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Biomarker Insights

Decreased Mitogen Inducible Gene 6 (MIG-6) Associated with Symptom Severity in Children with Autism

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ABSTRACT

BACKGROUND: Individuals with autism spectrum disorders (ASDs) demonstrate impairment in social interactions and problems in verbal and nonverbal communication. Autism spectrum disorders are thought to affect 1 in 88 children in the US. Recent research has shown that epidermal growth factor receptor (EGFR) activation is associated with nerve cell development and repair. Mitogen inducible gene 6 (MIG-6) is a 58-kDa non-kinase scaffolding adaptor protein consisting of 462 amino-acids, which has been shown to be a negative feedback regulator of EGFR and Met receptor tyrosine kinase (RTK) signaling.

SUBJECTS AND METHODS: In this study, we determined plasma levels of MIG-6, which suppresses the EGFR RTK pathway in autistic children, and compared MIG-6 levels with the EGFR ligand, epidermal growth factor (EGF), and the cMET ligand, hepatocyte growth factor (HGF). MIG-6 levels were also compared to the symptom severity of 19 different autistic behaviors.

Plasma MIG-6 concentration was measured in 40 autistic children and 39 neurotypical, age, and gender similar controls using an enzyme linked immunosorbent assay (ELISA). Plasma MIG-6 levels were compared to putative biomarkers known to be associated with EGFR and cMET and severity levels of 19 autism related symptoms [awareness, expressive language, receptive language, (conversational) pragmatic language, focus/attention, hyperactivity, impulsivity, perseveration, fine motor skills, gross motor skills, hypotonia (low muscle tone), tip toeing, rocking/pacing, stimming, obsessions/fixations, eye contact, sound sensitivity, light sensitivity, and tactile sensitivity].

RESULTS: In this study, we found that plasma MIG-6 levels in autistic children (182.41 ± 24.3 pg/ml) were significantly lower than neurotypical controls (1779.76 ± 352.5; $P = 1.76E - 5$). Decreased MIG-6 levels correlated with serotonin, dopamine, Tumor necrosis factor alpha (TNF-alpha), and urokinase receptor (uPAR) concentration, but not with other tested putative biomarkers. MIG-6 levels also correlated significantly with severity of expressive language, receptive language, tip toeing, rocking/pacing, and hand flapping/stimming.

CONCLUSIONS: These results suggest a relationship between decreased plasma MIG-6 levels, biomarkers associated with the EGFR pathway, and symptom severity in autism. A strong correlation between plasma MIG-6 and dopamine and serotonin levels suggest that decreased MIG-6 levels may be associated with abnormal neurotransmitter synthesis and/or action. A strong correlation between MIG-6 and uPAR and the inflammatory marker TNF-alpha suggests that low MIG-6 levels may be associated with the HGF/Met signaling pathway, as well as inflammation in autistic children.

KEYWORDS: MIG-6, EGFR, EGF, dopamine, serotonin, uPAR, TNF-alpha, autism, symptom severity

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Introduction

Individuals with autism spectrum disorders (ASDs) demonstrate impaired social interactions, deficits in communication, and repetitive and stereotyped behaviors.[1]

Mitogen inducible gene 6 (MIG-6) (also known as gene 33, ERRFI1, or RALT) produces MIG-6, a 58-kDa non-kinase scaffolding adaptor protein consisting of 462 amino-acids, which has been shown to be a negative feedback regulator of epidermal growth factor receptor (EGFR) and Met receptor tyrosine kinase (RTK) signaling. It is an immediate early-response protein that is expressed in various tissues and plays a critical role in many pathophysiological states.[2] Its expression can be induced by a broad spectrum of growth factors, hormones, or stress stimuli, and it is associated with various chronic conditions.[2,3]

One of the most prominent roles of the protein, MIG-6, regulating signal transduction, comes from its ability to directly interact with EGFR and other receptor tyrosine-protein kinase (ErbB) family members, inhibiting their phosphorylation and downstream signaling in a negative feedback fashion, ending in down regulation of extracellular signal-regulated kinase-1 and 2 (ERK1 and ERK2).[4–7] MIG-6 can be induced by hepatocyte growth factor (HGF) and functions as a negative feedback regulator of HGF-MNNG HOS transforming gene receptor (MET) signaling, and so, to summarize, can regulate both EGFR and MET signaling via a negative feedback loop.[8,9]

