Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format.

1. **Grantee Institution:** The Pennsylvania State University

2. **Reporting Period (start and end date of grant award period):** 1/1/2010 - 12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** John Anthony, MPA

4. **Grant Contact Person's Telephone Number:** 814 935 1081

5. **Grant SAP Number:** 4100050904

6. **Project Number and Title of Research Project:** 45. Mechanisms for km23-1 Control of Ras/TGFß Signaling in Human Colon Cancer and Noncancer Cells

7. **Start and End Date of Research Project:** 7/20/2012 - 12/31/2013

8. **Name of Principal Investigator for the Research Project:** Kathleen M Mulder, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 91,800

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
Mulder	PI	5%	4213.00
Jin	Research Assoc	10%	1344.00
Yi	Student Intern	25%	188.50
Wagner	Student Intern	20%	378.25
Luckenbaugh	Student Intern	70%	177.63

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project
Jin	Research Assoc	90%
Yi	Student Intern	75%
Wagner	Student Intern	80%
Luckenbaugh	Student Intern	30%

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
NONE		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes___X_____ No_____

If yes, please indicate the source and amount of other funds:

Department of Biochemistry and Molecular Biology $40,000 DC

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes____X_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
Directional Motility and ERM Scaffolding in Pathfinder Pancreatic Carcinoma Cells	x NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	3/2013	$420,746	$199,665

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes___X_____ No_____

If yes, please describe your plans:

The R21 listed above was funded. An additional RO1 is planned during 2014.

12. Future of Research Project. What are the future plans for this research project?

We plan to submit publications pertaining to the research described in section 17. For example, one manuscript in preparation is:

Jin, Q. and **Mulder, K.M.** Dynein-independent role of km23-1 in the regulation of ERK signaling in KRAS- and BRAF-mutant human CRC cells. In Prep, Int J Oncol, 2014.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes___X_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female	3			
Unknown				
Total	**3**			

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic	3			
Unknown				
Total	**3**			

	Undergraduate	Masters	Pre-doc	Post-doc
White	2			
Black				
Asian	1			
Other				
Unknown				
Total	**3**			

14. **Recruitment of Out-of–State Researchers**. Did you bring researchers into Pennsylvania to carry out this research project?

    Yes_____ No____X_____

    If yes, please list the name and degree of each researcher and his/her previous affiliation:

    The investigators were already working in the PI's laboratory, but the funds did help to sustain their positions, providing both salary support and research supplies for the project.

15. **Impact on Research Capacity and Quality**. Did the health research project enhance the quality and/or capacity of research at your institution?

    Yes____X_____ No_____

    If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

    The knowledge we have gained from this project will lead to collaborative research projects with others at the institution, as well as potential new collaborative research and translational grant applications and awards. For example, the new R21 grant involves a collaboration with Dr. Wei Ding, Pediatrics.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes____X_____ No_____

If yes, please describe the collaborations:

Dr. Murry Korc – Indiana University
Dr. Paul Timpson – Garvan Institute of Medical Research

16(B) Did the research project result in commercial development of any research products?

Yes_____ No_____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.
List the project goals, objectives and specific aims (as contained in the grant application's strategic plan). Summarize the progress made in achieving these goals, objectives and aims for the entire grant award period. Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Goals, objectives, and specific aims:

We have previously shown that Ras and various Ras effector pathways mediate production of the TGFß cytokines (Yue & Mulder, 2000; 2001; Hartsough et al, 1995-7; Liu et al, 2006b). Further, these signaling pathways are distinct in untransformed (i.e., non-cancer) TGFß-sensitive epithelial cells (UECs) compared to human carcinoma cells, permitting selectivity in targeting such events (Liu et al, 2006a). More recently, we have demonstrated that the TGFß receptor (TßR)-interacting protein km23-1 can function as a novel Ras regulator (Jin et al, 2012b). Further, knock-down (KD) of km23-1 inhibits Ras activity and blocks TGFß production in UECs, suggesting that km23-1 inhibitors may attenuate the constitutive TGFß production in human colorectal carcinoma (CRC) cells harboring KRas or BRAF mutations, as well as the associated phospho-extracellular signal-regulated kinase (ERK) signaling.

The project will address the mechanisms underlying km23-1's unique ability to block Ras/ERK activation in human CRC cells, thereby reducing production of TGFß1 and the associated paracrine tumor-enhancing effects. We will also examine the role of the dynein complex in regulating the altered Ras/ERK signaling in in KRAS-mutant and BRAF-mutant human CRC cells. This project should not only provide significant new information regarding km23-1 control of Ras and ERK in human carcinogenesis, but also facilitate efforts to develop km23-1 inhibitors for the treatment of human CRC.

