HHS Public Access

Author manuscript

Mol Carcinog. Author manuscript; available in PMC 2023 April 05.

Published in final edited form as:

Mol Carcinog. 2022 June ; 61(6): 537–548. doi:10.1002/mc.23399.

Transglutaminase 2 enhances hepatocyte growth factor signaling to drive the mesothelioma cancer cell phenotype

Warren Naselsky[4], **Gautam Adhikary**[1], **Suruchi Shrestha**[1], **Xi Chen**[1], **Geraldine Ezeka**[1], **Wen Xu**[1], **Joseph S Friedberg**[4,5], **Richard L. Eckert**[1,2,3,5]

[1]Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore, Maryland

[2]Department of Dermatology, University of Maryland School of Medicine, Baltimore, Maryland

[3]Department of Reproductive Biology, University of Maryland School of Medicine, Baltimore, Maryland

[4]Department of Surgery, Division of Thoracic Oncology, University of Maryland School of Medicine, Baltimore, Maryland

[5]Marlene and Stewart Greenebaum Comprehensive Cancer Center, University of Maryland School of Medicine, Baltimore, Maryland

Abstract

Transglutaminase 2 (TG2) is an important mesothelioma cancer cell survival protein. However, the mechanism whereby TG2 maintains mesothelioma cell survival is not well understood. We present studies showing that TG2 drives hepatocyte growth factor (HGF)-dependent MET receptor signaling to maintain the aggressive mesothelioma cancer phenotype. TG2 increases HGF and MET mRNA and protein levels to enhance MET signaling. TG2 inactivation reduces MET tyrosine kinase activity to reduce cancer cell spheroid formation, invasion and migration. We also confirm that HGF/MET signaling is a biologically important mediator of TG2 action. Reducing MET level using genetic methods or treatment with MET inhibitors reduces spheroid formation, invasion and migration and this is associated with reduced MEK1/2 and ERK1/2. In addition, MEK1/2 and ERK1/2 inhibitors suppress the cancer phenotype. Moreover, MET knockout mesothelioma cells form 10-fold smaller tumors compared to wild-type cells and these tumors display reduced MET, MEK1/2 and ERK1/2 activity. These findings suggest that TG2

Correspondence Richard L. Eckert, Ph.D., John F.B. Weaver Distinguished Professor, Chair - Department of Biochemistry and Molecular Biology, Associate Director - Basic Sciences Greenebaum Cancer Center, University of Maryland School of Medicine, 108 North Greene Street, Rm 103, Baltimore, Maryland 21201, Ph: 410-706-3220, reckert@umaryland.edu.

Author Contribution

Warren Naselsky: Experimental design of cell based and animal experiments and manuscript preparation
Gautam Adhikary: Performed cell based and animal experiments and manuscript preparation
Suruchi Shrestha: Performed experiment on cultured cell models
Xi Chen: Performed biochemistry studies
Geraldine Ezeka: Designed and completed immunohistology studies
Wen Xu: Derived knockdown and knockout studies
Joseph S Friedberg: Experimental design and disease relevance
Richard L. Eckert: Study design and supervision and manuscript preparation

Conflict of Interest The authors declare no conflict of interest.

maintains HGF and MET levels in cultured mesothelioma cells and tumors to drive HGF/MET, MEK1/2 and ERK1/2 signaling to maintain the aggressive mesothelioma cancer phenotype.

Keywords

mesothelioma; transglutaminase 2 (TGM2); hepatocyte growth factor (HGF); MAPK signaling; ERK1/2 signaling

Introduction

Mesothelioma is a cancer of the pleural and peritoneal mesothelial linings that develops in response to asbestos exposure [1,2]. Mesothelioma patients have a very limited life expectancy [1,2]. Cisplatin/pemetrexed chemotherapy with or without surgical resection is the standard of care; however, microscopic cancer ultimately leads to regrowth of aggressive drug-resistant disease [2]. This poor clinical experience speaks to the need for new therapeutic strategies [3]. We proposed that mesothelioma cancer stem-like (MCS) cells survive tumor excision/chemotherapy and give rise to aggressive tumor regrowth [4]. We further propose that effective therapies must target both MCS cells and non-stem cancer cells. We have identified transglutaminase 2 (TG2) as overexpressed and required for MCS cell survival [4]. Transglutaminase (TG2), which is an important pro-cancer signaling protein [4-10], is a novel transglutaminase family member that functions as a GTP binding signaling protein to drive cancer cell survival signaling [5-8,11-15]. Its expression is associated with increased metastasis and drug resistance [16-20], acquisition of cancer stem-cell traits [7,8,21-23] and resistance to chemotherapy [10].

TG2 activates a range of signaling pathways [10,13]. However, information is limited regarding the mechanism whereby TG2 maintains the mesothelioma cancer phenotype. Gene expression profile analysis of mesothelioma cell mRNA reveals that hepatocyte growth factor (HGF) mRNA is markedly reduced in the absence of TG2. HGF, also called scatter factor, interacts with the MET tyrosine kinase receptor to enhance cell signaling [24,25]. HGF/MET has been shown to stimulate the mesothelioma cancer phenotype [26]. However, little is known about mechanisms that maintain HGF/MET signaling.

We now show that TG2 maintains HGF mRNA and protein level to stimulate MET signaling which activates MEK1/2 and ERK1/2 to drive mesothelioma cell spheroid formation, invasion, migration and tumor formation. Interfering with HGF or MET attenuates the cancer phenotype and phenocopies the response to TG2 knockout. In addition, HGF treatment can partially restore the attenuated cancer phenotype observed in TG2 knockout cells suggesting that HGF/MET signaling is a biologically important downstream TG2 target.

Materials and Methods

Antibodies and reagents

RPMI1640 with L-glutamine (10-040-CV) and trypsin-EDTA 0.25% (25200-056) were purchased from Gibco (Grand Island, NY). Anti-β-actin (A5441), fetal calf serum

(FCS, F4135) and 4′,6-diamidino-2-phenylindole (DAPI, D9542) were obtained from Sigma (St. Louis, MO). Matrigel (354234) and BioCoat Millicell inserts (359097) were purchased from BD Bioscience (Franklin Lakes, NJ). Peroxidase-conjugated donkey anti-rabbit IgG (NA934V) and peroxidase-conjugated sheep anti-mouse IgG (NA931V) were purchased GE Healthcare (Piscataway, NJ) and used at a 1:5000 dilution. Antibodies for AKT (9272), phospho-ATK (9271), MET (8198S), HGF (5244S), CD31 (3528), phospho-MET (3077S), ERK1/2 (9102S), phospho-ERK1/2 (4370S), MEK1/2 (4694S), and phospho-MEK1/2 (9154S) were obtained from Cell Signaling Technology (Danvers, MA). AlexaFluor 488-conjugated donkey anti-mouse IgG secondary antibody (A-21202) was from ThermoFisher Scientific (Waltham, MA). TG2-siRNA (sc-37514) was obtained from Santa Cruz Biotechnology (Dallas, TX). Control- (D-001206-13-20), HGF- (M-006050-01-0005) and MET-siRNA (M-003156-02-0005) were purchased from Dharmacon (Lafayette, CO). ERK1/2-inhibitor LY3214996 (S8534) was obtained from Selleckchem (Houston, TX) and the MEK1/2-inhibitor U0126 (V1121) was purchased from Promega (Madison, WI). Anti-transglutaminase 2 (TG2) (MAB3839) and SGX-523 MET inhibitor (448106) were purchased from EMD Millipore Corp (Burlington, MA). The students t-test was used to assess significance. All values are presented as mean ± SD. Asterisks indicate a significant reduction and double asterisks indicate a significant increase.