Both the MET and EGFR genes have been implicated in autism.[10] Our lab has demonstrated that plasma HGF and epidermal growth factor (EGF) (the ligands for MET and EGFR, respectively) are significantly decreased in autistic children,[11,12] and in the above studies and recent research,[13] we have found that EGF is decreased and EGFR is increased, and EGF and EGFR levels, but not HGF levels, correlate with symptom severity in autistic children.[11–13]

MIG-6 can be induced by HGF and function as a negative feedback regulator of the MET pathway by inhibiting HGF-induced cell migration and proliferation.[8] Overall, the ERK cascade functions in cellular proliferation, differentiation, and survival, and its inappropriate activation is a common occurrence in human cancers. Specifically, low Mig-6 expression is associated with high levels of EGFR and ERK phosphorylation in certain cancers.[14] No physical interaction between MIG-6 and MET has been observed. Instead, direct regulation of the RTK pathway by MIG-6 appears to be unique to the EGFR family.

The urokinase receptor (uPAR) is a multidomain glycoprotein tethered to the cell membrane. It is a part of the plasminogen activation system. uPAR elicits a plethora of cellular responses that include cellular adhesion, differentiation, proliferation, and migration in a non-proteolytic fashion.[15] uPAR expression is elevated during inflammation, tissue remodeling, and in many human cancers.[16] Crosstalk between uPAR and the EGFR is extensive. uPA binding to uPAR results in EGFR transactivation, which may be important for ERK phosphorylation.[17,18]

Tumor necrosis factor alpha (TNF-alpha) is involved in systemic inflammation and is a member of a group of cytokines that stimulate the acute phase reaction. TNF-alpha is produced chiefly by activated macrophages, CD4+ lymphocytes, NK cells, and neurons. Dysregulation of TNF production has been implicated in a variety of human diseases including Alzheimer's disease,[19] cancer,[20] major depression,[21] and inflammatory bowel disease (IBD).[22] TNF-alpha has been found to induce upregulation of EGFR expression.[23,24]

The growth factors, HGF and EGF, signal the MET and EGFR RTKs, and through a cascade of reactions, modulate the ERK and PI3K intracellular regulatory pathways. ERK and PI3K increase mRNA translation associated with the cell cycle, cell survival, differentiation, and motility. These same RTKs have been implicated in risk of ASD.[10]

MIG-6 was originally identified as a mitogen-inducible gene and has been implicated in the feedback regulation of a variety of signaling processes, including the EGFR pathway.[3,25–27] Ablation of MIG-6 has been shown to induce tumor formation in various tissues, supporting the tumor suppressor function of MIG-6.[9,28,29] However, the role of MIG-6, and its potential effect on the EGFR pathway associated with the etiology of autism, is relatively unknown.

Because of the direct interaction and regulatory effect of MIG-6 on EGFR and the apparent importance of EGFR levels, as it might relate to symptom severity in autism, this study established MIG-6 levels in autistic children and compared those levels with putative biomarkers and symptom severity.

Materials and Methods

Subjects. Plasma MIG-6 concentration was measured in 40 autistic children (mean age 10.6 ± 3.2; 32 males) and 39 neurotypical controls (mean age 9.2 ± 4.5; 30 males), using an enzyme linked immunosorbent assay (ELISA). Plasma MIG-6 levels were compared to putative biomarkers known to be associated with EGFR and cMET [serotonin, dopamine, TNF-alpha, uPAR, EGF, HGF, gamma-aminobutyric acid (GABA), glutamic acid decarboxylase 2 (GAD2), uPA, HMGB1] and severity levels of 19 autism related symptoms [tactile sensitivity, expressive language, receptive language, (conversational) pragmatic language, focus/attention, hyperactivity, impulsivity, perseveration, awareness, fine motor skills, gross motor skills, hypotonia (low muscle tone), tip toeing, rocking/pacing, stimming, obsessions/fixations, eye contact, sound sensitivity, and light sensitivity]. It should be noted that not all the autistic individuals tested for MIG-6 were also tested for the other biomarkers. This could have been for a variety of reasons, including plasma not being available. However, the choice of patients tested for each biomarker was completely random.

The diagnostic criteria used in this study were defined by DSM-IV criteria, which were changed to DSM-V in 2012, when separate diagnostic labels of autistic disorder, Asperger's disorder, and PDD-NOS were replaced by the term "Autism Spectrum Disorder."

Plasma from consecutive individuals with diagnosed autism (*n* = 40; 32 male; mean age 10.2 years) and controls (*n* = 39; 29 male; mean age 9.8 years) was obtained from patients presenting at the Health Research Institute (HRI).* Neurotypical control plasma was obtained from HRI and the

Autism Genetic Resource Exchange (AGRE).† All the autistic individuals in this study met the DSM-IV criteria and were diagnosed using The Autism Diagnostic Interview-Revised – ADI-R – before presenting to the HRI. Plasma from HRI and AGRE were treated (as above) in an identical fashion.

