Edman Degradation Confirms the N-Terminal Sequence of a Novel Osteogenic Protein

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Our role: Creative Proteomics performed N-terminal sequencing of purified HKUOT-S2 by Edman degradation, identifying the sequence IKITTYRQ and providing direct residue-level evidence that complemented MS-based protein characterization.

Background & Significance

Natural sources contain bioactive proteins with potential value in tissue-regeneration research. However, confident characterization can be difficult when purified proteins have no reliable match in existing sequence databases.

In this study, researchers isolated HKUOT-S2, an approximately 32 kDa protein from *Dioscorea opposita* Thunb, and investigated its ability to promote osteogenesis and bone-defect repair.

Analytical Challenge

LC–MS/MS-derived peptide sequences from HKUOT-S2 did not match known proteins in the searched plant databases.

A direct N-terminal sequence readout was therefore needed to:

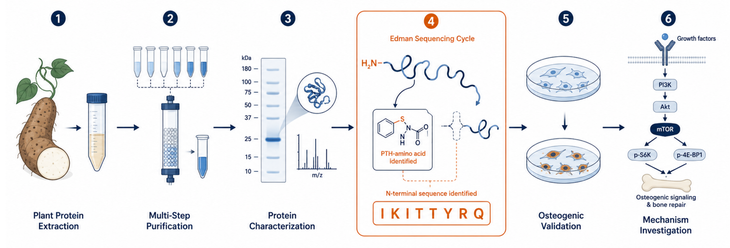
- Strengthen protein identity characterization
- Provide evidence independent of database matching
- Establish a sequence anchor for downstream functional studies

Study Objective

Determine the N-terminal sequence of HKUOT-S2 and integrate sequence evidence with cellular, molecular, and in vivo evaluation of its osteogenic activity.

Workflow

ST6GalNAc-I perturbation in LUAD models → MS-based glycomic/proteomic profiling → candidate glycoprotein identification → immune and endothelial functional validation → orthotopic in vivo confirmation



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Key Findings

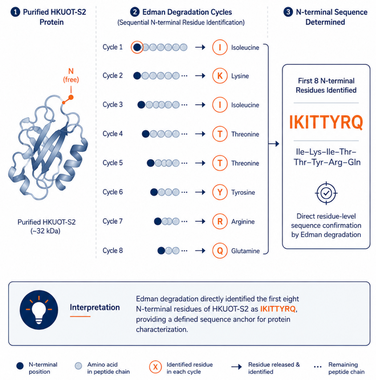
Finding 1 | Direct N-Terminal Sequencing Identified IKITTYRQ

The purified 32 kDa HKUOT-S2 protein was analyzed by Edman degradation, directly resolving its first eight N-terminal residues as:

Ile–Lys–Ile–Thr–Thr–Tyr–Arg–Gln

IKITTYRQ

This residue-by-residue readout complemented LC–MS/MS and de novo peptide evidence, providing a direct sequence anchor when database searches did not identify a matching known plant protein.



Edman degradation directly identified the first eight N-terminal residues of HKUOT-S2 as IKITTYRQ.

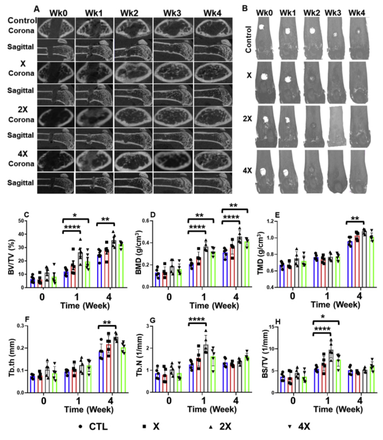
Finding 2 | ST6GalNAc-I promotes immune evasion through the NECTIN2/TIGIT axis

In a murine femoral-defect model, HKUOT-S2 treatment promoted structural repair and new bone formation.

Micro-CT analysis showed increased:

- Bone volume fraction
- Bone mineral density
- Trabecular structure
- Bone formation at the defect site

These results established the osteogenic activity of the sequence-characterized HKUOT-S2 protein in vivo.



HKUOT-S2 increased bone volume, mineral density, and structural repair in a murine femoral-defect model.

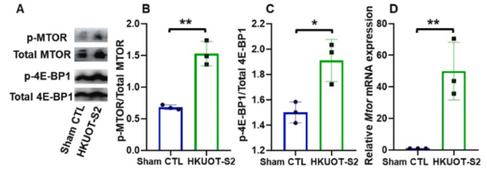
Finding 3 | HKUOT-S2 Activated mTOR-Associated Signaling In Vivo

At day 7 after surgery, the 2.18 mg/kg HKUOT-S2 treatment increased mTOR pathway activity in bone-defect tissue.

The analysis demonstrated:

- Increased p-mTOR/total mTOR
- Increased p-4E-BP1/total 4E-BP1
- Increased Mtor mRNA expression

These findings linked HKUOT-S2 treatment with activation of an mTOR-associated osteogenic signaling program during bone repair.



HKUOT-S2 activated mTOR and 4E-BP1 signaling in bone-defect tissue.

Our Contribution (Creative Proteomics)

Creative Proteomics delivered direct N-terminal sequence information for purified HKUOT-S2 using Edman degradation.

The analysis:

- Identified the first eight N-terminal amino acids
- Provided direct intact-protein sequence evidence
- Complemented MS-based protein characterization
- Supported confident interpretation of subsequent functional studies

What This Case Demonstrates

- Edman degradation can support characterization of novel proteins without relying solely on reference-database matches
- Direct residue-level sequencing provides an orthogonal method for confirming protein N termini
- N-terminal sequence confirmation strengthens the analytical foundation for downstream biological validation
- Sequence-level evidence can connect protein purification with functional and mechanistic research

Why Choose Creative Proteomics

- Direct Sequence Readout: Sequential identification of N-terminal amino acids provides clear residue-level information without requiring enzymatic digestion.
- Multiple Sample Formats: Analysis is available for purified proteins, peptides, liquid samples, lyophilized samples, and PVDF-bound protein bands.
- Sensitive Residue Detection: Low-picomole detection supports sequence analysis when only limited purified material is available.
- Orthogonal Protein Confirmation: Edman degradation complements LC–MS/MS and de novo sequencing, strengthening protein identity and terminal-sequence verification.

Need Direct Sequence Confirmation for a Purified or Novel Protein?

Creative Proteomics provides Edman-based N-terminal sequencing to support protein identity verification, terminal processing analysis, and orthogonal confirmation alongside MS-based characterization.

Contact us to discuss the most suitable N-terminal sequencing strategy for your sample.

Web: www.creative-proteomics.com

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