Spheroid formation, invasion and migration assays

Meso-1 [27] and NCI-Meso-17 [28] are malignant cell lines derived, respectively, from peritoneal and pleural mesothelioma. Cell lines were maintained in RPMI1640 supplemented with 2 μM L-glutamine and 10% FCS. Spheroid assays were used to derive highly enriched cancer stem cell populations and to measure the impact of TG2 and HGF/MET on cell function [4]. For spheriod assay, 20,000 cells in 3 ml were plated in six well ultra-low attachment cluster dishes [4]. Spheroids, defined as a collection of cells with diameter ≥ 25 μM, were counted after 0 - 5 d. For invasion assay, BioCoat Millicell inserts (1 cm diameter, 8 μM pores), coated with 120 μL of 250 μg/mL Matrigel (BD Biolabs) diluted in 0.01 M Tris-HCl/0.7% NaCl, were placed individually into wells of a flat-bottom 24-well plate. Cells (20,000) were seed atop the Matrigel in growth medium containing 1% FCS, while the bottom chamber contained growth medium containing 10% FCS. Cell migration was monitored at 18 h. Invaded cells were fixed in 4% paraformaldehyde prior to staining with DAPI (1:5000) for fluorescent visualization. For migration, confluent monolayer cultures were wounded using a 10 μl pipette tip, washed to remove excess cells, and wound closure was monitored from 0 - 24 h.

Gene knockdown

Cells were grown to 80% confluence and harvested with trypsin and replated the night before the electroporation. Cells (1.2 million/group) were harvested with trypsin to produce single cells and resuspended in 100 μL of Lonza VPD-1002 nucleofection reagent (Wakersville, MD) containing 3 μg of siRNA and electroporated using an AMAXA electroporator on the T-018 setting [29,30]. After 48 h, the electroporation was repeated and the cells were permitted to recover for 12 h before use in biological assays.

Creating MET knockout cells

MET-specific CRISPR guide RNA, forward (5'-caccGTCATACTGCTGACATACAGT) and reverse (5'-aaacACTGTATGTCAGCAGTATGAC), were identified at http://crispr.technology and cloned in the U6-driven pSpCas9(BB)-2A-Puro (PX459) V2.0 vector from Addgene (#2429). Meso-1 cells were electroporated with 3 μg of plasmid using the AMAXA electroporator. At 48 h post-electroporation cells were treated with 2 μg/ml puromycin for 24 h and single cell-derived MET knockout clones were selected by dilution cloning.

Immunoblot

Cell lysates were prepared in modified Laemmli buffer (0.063 M Tris-HCl, pH 7.5, 10% glycerol, 5% SDS, 5% β-mercaptoethanol). Equivalent amounts of protein were electrophoresed on denaturing and reducing 10% polyacrylamide gels prior to transfer to nitrocellulose membrane. Membranes were blocked in 5% non-fat dry milk for 1 h and then incubated with primary antibodies (1:1000). After a 12 h incubation, membranes were washed and soaked in the appropriate secondary antibody (1:5000) for 2 h. Antibody binding was then monitored by chemiluminescence detection.

Tumor xenografts

Cancer cells were grown as spheroids for 6 d and harvested to prepare a single cell suspension. Cells (3 million) were resuspended 100 μl PBS containing 30% Matrigel and injected subcutaneously using a 26.5-gauge needle into each front flank of five eight-week-old female NOD/scid/IL-2 receptor gamma chain (NSG) knockout mice [4]. Tumor growth was monitored using a caliper and was reported as tumor volume = 4/3 x π x (diameter/2)^3. Tumors were harvested and sections and lysates were prepared for immunostain and immunoblot. All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Maryland Baltimore.

Immunostaining

To detect CD31 using immunofluorescence, paraffin-embedded tumor sections were incubated overnight at 4 C with mouse anti-CD31 primary antibody (1:500), followed by a 2 h incubation with AlexaFluor 488-conjugated donkey anti-mouse IgG secondary antibody (1:400). Following secondary antibody incubation, the slides were co-stained with Hoescht for 5 minutes at room temperature and the fluorescence signal was detected using an inverted confocal microscope.

Results

TG2 maintains HGF/MET signaling and the cancer phenotype

We recently showed that TG2 is required for mesothelioma cell survival and tumor formation [4]. However, we have limited information regarding how TG2 modulates downstream targets to maintain the cancer phenotype. To assess this, we used RNA-seq transcriptomic analysis to compare the gene expression profiles of wild-type and TG2 knockout (TG2-KO) Meso-1 cells. Meso-1 cells are derived from peritoneal mesothelioma

[27]. This anaylsis revealed that HGF mRNA levels are markedly reduced in TG2-negative cells, suggesting that HGF/MET may have a role in mediating TG2 maintainence of the aggressive cancer phenotype. Consistent with this finding, qRT-PCR analysis confirms a marked reduction in HGF mRNA in TG2-null Meso-1 cells as compared to wild-type cells (Fig. 1A). We next examined the relationship between TG2 and HGF/MET. If HGF is a key downstream TG2 target, treatment of TG2 knockout cells with HGF should partially restore MET signaling and TG2-dependent biological responses. Fig. 1B shows that MET activity is low in Meso-1 and Meso1-TG2-KOc4 cells and that HGF treatment increases wild-type Meso-1 cell MET activity but that activity is much less efficiently activated in the TG2 knockout cells. Moreover, the TG2 knockout associated reduction in spheroid formation and invasion is also only partially reversed by HGF treatment (Fig. 1C/D). Consistent with this, Fig. 1E/F shows a very similar patten of regulation for NCI-Meso-17 cells, which are derived from a patient with pleural mesothelioma. HGF only partially restores MET receptor activity (Fig. 1E) and cell invasion (Fig. 1F) in cells treated with TG2-siRNA as compared to control-siRNA treated cells.

To examine the impact of TG2 HGF/MET function in tumors, we used a xenograft model. TG2 knockout tumors grow slowly (Fig. 1G) and this is associated with reduced HGF and MET mRNA and reduced MET protein (Fig. 1H/I). Moreover, an important observation is that the TG2 knockout tumors display reduces ERK1/2 activity which is a MET responsive target [24]. The importance of the reduction in ERK1/2 activity in TG2-null cells will become apparent later in the manuscript. These findings suggest that TG2 maintains HGF and MET mRNA expression to increase HGF/MET signaling via a mechanism that may involve ERK1/2 signaling to drive an aggressive cancer phenotype.

HGF signaling and the cancer cell phenotype

Our next goal was to determine if the HGF/MET signaling pathway is a functionally important TG2 target. This required characterizing the role of HGF/MET in regulating the cancer phenotype. Fig. 2A/B show that HGF knockdown reduces Meso-1 cell invasion and ability to close a scratch wound. To examine the impact of MET knockdown on cell signaling, Meso-1 cells were treated with control- or MET-siRNA and extracts were prepared to monitoring signal transduction. Fig. 2C confirms that MET knockdown reduces MET-*P* and Fig. 2D/E shows that this is associated with reduced invasion and migration.

