Hepatocyte growth factor (HGF) promotes regeneration of damaged peripheral nerves by directly interacting with sensory neurons and Schwann cells: Implication for developing novel treatment methods for peripheral neuropathy

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ABSTRACT

We found that the HGF/c-Met pathway plays a critical role in axon regeneration by regulating sensory neurons and Schwann cells (SCs). Upon a nerve crush in a mouse model, HGF expression increased in injury and distal sites with bell-shaped kinetics showing a peak level as early as one day post-injury. Phosphorylated c-Met, the only receptor for HGF, highly increased in the peripheral axons around the injury site and were co-localized with SCs. A tyrosine kinase inhibitor (PHA665752) of the c-Met receptor affected myelin thickness during regeneration and suppressed axon regrowth, implying the importance of the HGF/c-Met signaling. In in vitro experiments with primary SCs (from rat sciatic nerves) and sensory neurons (from embryonic DRG), HGF treatment induced migration and proliferation of SCs and neurite outgrowth of sensory neurons. Even with the same growth factor, the activated signaling pathways appeared different depending on target cells. Erk signaling seems to be important in SCs, while Jun may act as a key player without Erk activation in sensory neurons. Moreover, we reanalyzed gene expression profiles and reinterpreted cellular changes during nerve repair processes using a single-cell transcriptome dataset. HGF expression is unique at M2-polarized macrophages in the late stage of repairing except for mesenchymal-like cells. c-Met expression is relatively high at both Schwann cells and endothelial cells. The non-myelinated and repairing subtype of Schwann cells showed a high c-Met expression level. Taken together, our results suggested that **HGF/c-Met signaling is highly involved in the regeneration process of damaged peripheral nerves and thus may serve as a platform for devising novel treatment strategies for peripheral neuropathy.**

INTRODUCTION

HGF (Hepatocyte Growth Factor)

- Originally discovered as a growth factor for hepatocytes
- Later found to contain multiple bioactivities:
  - Angiogenic
  - Neurotrophic
  - Anti-apoptotic
  - Anti-fibrotic
  - Anti-inflammatory

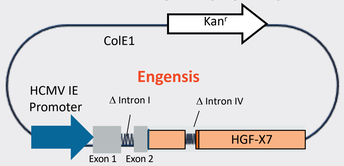
HGF's roles and functions in CNS and PNS

- Neurite outgrowth in the developmental stage
- Enhances survival and maturation of neurons in CNS
- Promotes remyelination of Schwann cells in PNS
- ★The role(s) of HGF/c-Met the pathway in nerve regeneration during the adult stage remains largely unknown.

c-Met cellular receptor expressed in

- Endothelial cells
- Smooth muscle cells
- Skeletal muscle cells
- Motor neurons
- Sensory neurons
- Schwann cells

Plasmid DNA encoding HGF (Engensis)

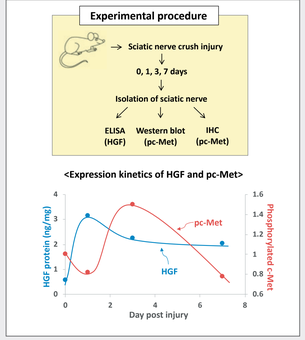


Clinical studies (Phase I, II, and III) had been conducted for diabetic peripheral neuropathy. Data suggested:

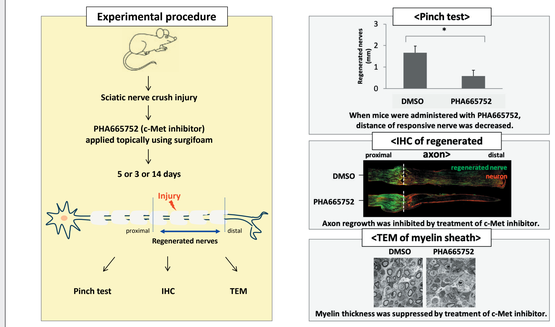
- Excellent safety profile
- Strong analgesic effect for an extended period (in the absence of Engensis DNA and HGF protein expression)
- Sensory function might return as measured by monofilament tests.

RESULTS

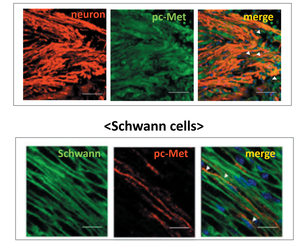
1. The levels of HGF protein and phosphorylated (activated) c-Met receptor were highly increased after nerve injury.



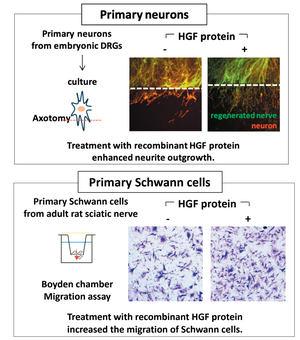
2. When phosphorylation of c-Met receptor was blocked by a specific chemical inhibitor (PHA665752), nerve regeneration process was effectively suppressed.



3. Phosphorylated c-Met was found mostly in axons and Schwann cells.

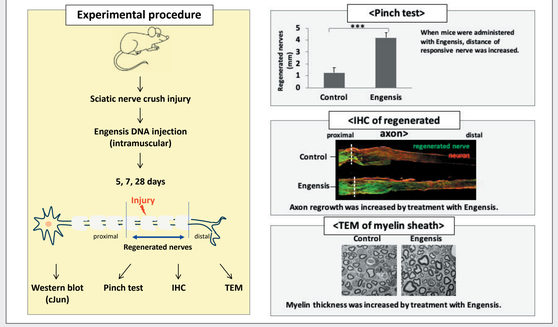


4. HGF interacts with sensory neurons and Schwann cells independently.

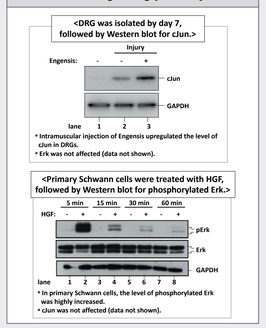


RESULTS

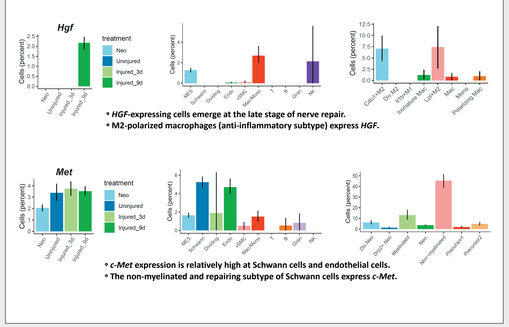
5. Exogenously added HGF (by intramuscular injection of Engensis DNA) can induce nerve regeneration.



6. In the context of HGF, sensory neurons and Schwann cells may use different signaling pathways.



7. *In silico* analyses identified HGF- and c-Met-expressing cell types.



CONCLUSIONS

- HGF plays important roles in the nerve regeneration process by interacting with two key cell types, sensory neurons and Schwann cells, to promote axon regrowth and remyelination.
- Intramuscular injection of plasmid DNA-encoding HGF can help nerve regeneration.
- Engensis has the potential of a disease-modifying drug and/or regenerative medicine.

References: Ko et al., (2018), *Scientific Reports* 8: 8316-8329 | Nho et al., (2018), *The FASEB Journal* 32: 5119-5131 | Kessler et al., (2015), *Annals of Clinical and Translational Neurology* 2: 465-478

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