Patient consent was obtained from all patients involved in this study, and this study was approved by the IRB of the HRI. The research was conducted in accordance with the principles of the Declaration of Helsinki.

Severity of disease. An autism symptom severity questionnaire was used to evaluate symptoms. The questionnaire (Pfeiffer questionnaire) asked parents or caregivers to assess the severity of the following symptoms: Awareness, expressive language, receptive language, (conversational) pragmatic language, focus, attention, hyperactivity, impulsivity, perseveration, fine motor skills, gross motor skills, hypotonia (low muscle tone), tip toeing, rocking/pacing, stimming, obsessions/fixations, eye contact, sound sensitivity, light sensitivity, and tactile sensitivity. The symptoms were rated by parents/guardians on a scale of 0–5 (5 being the highest severity) for each of these behaviors.

Plasma. All plasmas, experimental and controls, were treated in an identical fashion, and chosen for this study in a blind fashion. Plasma was frozen at −70 °C immediately after collection until thawed for use in ELISAs. MIG-6 levels remained stable over several months, as indicated by consistent results in multiple assays over time.

ELISAs. ELISAs were used to measure plasma MIG-6 and other putative biomarkers (see above) (ELISA kits, R&D Systems, Minneapolis, MN and USCN Life Sciences, Wuhan, China), as reported previously.[11]

Briefly, the plate, previously coated with appropriate capture antibody, was coated with 100 µL of the diluted plasma, then incubated at 4 °C overnight. The plate was then washed 3 × with PBS-Tween 20. One hundred microliter of biotin conjugated antibody, specific for antigen, was added to each well and the plate was incubated for 30–60 minutes at room temperature (RT). Plate was washed as above and 100 µL HRP conjugated avidin was added to each well. Plate was incubated for 30 minutes at RT. One hundred microliter of HRP substrate was added to each well and the plate was incubated until appropriate color change. Fifty microliter of stop solution was added to each well and plate was read plates on an ELISA plate reader at 450 nm (BioRad Laboratories, Inc., Hercules, CA, USA).

Statistics. Inferential statistics were derived from unpaired *t*-test and odds ratios with 95% confidence intervals. Pearson moment correlation test was used to establish degree of correlation between groups. It should be noted that our statistical analysis has limitations based on multiple hypothesis testing.

*The Health Research Institute is a comprehensive treatment and research center, specializing in the care of neurological disorders, including autism.

†The Autism Genetic Resource Exchange (AGRE) is a repository of biomaterials and phenotypic and genotypic data to aid research on ASDs.

Results

In this study, we found plasma MIG-6 levels in autistic children (182.41 ± 24.3 pg/ml) significantly lower than neurotypical controls (1779.76 ± 352.5). ($p < 0.0001$) (Fig. 1).

In the group of autistic individuals, decreased MIG-6 levels correlated with serotonin ($N = 15$, $r = -0.52$, $p = 0.02$) (Fig. 2), dopamine ($N = 12$, $r = -0.62$, $p = 0.01$) (Fig. 3), TNF-alpha ($N = 18$, $r = 0.43$, $p = 0.03$), and uPAR ($N = 20$, $r = 0.47$, $p = 0.01$) concentration, but not with other tested biomarkers, EGF ($N = 21$, $r = -0.1$, $p = 0.31$), HGF ($N = 20$, $r = 0.22$, $p = 0.18$), GABA ($N = 16$, $r = -0.12$, $p = 0.31$), GAD2 ($N = 22$, $r = -0.2$, $p = 0.19$), uPA ($N = 21$, $r = -0.09$, $p = 0.35$), and HMGB1 ($N = 18$, $r = -0.12$, $p = 0.31$).

Also, in the group of autistic individuals, MIG-6 levels correlated significantly with severity of expressive language ($N = 25$, $r = 0.39$, $p = 0.02$), receptive language ($N = 24$, $r = 0.4$, $p = 0.02$), tip toeing ($N = 24$, $r = 0.55$, $p = 0.002$), rocking/pacing ($N = 24$, $r = 0.36$, $p = 0.03$), and hand flapping/stimming ($N = 24$, $r = 0.33$, $p = 0.05$), but not with severity of other symptoms, awareness ($N = 25$, $r = 0.21$, $p = 0.19$), conversational language ($N = 25$, $r = 0.16$, $p = 0.2$) focus/attention ($N = 25$, $r = -0.19$, $p = 0.17$), hyperactivity ($N = 24$, $r = -0.2$, $p = 0.16$), impulsivity ($N = 23$, $r = -0.07$, $p = 0.3$), perseverance ($N = 24$, $r = -0.08$, $p = 0.3$), fine motor skills ($N = 25$; $r = 0.04$; $p = 0.43$), and gross motor skills ($N = 24$; $r = 0.04$, $p = 0.4$).