HGF/MET signaling is known to regulate a wide range of downstream kinases, including PI3K/Akt, Ras, FAK/Src, JNK and ERK1/2, in a cell type-dependent manner [25,31]. We therefore measured the impact of MET knockdown on a host of downstream kinases and found that only ERK1/2 activity is consistently reduced (Fig. 2C). It is important to note that the reduction in ERK1/2 activity in MET knockdown cells phenocopies the reduction in ERK1/2 activity observed following TG2 knockout (Fig. 1I). To further confirm a role for HGF/MET signaling, we treated cells with SGX-523, a MET inhibitor [32], which we show reduces MET and ERK1/2 activity (Fig. 2F) and spheroid size, invasion and migration (Fig 2G/H/I). We next tested the impact of HGF treatment on MET signaling and biological response. We show that HGF treatment activates MET, MEK1/2 and ERK1/2 (Fig. 1**J**) and increases cell invasion and migration (Fig. 2K/L).

We repeated these experiments using pleural mesothelioma-derived NCI-Meso-17 cells [28]. Fig. 3A/B show that HGF knockdown reduces cell invasion and migration. Fig. 3C/D/E show that MET knockdown reduces MET and ERK1/2 activity, and cell invasion and migration. In addition, SGX-523 treatment reduces MET activity (Fig. 3F) and inhibits spheriod formation, invasion and migration (Fig. 3G/H/I). These findings predict that HGF treatment should stimulate an aggressive cancer cell phenotype. Indeed, HGF stimulates MET signaling (Fig. 3J) and invasion and migration (Fig. 3K/L). Taken together, these finding suggest that HGF/MET activation phenocopies the TG2 stimulation of the mesothelioma cancer phenotype.

To further assess the role of MET as a mediator of HGF action, we suppressed MET level using siRNA in Meso-1 and NCI-Meso-17 cells and monitored the impact of HGF treatment. Fig. 4A/B show that HGF treatment increases control-siRNA treated cell invasion and migration but minimally impacts cells treated with MET-siRNA. MET knockdown also prevents HGF-stimulated NCI-Meso-17 cell invasion (Fig. 4C), and eliminates MET and MEK1/2 activity, and attenuates ERK1/2 activity (Fig. 4D). We next examined the impact of HGF on MET knockout Meso-1 cells. Fig. 4E confirms the absence of MET receptor in two clonal MET knockout cells lines. Fig. 4F/G/H/I show that the MET knockout clones display reduced cell proliferation, invasion and migration. Moreover, treatment of wild-type cells with HGF increases invasion (Fig. 4I) and MEK1/2 and ERK1/2 activity (Fig. 4J), but these responses are absent in the Meso1-MET-KOc1-4 cells.

MEK1/2 and ERK1/2 are required to maintain the aggressive cancer phenotype

We next examined downstream signaling events. HGF/MET can activate a host of signaling cascades, including PKC, Src/FAK, STAT3, Ras/Raf, PI3K/Akt and MAPK (MEK1/2, ERK1/2) [24,25]. We screened for changes in these signaling cacades in response to interfering with TG2 function and found that only ERK1/2 activity was reduced (Fig. 1I). MEK1/2 is the immediate upstream regulator of ERK1/2 [33]. To determine if MEK1/2 and ERK1/2 are essential mediators, we used inhibitors. Treatment with MEK1/2 inhibitor (U0126) reduces ERK1/2 activity as evidenced by reduced phosphorylation of the ERK1/2 activation loop (reduced ERK1/2-*P*) (Fig. 5A). The ERK1/2 inhibitor (LY3214996) inhibts ATP binding to the ERK1/2 catalytic site to inhibit ERK1/2 modification of downstream targets, but does not inhibit MEK1/2 labeling of the ERK1/2 activation loop [34]. Thus, although ERK1/2 is inhibited, the ERK1/2 activation loop can still be phosphorylated via feedback mechanisms as is evident in Fig. 5A. Fig. 5B/C/D show that treatment with U0126 or LY3214996 reduces Meso-1 cell spheroid formation, invasion and migration, suggesting that MEK1/2 and ERK1/2 activity are required to maintain the cancer phenotype.

MET signaling in tumors

We next assessed the impact of MET knockout on tumor formation. Meso-1 cells, grown as unattached spheroids to enrich for MCS cells, were injected subcutaneously in NSG mice [4]. Fig. 5E/F show that the MET knockout cells are severely attenuated in tumor formation, compared to wild-type cells, and this is associated with reduced MET, MEK1/2 and ERK1/2 activity. These responses and the reduction in tumor formation for MET knockout cells mimic the signaling changes and reduction in tumor size observed for TG2 knockout cells

(Fig. 1G/H/I). In addition, MET knockout reduces vascularization which is confirmed by reduced levels of CD31, as measured by immunostaining and immunoblot (Fig. 5G).

Discussion

TG2 acts via HGF/MET to maintain the cancer phenotype

TG2 is a pro-cancer regulator that activates a range of signaling cascades [9,13,35]. TG2 maintains VEGF [36], NRP1 [37], GIPC1/SYX/RhoA/p38 [37], α6/β4-integrin [15,38] and YAP1/TAZ [15] pro-cancer signaling in various cancer cell types. However, very little is known about the role of TG2 in mesothelioma other than that it is highly expressed, maintains the MCS cell phenotype and promotes EMT [4,39]. To study the mechanism of TG2 action, we performed RNA-seq transcriptomic analysis to identify TG2 specific changes in gene expression. This analysis showed a marked reduction in HGF mRNA in TG2 knockout Meso-1 cells, suggesting that HGF/MET signaling is a mediator of TG2 action. We confirmed these finding by showing that HGF/MET signaling is reduced along with spheroid formation and invasion in TG2 knockdown Meso-1 and NCI-Meso-17 cells. In addition, TG2 knockout tumors, which grow slowly, display reduced HGF and MET mRNA, reduced MET protein level and reduced ERK1/2 signaling. These findings clearly implicate HGF/MET as potential mediators of TG2 pro-cancer action in mesothelioma.

HGF/MET regulates the cancer phenotype

Of course it is possible that the reduction in HGF/MET activity in TG2 knockdown cells may not be biologically important. To address this issue, we performed experiments to directly demonstrate a biological role for HGF/MET. These studies show that HGF/MET knockout/knockdown or treatment with MET inhibitor reduces mesothelioma cell spheroid formation, invasion and migration in both Meso-1 (peritoneal) and NCI-Meso-17 (pleural) mesothelioma cells. Moreover, these responses require HGF interaction with MET, as inhibiting MET action attenuates the response to HGF. The finding that HGF/MET is important in mesothelioma is consistent with previous studies showing that HGF is highly expressed in mesothelioma and is secreted to produce autocrine and paracrine actions [26,40,41]. Moreover, HGF acts as a mesothelioma cell chemoattractant [26,42], stimulates microvessel formation [43], modulates metalloproteinase function [42] and serves as a therapy target [44]. However, less is known about mechanisms that maintain HGF/MET signaling in mesothelioma. The present studies identify the TG2 cancer cell survival protein as an upstream factor that maintains HGF and MET levels, and MET signaling, in mesothelioma cancer cells.

