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Antiangiogenic Isoforms of Hepatocyte Growth Factor (HGF) Confound Its Detection as a Biomarker for Risk of Stroke

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Introduction

Hepatocyte growth factor (HGF) has been associated with risk of stroke in several studies including the *Women's Health Initiative*, the *MESA (Multi-Ethnic Study of Atherosclerosis)* and the *ANFIS (Angiogenic Factors in Intracranial Stenosis)*. HGF is an alpha-beta heterodimer, which is only proangiogenic in this form, as shown in Figure 1 below. However, naturally occurring and artificially produced fragments of the alpha subunit are antiangiogenic. The precise location of the epitope targeted by the antibodies of the commercially available immunoassays is unknown. Therefore, there is a possibility of cross-reactivity or interference caused by antiangiogenic isoforms of HGF, which has never been assessed. We tested the hypothesis that the highly antiangiogenic fragment of HGF, NK4, is detected by immunoassays as HGF.

Learning Objectives

To discuss the potential role of antiangiogenic HGF isoforms in stroke

Methods

Commercially available ELISA plates for HGF were used to quantify sequential dilutions of decreasing known concentrations of HGF and equimolar NK4. We compared the measured results (HGF-m) with the actual HGF and NK4 (HGF-a and NK4-a) concentrations. The affinity of the HGF ELISA antibodies to HGF and NK4 was evaluated in terms of correlation between HGF-m and HGF-a and NK4-a, and the reliability of the tests was tested with standard Cronbach's alpha. We calculated the sensitivity, specificity, and accuracy of HGF-m to detect NK4-a and HGF-a.

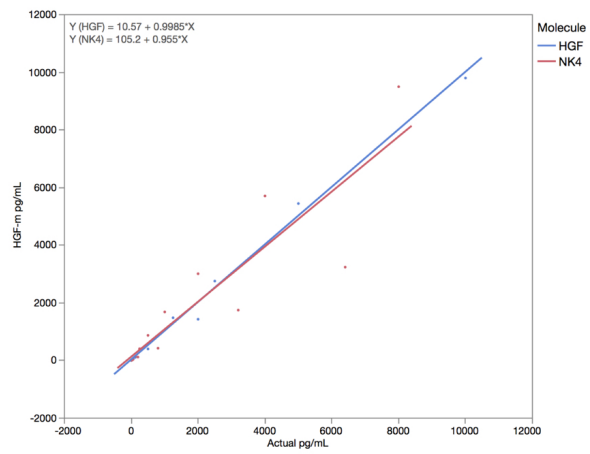
Results

HGF-m on ELISA detected NK4-a levels "as HGF" at all concentrations tested (Range 0.1 to 10,000 pg/mL) with a Spearman's rho of 0.98 ($p<0.001$). This was indistinguishable from the HGF-a detection (Figure 1), with a very high reliability Cronbach's alpha of 0.83. Immunoassays had reduced sensitivity (0.26), specificity (0.56), and global accuracy (0.53) to differentiate HGF from NK4.

Conclusions

The detection of anti-angiogenic NK4 with immunoassays for HGF opens the possibility that elevated concentrations of measured HGF associated with stroke may be caused by circulating antiangiogenic splicing variants of HGF such as NK fragments. Further evaluation of the role of these antiangiogenic isoforms in stroke is necessary.

Figure 1



The graphic illustrates the detection of NK4 and HGF using HGF ELISA. ELISA detected NK4 (antiangiogenic isoform) as HGF at all concentrations. Elevated concentrations of HGF reported previously in association with stroke may be caused by circulating antiangiogenic splicing variants as NK fragments.

References

1- Bell EJ, Larson NB, Decker PA, Pankow JS, Tsai MY, Hanson NQ, Wassel CL, Longstreth WT Jr, Bielinski SJ. Stroke. 2016 Nov;47(11):2689-2694. Epub 2016 Oct 11.

2- Rajpathak SN1, Wang T, Wassertheil-Smoller S, Strickler HD, Kaplan RC, McGinn AP, Wildman RP, Rosenbaum D, Rohan TE, Scherer PE, Cushman M, Ho GY. Stroke. 2010 May;41(5):857-62. doi: 10.1161 /STROKEAHA. 109.567719. Epub 2010 Mar 4.

3- Gonzalez NR, Liou R, Kurth F, Jiang H, Saver J. Angiogenesis. 2017 Oct 9. doi: 10.1007/s10456-017-9578 -1. [Epub ahead of print]