Discussion

Our lab has demonstrated that plasma HGF and EGF (the ligands for MET and EGFR, respectively), are significantly decreased in autistic children and we have found that EGFR is increased. We have also found that EGF and EGFR levels, but not HGF levels, correlate with symptom severity in autistic children.[10–13] The data reported in this study demonstrate that a population of autistic children has significantly decreased MIG-6. Mig-6 mRNA expression is induced by EGF. Upon EGF stimulation, Mig-6 binds to the EGFR. Overall, Mig-6 over-expression results in reduced activation of the EGFR

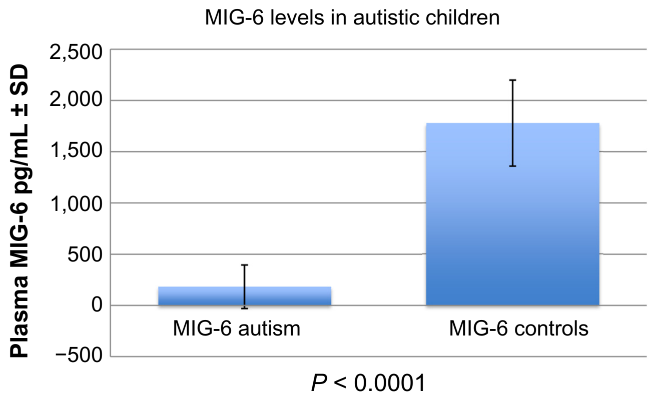

Figure 1. MIG-6 plasma concentration in autistic children (N = 40) is significantly lower than in controls (N = 39, $P < 0.0001$).

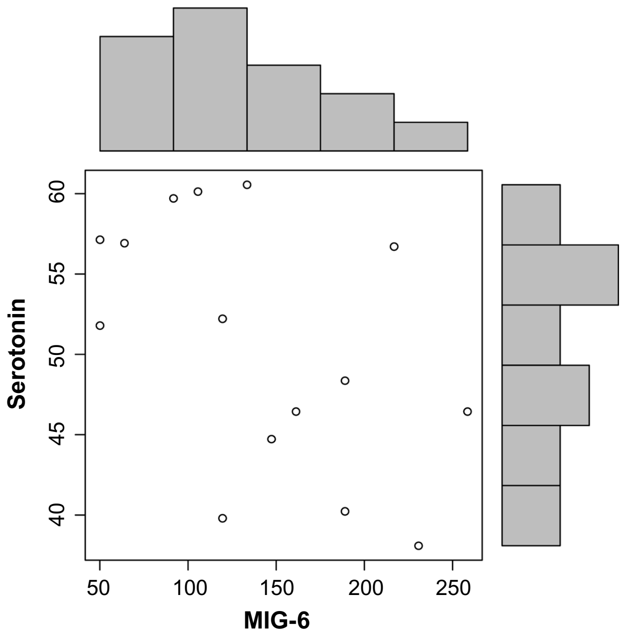

Figure 2. Correlation between MIG-6 and serotonin in autistic children (N = 15, r = −0.52, $P = 0.02$).

cascade resulting in reduced activation of protein kinase ERK2.[7] Although we did not find a correlation between EGF and MIG-6 levels in this study, as EGF induces MIG-6 expression, it is conceivable that decreased EGF, seen in autistic children,[11] is a reason for our observed low MIG-6 levels. This may, however, further alter regulation of the EGFR pathway.

MIG-6 is also associated with the inhibition of Met signaling, but does not bind Met directly, as it does EGFR.