The role of MEK1/2 and ERK1/2 signaling

HGF/MET signaling is known to regulate a range of downstream kinases including PI3K/Akt, Ras, FAK/Src, JNK and ERK1/2 in a cancer cell type-dependent manner [24,25,31] and can also crosstalk with and activate EGFR signaling [26]. For example, HGF/MET activates PI3K/MEK5/Fra-1 [45,46], Akt [47,48] and other signalling pathways [26].

A role for MEK/ERK signaling in mesothelioma has been documented. Agents that induce mesothelima, such as aronite [49] and asbestos [50], induce ERK1/2 activity. Cisplatin treatment

can induce ERK1/2 activity as a resistance mechanism [51]. In contrast, gallic acid [52], Gefitinib (an EGFR inhibitor) [53] and U0126, a MEK1/2 inhibitor, reduce ERK1/2 signaling and attentuate mesothelioma cell proliferation [54]. In addition, HGF/MET activity can crosstalk with and activate EGFR signaling which can contribute to ERK1/2 activation [26]. ERK1/2 signaling also stimulates mesothelioma cell proliferation [55], makes cells resistant to chemotherapy [56] and confers drug resistance [53]. Our studies show that TG2 triggers HGF/MET activity to increase MEK1/2 and ERK1/2 signaling as a mechanism to maintain the cancer phenotype.

TG2 and HGF/MET signaling in tumors

It is important to confirm that the TG2, HGF/MET, MEK1/2 and ERK1/2 pro-cancer pathway is relevant in tumors. To assess this, we monitored wild-type and MET knockout cell tumors for expression of these proteins. These experiments show that wild-type mesothelioma cells initiate rapid tumor growth at six weeks with optimal tumor size at eight weeks, but that MET knockout tumors grow slowly and at eight weeks are only 10% the size of wild-type tumors. Thus, MET loss markedly reduces tumor formation. Moreover, consistent with the cell culture findings, the MET knockout tumors display reduced MET, MEK1/2 and ERK1/2 activity. MET loss also reduces tumor vascularization, as evidenced by reduced CD31 levels. It is important to note that these responses in MET knockout cell tumors phenocopy the responses observed for TG2 knockout cells, confirming a close relationship between TG2 presence and activation of HGF/MET signaling in mesothelioma.

A TG2, HGF/MET and MEK/ERK pro-cancer signaling cascade

Knowledge is limited regarding upstream mechanisms that regulate and maintain HGF/MET levels and signaling. Our study points to a novel role for TG2 in maintaining HGF and MET gene expression and HGF/MET signaling to drive the mesothelioma cancer phenotype. This is of interest from a therapy standpoint, as TG2 interacts with a host of membrane-associated receptors to maintain pro-cancer signalling cascades [15,36-38] suggesting that inhibiting TG2 may facilitate inactivation of multiple cascades that could drive mesothelioma cancer cell survival. Based on the present studies, we propose that TG2 maintains HGF and MET gene expression and HGF/MET activity which stimulates MEK1/2-ERK1/2 signaling to drive cancer cell invasion, migration and tumor formation (Fig. 5H). The fact that TG2 maintains the HGF/MET pro-cancer signaling cascade [9,13] suggests that inhibition of TG2 may be a useful mesothelioma treatment strategy.

Acknowledgements

This work was supported by a grant from the Kazan McClain Partners' Foundation to RLE and JSF. Warren Naselsky was supported by the Cancer Biology T32 Training Grant (T32 CA154274) an International Lung Cancer Foundation Young Investigator Award, and a 2021 iMIG Young Investigator Microgrant Award sponsored by Kazan, McClain, Satterley & Greenwood PLC. This work utilized the facilities of the Greenebaum Comprehensive Cancer Center (P30 CA134274) at the University of Maryland School of Medicine.

Data Availability

The author elect to not share data

Abbreviations:

HGF	hepatocyte growth factor
TG2	Transglutaminase 2
MCS cells	mesothelioma cancer stem-like cells

Reference List

1. Cao S, Jin S, Cao J, et al. Advances in malignant peritoneal mesothelioma. Int J Colorectal Dis. 2015;30(1):1–10. [PubMed: 25331029]
2. Hassan R, Alexander R, Antman K, et al. Current treatment options and biology of peritoneal mesothelioma: meeting summary of the first NIH peritoneal mesothelioma conference. Ann Oncol. 2006;17(11):1615–1619. [PubMed: 16600983]
3. Vogelzang NJ, Rusthoven JJ, Symanowski J, et al. Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. J Clin Oncol. 2003;21(14):2636–2644. [PubMed: 12860938]
4. Adhikary G, Grun D, Alexander HR, et al. Transglutaminase is a mesothelioma cancer stem cell survival protein that is required for tumor formation. Oncotarget. 2018;9(77):34495–34505. [PubMed: 30349644]
5. Zhang J, Lesort M, Guttmann RP, Johnson GV. Modulation of the in situ activity of tissue transglutaminase by calcium and GTP. J Biol Chem. 1998;273(4):2288–2295. [PubMed: 9442073]
6. Chhabra A, Verma A, Mehta K. Tissue transglutaminase promotes or suppresses tumors depending on cell context. Anticancer Res. 2009;29(6):1909–1919. [PubMed: 19528447]
7. Kumar A, Gao H, Xu J, Reuben J, Yu D, Mehta K. Evidence that aberrant expression of tissue transglutaminase promotes stem cell characteristics in mammary epithelial cells. PLoS One. 2011;6(6):e20701. [PubMed: 21687668]
8. Kumar A, Xu J, Sung B, et al. Evidence that GTP-binding domain but not catalytic domain of transglutaminase 2 is essential for epithelial-to-mesenchymal transition in mammary epithelial cells. Breast Cancer Res. 2012;14(1):R4. [PubMed: 22225906]
9. Eckert RL. Transglutaminase 2 takes center stage as a cancer cell survival factor and therapy target. Mol Carcinog. 2019;58(6).
10. Eckert RL, Kaartinen MT, Nurminskaya M, et al. Transglutaminase regulation of cell function. Physiol Rev. 2014;94(2):383–417. [PubMed: 24692352]
11. Fesus L, Piacentini M. Transglutaminase 2: an enigmatic enzyme with diverse functions. Trends Biochem Sci. 2002;27(10):534–539. [PubMed: 12368090]
12. Belkin AM. Extracellular TG2: emerging functions and regulation. FEBS J. 2011;278(24):4704–4716. [PubMed: 21902810]
13. Eckert RL, Fisher ML, Grun D, Adhikary G, Xu W, Kerr C. Transglutaminase is a tumor cell and cancer stem cell survival factor. Mol Carcinog. 2015;54(10):947–958. [PubMed: 26258961]
14. Fisher ML, Adhikary G, Xu W, Kerr C, Keillor JW, Eckert RL. Type II transglutaminase stimulates epidermal cancer stem cell epithelial-mesenchymal transition. Oncotarget. 2015;6(24):20525–20539. [PubMed: 25971211]
15. Fisher ML, Kerr C, Adhikary G, et al. Transglutaminase Interaction with alpha6/beta4-Integrin Stimulates YAP1-Dependent DeltaNp63alpha Stabilization and Leads to Enhanced Cancer Stem Cell Survival and Tumor Formation. Cancer Res. 2016;76(24):7265–7276. [PubMed: 27780825]
16. Mehta K, Fok J, Miller FR, Koul D, Sahin AA. Prognostic significance of tissue transglutaminase in drug resistant and metastatic breast cancer. Clin Cancer Res. 2004;10(23):8068–8076. [PubMed: 15585642]
17. Budillon A, Carbone C, Di GE. Tissue transglutaminase: a new target to reverse cancer drug resistance. Amino Acids. 2011.

