capacity	30.39 ± 2.294	59.56 ± 5.052	< 0.0001
Proton Leak	5.365 ± 0.4699	3.811 ± 0.6781	0.0689
Coupling Efficiency (%)	71.33 ± 0.8852	79.86 ± 2.952	0.0088

Table 2. CTHRC1 increases mitochondrial respiration. Mitochondrial respiration profiles of control (ß-gal) and Cthrc1 transduced HUVECs using Seahorse. CTHRC1 significantly increases mitochondrial respiratory capacity and coupling efficiency.

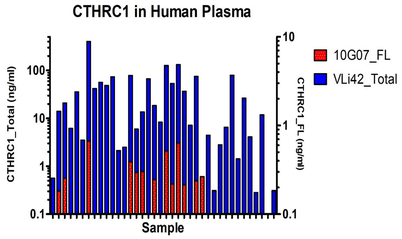

Fig 4. CTHRC1 levels in human plasma samples. Full length (red) and total (blue) CTHRC1 levels are shown for each sample.

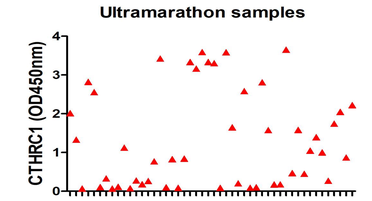

Fig 5. Relative CTHRC1 levels in human endurance runners. ELISA results from each runner are shown for samples taken before the race.

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