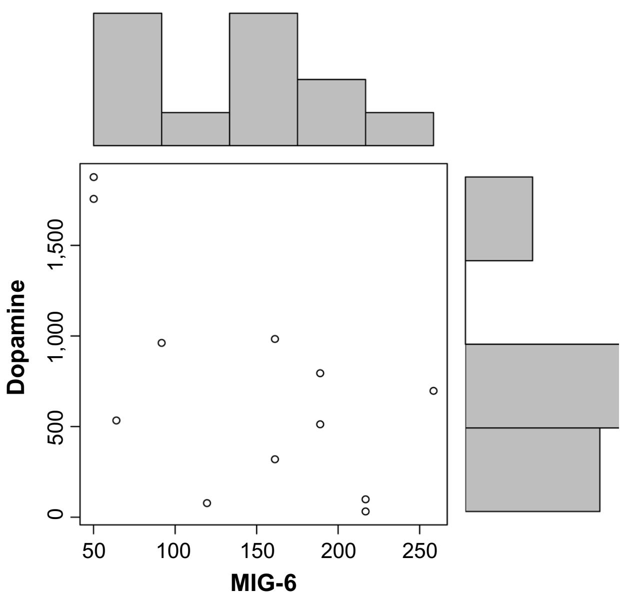

Figure 3. Correlation between MIG-6 and dopamine in autistic children (N = 12, r = −0.62, $P = 0.01$).

MIG-6 is induced by HGF stimulation, It is, however, a part of a negative feedback loop that attenuates Met signaling in a variety of cellular functions.[26] Although in this study, we did not find a correlation between HGF and MIG-6 levels, because our lab and others have found decreased HGF levels in autistic children,[12] we suggest that as MIG-6 is induced by HGF, low levels of HGF may be a reason that MIG-6 is decreased in autistic children.

EGFR levels and structure affect MIG-6 levels as well. MIG-6 is highly expressed in mutated EGFR cells.[30] However, mutation and down regulation of MIG-6 are often observed in human lung cancer cell lines[9] and also correlate with a reduced survival rate in breast cancer patients.[31,32] Mutation, as well as our observed down regulation, may be observed in behavioral disorders such as autism.

There is much crosstalk between uPAR and EGFR, once uPAR is signaled by its ligand, uPA. This crosstalk has been shown to result in ERK activation. We found a strong correlation between MIG-6 and uPAR levels in this population of autistic children, suggesting that these markers may both be associated with EGFR pathway dysfunction.

These data show a strong correlation between TNF-alpha levels and MIG-6. Recently, this can be explained by the fact that TNF-alpha-dependent signaling in neuroendocrine cells has been shown to lead to a unique, persistent mode of NF-kappa B activation that features long-lasting transcription of both I kappa B and MIG-6. This may play a role in the long-lasting effects of TNF-alpha in regulating neuropeptide output from the adrenal, which is a potentially important feedback station for modulating long-term cytokine effects in inflammation.[33] In a recent study, profiling proteins in adults with Asperger syndrome, the males showed changes predominantly in inflammation signaling, including TNF-alpha.[34]

These data also show a strong correlation between low MIG-6 levels and high dopamine and serotonin levels in autistic children. Both dopamine and serotonin levels have been found to be abnormal in autistic children.[35] Elevated whole blood serotonin 5-HT, or hyperserotonemia, is a common biomarker in ASD[36] and dopamine levels are elevated in autistic children as well as mouse models of ASD.[37]

It is not surprising that RTKs are associated with autism, because other neuro-behavioral and mood disorders such as bipolar disorder and schizophrenia are linked to altered markers associated with these pathways.[32,33,35]

Signaling by EGFR must be controlled tightly, in part because aberrant EGFR activity may cause cell transformation. MIG-6 is a feedback inhibitor of EGFR. It is plausible that higher EGFR levels found in autistic children are the result of altered RTK suppressor proteins such as MIG-6.

In summary, there is compelling evidence, including data from our lab that the EGFR/ERK pathway, associated with cell growth, differentiation, and division, may be associated with the etiology of autism. High EGFR levels are associated with many cancers. These increased levels are, in turn, associated with unregulated cell division. The data reported in this study show that the EGFR suppressor protein, MIG-6, is decreased in a population of autistic children, which may influence higher EGFR levels in these individuals. These results also suggest a relationship between low MIG-6 and neurotransmitter levels (dopamine and serotonin), the receptor uPAR as well as the inflammatory marker TNF-alpha.

Author Contributions

AR carried out the immunoassays and performed the statistical analysis. AR conceived of the study, and participated in its design and coordination. AR drafted and approved the final manuscript.

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Abbreviations

MIG-6, mitogen inducible gene 6; GAD, glutamic acid decarboxylase; μPA, urokinase plasminogen activator; μPAR, PLAUR, urokinase plasminogen activator receptor; ELISA, enzyme linked immunosorbent assay; c-Met, MET or MNNG HOS transforming gene; PDD, pervasive developmental disorder; ERK, extracellular signal-regulated kinases; RTK, receptor tyrosine kinases; EGFR, epidermal growth factor receptor; HGF, hepatocyte growth factor; GABA, gamma-aminobutyric acid.

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