18. Mangala LS, Fok JY, Zorrilla-Calancha IR, Verma A, Mehta K. Tissue transglutaminase expression promotes cell attachment, invasion and survival in breast cancer cells. Oncogene. 2007;26(17):2459–2470. [PubMed: 17043648]
19. Oh K, Ko E, Kim HS, et al. Transglutaminase 2 facilitates the distant hematogenous metastasis of breast cancer by modulating interleukin-6 in cancer cells. Breast Cancer Res. 2011;13(5):R96. [PubMed: 21967801]
20. Ai L, Kim WJ, Demircan B, et al. The transglutaminase 2 gene (TGM2), a potential molecular marker for chemotherapeutic drug sensitivity, is epigenetically silenced in breast cancer. Carcinogenesis. 2008;29(3):510–518. [PubMed: 18174247]
21. Kumar A, Xu J, Brady S, et al. Tissue transglutaminase promotes drug resistance and invasion by inducing mesenchymal transition in mammary epithelial cells. PLoS One. 2010;5(10):e13390. [PubMed: 20967228]
22. Shao M, Cao L, Shen C, et al. Epithelial-to-mesenchymal transition and ovarian tumor progression induced by tissue transglutaminase. Cancer Res. 2009;69(24):9192–9201. [PubMed: 19951993]
23. Cao L, Shao M, Schilder J, Guise T, Mohammad KS, Matei D. Tissue transglutaminase links TGF-beta, epithelial to mesenchymal transition and a stem cell phenotype in ovarian cancer. Oncogene. 2012;31(20):2521–2534. [PubMed: 21963846]
24. Organ SL, Tsao MS. An overview of the c-MET signaling pathway. Ther Adv Med Oncol. 2011;3(1 Suppl):S7–S19. [PubMed: 22128289]
25. Maroun CR, Rowlands T. The Met receptor tyrosine kinase: a key player in oncogenesis and drug resistance. Pharmacol Ther. 2014;142(3):316–338. [PubMed: 24384534]
26. Gaudino G, Yang H, Carbone M. HGF/Met Signaling Is a Key Player in Malignant Mesothelioma Carcinogenesis. Biomedicines. 2014;2(4):327–344. [PubMed: 28548074]
27. Varghese S, Whipple R, Martin SS, Alexander HR. Multipotent cancer stem cells derived from human malignant peritoneal mesothelioma promote tumorigenesis. PLoS One. 2012;7(12):e52825. [PubMed: 23285196]
28. Kalra N, Zhang J, Thomas A, et al. Mesothelioma patient derived tumor xenografts with defined BAP1 mutations that mimic the molecular characteristics of human malignant mesothelioma. BMC Cancer. 2015;15(1):376. [PubMed: 25952750]
29. Adhikary G, Chew YC, Reece EA, Eckert RL. PKC-delta and -eta, MEKK-1, MEK-6, MEK-3, and p38-delta Are Essential Mediators of the Response of Normal Human Epidermal Keratinocytes to Differentiating Agents. J Invest Dermatol. 2010.
30. Chew YC, Adhikary G, Wilson GM, Reece EA, Eckert RL. PKCdelta suppresses keratinocyte proliferation by increasing p21CIP1 level by a KLF4-dependent mechanism. J Biol Chem. 2011;286:28771–28782.
31. Arnold L, Enders J, Thomas SM. Activated HGF-c-Met Axis in Head and Neck Cancer. Cancers (Basel). 2017;9(12).
32. Buchanan SG, Hendle J, Lee PS, et al. SGX523 is an exquisitely selective, ATP-competitive inhibitor of the MET receptor tyrosine kinase with antitumor activity in vivo. Mol Cancer Ther. 2009;8(12):3181–3190. [PubMed: 19934279]
33. Cobb MH. MAP kinase pathways. Prog Biophys Mol Biol. 1999;71(3-4):479–500. [PubMed: 10354710]
34. Bhagwat SV, McMillen WT, Cai S, et al. ERK Inhibitor LY3214996 Targets ERK Pathway-Driven Cancers: A Therapeutic Approach Toward Precision Medicine. Mol Cancer Ther. 2020;19(2):325–336. [PubMed: 31744895]
35. Mehta K, Kumar A, Kim HI. Transglutaminase 2: a multi-tasking protein in the complex circuitry of inflammation and cancer. Biochem Pharmacol. 2010;80(12):1921–1929. [PubMed: 20599779]
36. Grun D, Adhikary G, Eckert RL. VEGF-A acts via neuropilin-1 to enhance epidermal cancer stem cell survival and formation of aggressive and highly vascularized tumors. Oncogene. 2016;35:4379–4387. [PubMed: 26804163]
37. Grun D, Adhikary G, Eckert RL. NRP-1 interacts with GIPC1 and SYX to activate p38 MAPK signaling and cancer stem cell survival. Mol Carcinog. 2019;58(4):488–499. [PubMed: 30456845]

38. Fisher ML, Keillor JW, Xu W, Eckert RL, Kerr C. Transglutaminase is required for epidermal squamous cell carcinoma stem cell survival. Mol Cancer Res. 2015;13:1083–1094. [PubMed: 25934691]
39. Zonca S, Pinton G, Wang Z, et al. Tissue transglutaminase (TG2) enables survival of human malignant pleural mesothelioma cells in hypoxia. Cell Death Dis. 2017;8(2):e2592. [PubMed: 28151477]
40. Harvey P, Warn A, Dobbin S, et al. Expression of HGF/SF in mesothelioma cell lines and its effects on cell motility, proliferation and morphology. Br J Cancer. 1998;77(7):1052–1059. [PubMed: 9569039]
41. Klominek J, Baskin B, Liu Z, Hauzenberger D. Hepatocyte growth factor/scatter factor stimulates chemotaxis and growth of malignant mesothelioma cells through c-met receptor. Int J Cancer. 1998;76(2):240–249. [PubMed: 9537587]
42. Harvey P, Clark IM, Jaurand MC, Warn RM, Edwards DR. Hepatocyte growth factor/scatter factor enhances the invasion of mesothelioma cell lines and the expression of matrix metalloproteinases. Br J Cancer. 2000;83(9):1147–1153. [PubMed: 11027427]
43. Tolnay E, Kuhnen C, Wiethege T, König JE, Voss B, Müller KM. Hepatocyte growth factor/scatter factor and its receptor c-Met are overexpressed and associated with an increased microvessel density in malignant pleural mesothelioma. J Cancer Res Clin Oncol. 1998;124(6):291–296. [PubMed: 9692834]
44. Mukohara T, Civiello G, Davis IJ, et al. Inhibition of the met receptor in mesothelioma. Clin Cancer Res. 2005;11(22):8122–8130. [PubMed: 16299245]
45. Ramos-Nino ME, Blumen SR, Sabo-Attwood T, et al. HGF mediates cell proliferation of human mesothelioma cells through a PI3K/MEK5/Fra-1 pathway. Am J Respir Cell Mol Biol. 2008;38(2):209–217. [PubMed: 17872495]
46. Ramos-Nino ME, Scapoli L, Martinelli M, Land S, Mossman BT. Microarray analysis and RNA silencing link fra-1 to cd44 and c-met expression in mesothelioma. Cancer Res. 2003;63(13):3539–3545. [PubMed: 12839939]
47. Altomare DA, You H, Xiao GH, et al. Human and mouse mesotheliomas exhibit elevated AKT/PKB activity, which can be targeted pharmacologically to inhibit tumor cell growth. Oncogene. 2005;24(40):6080–6089. [PubMed: 15897870]
48. Xiao GH, Jeffers M, Bellacosa A, Mitsuuchi Y, Vande Woude GF, Testa JR. Anti-apoptotic signaling by hepatocyte growth factor/Met via the phosphatidylinositol 3-kinase/Akt and mitogen-activated protein kinase pathways. Proc Natl Acad Sci U S A. 2001;98(1):247–252. [PubMed: 11134526]
49. Bertino P, Marconi A, Palumbo L, et al. Erionite and asbestos differently cause transformation of human mesothelial cells. Int J Cancer. 2007;121(1):12–20. [PubMed: 17354240]
50. Kroczynska B, Cutrone R, Bocchetta M, et al. Crocidolite asbestos and SV40 are cocarcinogens in human mesothelial cells and in causing mesothelioma in hamsters. Proc Natl Acad Sci U S A. 2006;103(38):14128–14133. [PubMed: 16966607]
51. Muscella A, Vetrugno C, Antonaci G, Cossa LG, Marsigliante S. PKC-δ/PKC-α activity balance regulates the lethal effects of cisplatin. Biochem Pharmacol. 2015;98(1):29–40. [PubMed: 26300055]
52. Demiroglu-Zergeroglu A, Candemir G, Turhanlar E, Sagir F, Ayvali N. EGFR-dependent signalling reduced and p38 dependent apoptosis required by Gallic acid in Malignant Mesothelioma cells. Biomed Pharmacother. 2016;84:2000–2007. [PubMed: 27847212]
53. Shukla A, Barrett TF, MacPherson MB, et al. An extracellular signal-regulated kinase 2 survival pathway mediates resistance of human mesothelioma cells to asbestos-induced injury. Am J Respir Cell Mol Biol. 2011;45(5):906–914. [PubMed: 21454801]
54. Cole GW Jr., Alleva AM, Zuo JT, et al. Suppression of pro-metastasis phenotypes expression in malignant pleural mesothelioma by the PI3K inhibitor LY294002 or the MEK inhibitor UO126. Anticancer Res. 2006;26(2a):809–821. [PubMed: 16619474]
55. Shukla A, Hillegass JM, MacPherson MB, et al. ERK2 is essential for the growth of human epithelioid malignant mesotheliomas. Int J Cancer. 2011;129(5):1075–1086. [PubMed: 21710492]

56. Shukla A, Hillegass JM, MacPherson MB, et al. Blocking of ERK1 and ERK2 sensitizes human mesothelioma cells to doxorubicin. Mol Cancer. 2010;9:314. [PubMed: 21159167]

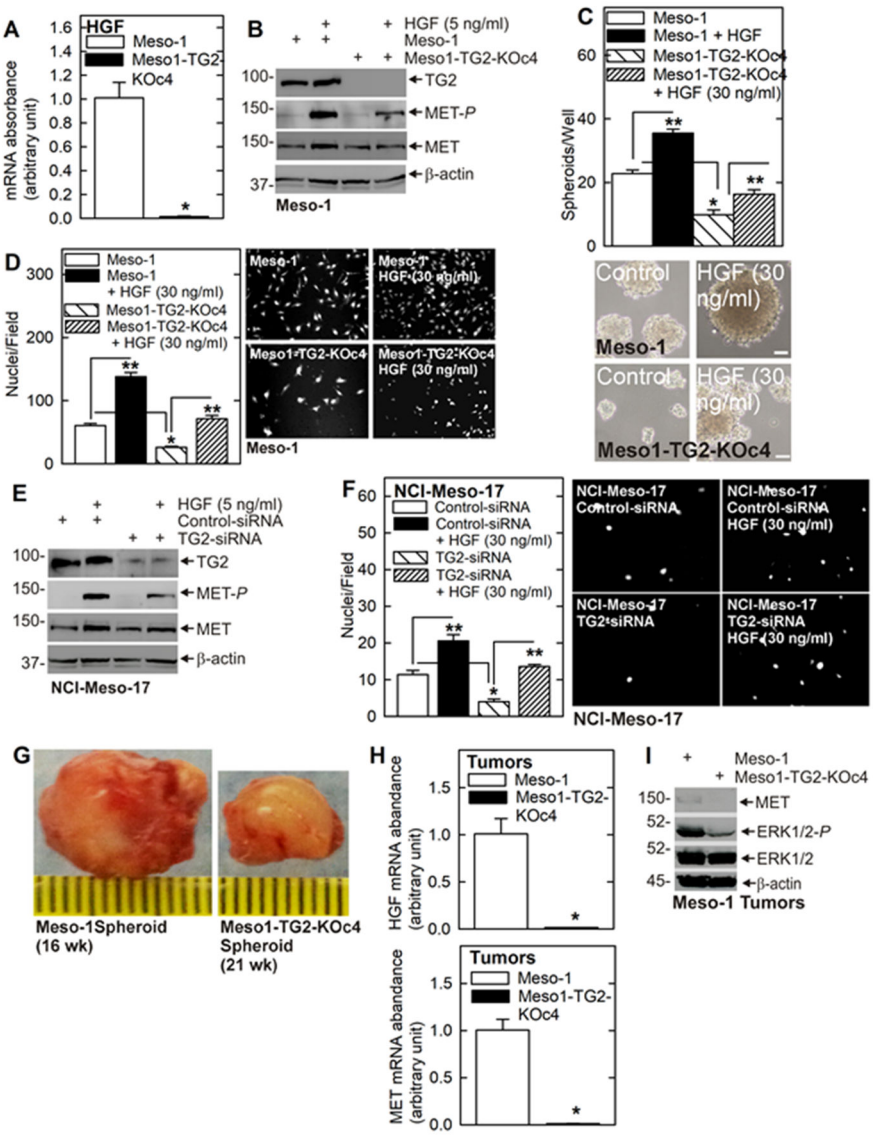

Fig. 1.

TG2 maintains the cancer phenotype via HGF/MET receptor. **A** TG2 knockout reduces HGF mRNA transcript levels. **B/C/D** TG2 knockout attenuates MET and ERK1/2 signaling, and spheroid formation and invasion. HGF treatment partially restores MET and ERK1/2 signaling and biological responses. **E/F** TG2 knockdown reduces NCI-Meso-17 MET and ERK1/2 signaling and invasion, and these responses are partially reversed by HGF treatment. **G/H/I** TG2 knockout reduces tumor formation, HGF and MET mRNA, MET protein level and ERK1/2 activity. Single asterisks indicate a significant decrease ($n = 3$, $p \leq 0.001$) and double asterisks indicate a significant increase ($n = 3$, $p \leq 0.001$).

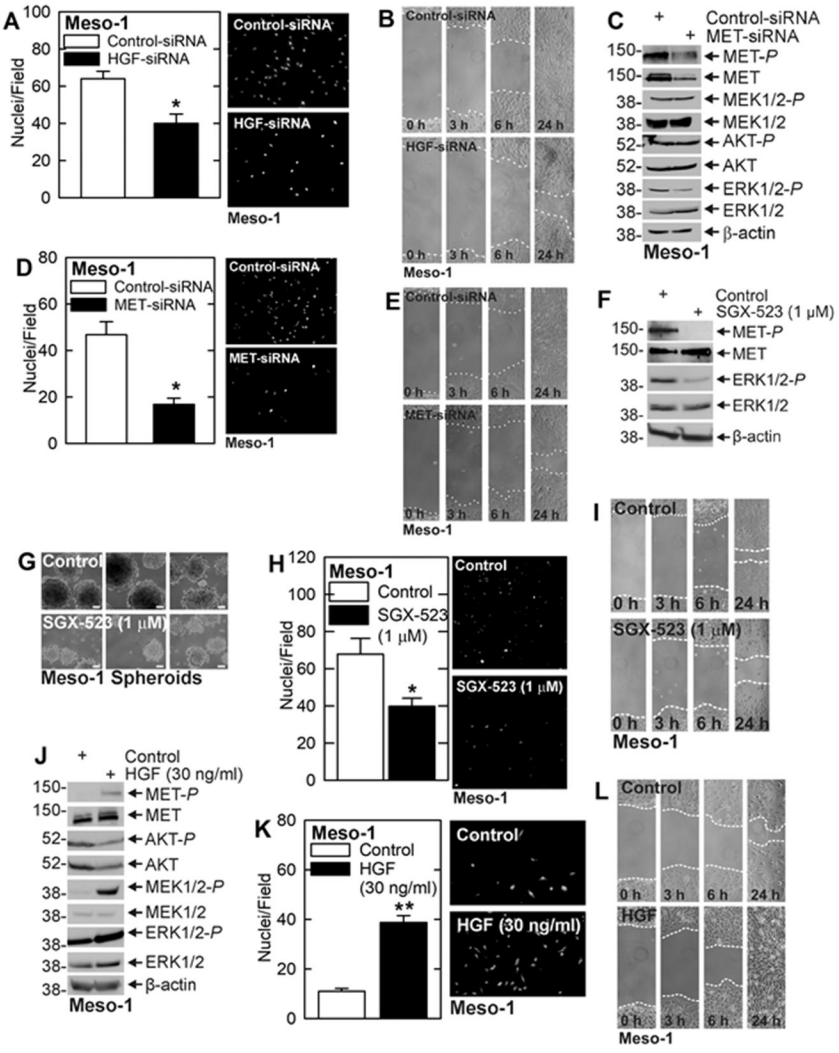

Fig. 2.

HGF/MET enhances the Meso-1 cell cancer phenotype. **A/B** HGF knockdown reduces Meso-1 cell invasion and migration. **C/D/E** Meso-1 cell MET knockdown reduces MET and ERK1/2 activity which is associated with reduced invasion and migration. **F/G/H/I** MET-inhibitor SGX-523 reduces Meso-1 ERK1/2 activity and spheroid formation, invasion, and migration. **J/K/L** HGF treatment activates MET, MEK1/2 and ERK1/2 signaling and increases invasion and migration. Single asterisks indicate a significant decrease ($n = 3$, $p \leq 0.001$).

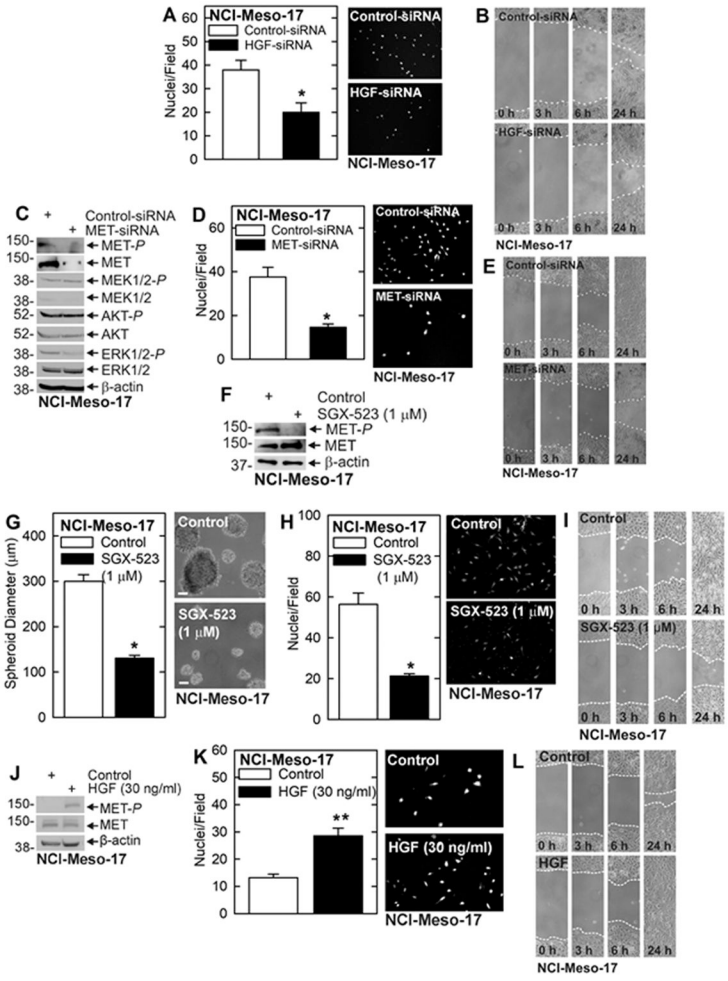

Fig. 3.

HGF/MET maintains the NCI-Meso-17 cell cancer phenotype. **A/B** HGF-knockdown reduces NCI-Meso-17 cell invasion and migration. **C/D/E** MET-knockdown reduces ERK1/2 activity and cell invasion and migration. F/**G/H/I** Treatment with the SGX-523 MET inhibitor reduces spheroid formation, invasion and migration. **J/K/L** HGF treatment increases MET signaling and cell invasion and migration. Single asterisks indicate a significant decrease ($n = 3$, $p \leq 0.001$) and double asterisks indicate a significant increase ($n = 3$, $p \leq 0.001$).

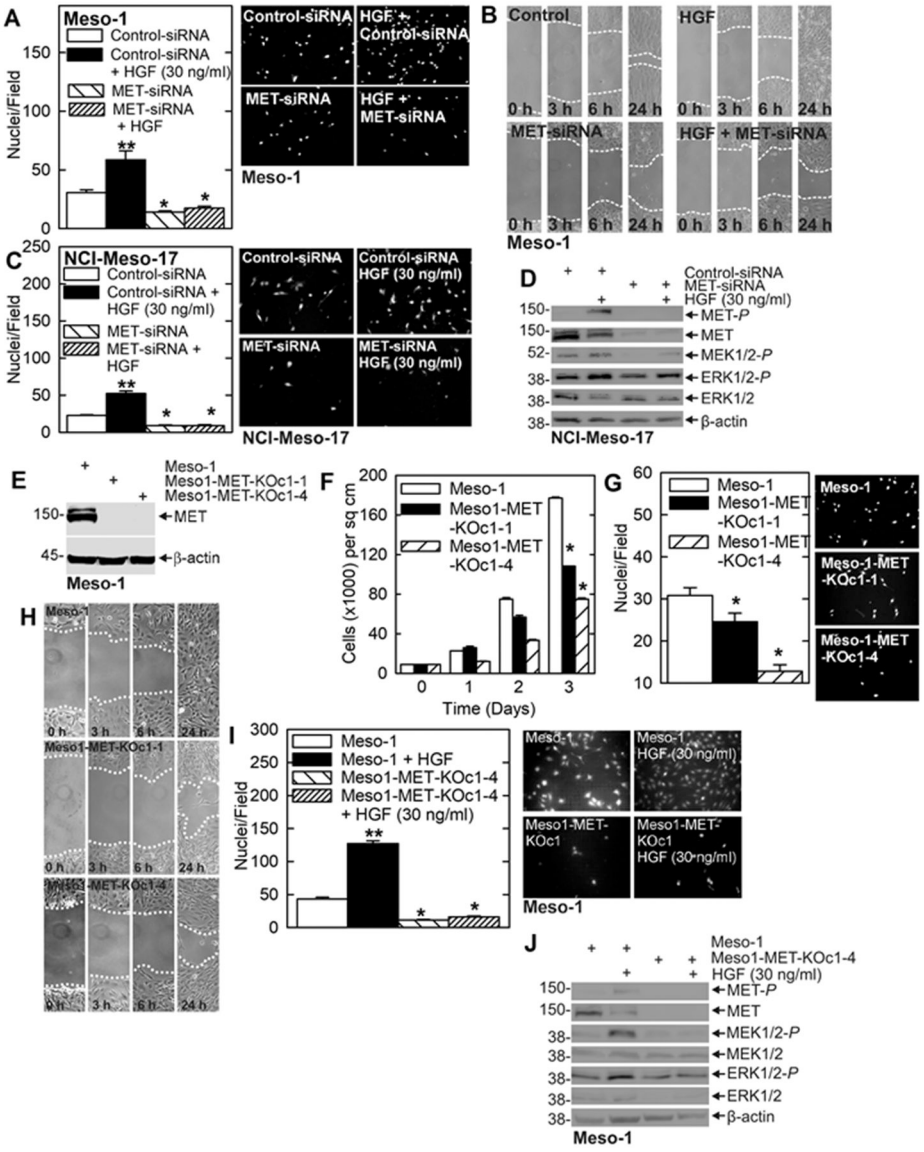

Fig. 4.

HGF activation of MET enhances the cancer phenotype. **A/B** HGF stimulates Meso-1 cell invasion and migration and this response requires MET receptor. **C/D** HGF stimulates NCI-Meso-17 cell MET, MEK1/2 and ERK1/2 activity and increases invasion, and these responses require MET receptor. **E/F/G/H** MET knockout cell lines display reduced cell proliferation, invasion and migration. **I/J** HGF treatment does not increase MEK1/2 or ERK1/2 activity in Meso1-MET-KOc1-4 cells. Single asterisks indicate a significant decrease ($n = 3$, $p \leq 0.001$) and double asterisks indicate a significant increase ($n = 3$, $p \leq 0.001$).

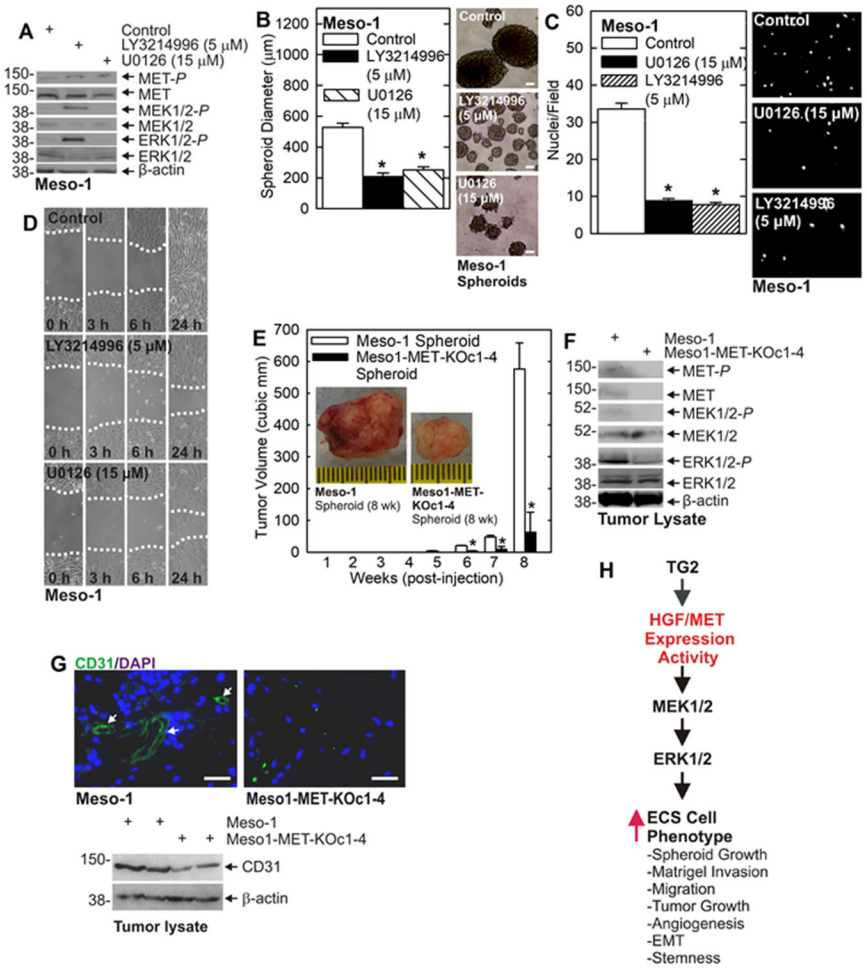

Fig. 5.

ERK1/2 and MEK1/2 signaling is critical for MET-dependent activation of the cancer phenotype. **A/B/C/D** Treatment with MEK1/2 (U0126) or ERK1/2 (LY3214996) inhibitor reduces Meso-1 cell MEK1/2 or ERK1/2 activity leading to reduced spheroid formation, invasion and migration. **E/F/G** MET knockout cells display reduced tumor formation and these tumors display reduced MET, MEK1/2 and ERK1/2 activity and reduced levels of CD31. Bars = 50 microns. **H** These studies show that TG2 maintains HGF/MET mRNA and protein levels to facilitate HGF/MET signaling which increases MEK1/2 and ERK1/2 activity to drive the aggressive cancer phenotype. Single asterisks indicate a significant decrease ($n = 3$, $p \leq 0.001$).