Our results have shown that km23-1 regulates, among other Ras effector pathways, both the Jun N-terminal kinase (JNK) and ERK mitogen-activated kinase (MAPK) cascades, with km23-1 functioning as a novel adaptor linking TßRs to Ras activation in lipid rafts after TGFβ treatment (Jin et al, 2012b). Of particular interest with regard to km23-1's role as a Ras adaptor are recent reports of a structural homolog of km23-1 in bacteria (MglB) that interacts with a Ras-like small G protein (MglA) (Miertzschke et al, 2011; Leonardy et al, 2010; Zhang et al, 2010). km23-1 is actually part of an ancient superfamily that is widely represented in archaea and bacteria (Koonin & Aravind, 2000). This superfamily appears to be involved in regulating NTPase activity. By analogy to MglA/B, km23-1 would be expected to regulate the activity and biological functions of Ras family proteins. In contrast to km23-1, however, MglB functions as a GTPase-activating protein (GAP) that regulates MglA in bacteria (Zhang et al, 2010). Given the higher evolutionary level of km23-1, many of its functions would be expected to be far more complex than those of

its bacterial counterparts, presumably involving additional positive and negative regulatory factors. Thus, additional studies of the novel role of km23-1 in Ras/ERK signaling are warranted.

The Specific Aims are:
1. To determine whether km23-1's ability to regulate Ras and TßR signaling is dynein-dependent, as well as how these events differ between UECs and human CRC cells.
2. To determine how km23-1 regulation of Ras/MAPK scaffolding is altered in human CRC cells, as well as the mechanism by which km23-1 inhibition can reduce the malignant potential of Ras- and Raf-mutant human CRC cells in vitro and in vivo.

Progress made and methods used:
Specific Aim 1. We have used both immunofluorescence (IF) and biochemical methods (fast protein liquid chromatography [FPLC] and sucrose density gradient centrifugation) to address whether dynein was associated with the km23-1-regulated Ras/MAPK events in UECs and in KRAS- and BRAF-mutant human CRC cells. Of interest, these events appeared to be dynein motor protein complex-independent, in contrast to the TGFß/Smad events we have previously reported (ie, Jin et al, 2007; 2009). Further, since the RKO and HCT116 human CRC cells used are deficient in TGFß receptor RII, the regulation of Ras/MAPK signaling was ligand-independent, being uncoupled from the TßRII regulatory events we reported for UECs (Jin et al, 2012b).

Specific Aim 2. Part A -- While other scaffolds may be involved in km23-1 regulation of Ras/MAPK signaling events (ie, KSR1, MLK3), we have focused on the Ras/MAPK scaffold Ezrin as a key scaffold in transmitting signals from growth factors and the actin cytoskeleton to downstream effectors of Ras/MAPKs. It is also highly expressed in human CRC compared to normal cells (Nowak et al, 2010). While Ezrin is a scaffold that connects F-actin to focal adhesions, it is also an A-kinase anchoring protein (AKAP) that can target protein kinase A (PKA) type I to lipid rafts (Ruppelt et al, 2007; Neisch & Fehon, 2011). This Ezrin function is particularly relevant to our project, due to another recent report of ours describing the ability of protein kinase A (PKA) to regulate km23-1 (Jin et al, 2013a). We have used immunoprecipitation (IP)/blot and IF methods to demonstrate that while km23-1 does not directly form a complex with Ezrin in the cytosol, km23-1 KD can regulate Ezrin expression in invading human CRC cells. Further, another km23-1-interacting protein that we have identified (cofilin; Jin et al, 2012c) can regulate Ezrin's functional complex with its binding partner ERM binding phospho-protein 50 (EBP50), also called Na+/H+-exchanger 3 regulatory factor 1 (NHE1)], thereby potentially decreasing other Ezrin functions in human CRC progression. Thus, we have successfully examined the role of km23-1 in regulating this altered scaffolding in human CRC cells.

Part B -- We have shown that km23-1 KD diminished the malignant potential of KRAS- and BRAF-mutant human CRC cells in vitro and in vivo via several mechanisms. These include a reduction in constitutive ERK and Elk-1 activity, diminished production of TGFß1, decreased paracrine effects of CRC cell-secreted factors on fibroblast mitogenesis and migration, and attenuated CRC motility and invasion (Jin et al, 2013c). We had previously shown that an ERK/Elk-1/c-Fos pathway mediates TGFß1 production in human CRC cells, which can be attenuated by c-Fos KD, thereby reducing the in vivo growth of human CRC cells (Pandey et al,

2012). Our data from the current project, therefore, support and extend this previous work. They also demonstrate that km23-1 plays a key role in the progression and invasion of human CRC cells.

Detailed results of the project: Aim 1 was addressed by abstract 1 below, Aim 2A was addressed by abstract 2A below, and Aim 2B was addressed by abstract 2B below.

1. km23-1 regulation of MEK/ERK activation in human colorectal carcinoma (CRC) cells independent of the dynein complex (Jin, Q. & Mulder, K.M., 2014). We have previously shown that the dynein light chain (DLC) km23-1 is required for TGFβ1 auto-induction through Ras/ERK/Jun N-terminal kinase (JNK) pathways in TGFβ-sensitive epithelial cells (UECs). In contrast to the requirement for dynein motor activity in TGFβ-mediated Smad2 nuclear translocation by TGFß (Jin et al, 2007), activation of MEK and JNK by TGFß did not appear to be strictly dynein-dependent (Fig. 1). In addition, we show that depletion of km23-1 in human colorectal carcinoma (CRC) cells inhibited constitutive MEK activation (Fig. 2). Size exclusion chromatography experiments revealed that BRAF, ERK, and p-ERK were not recruited into the large dynein enzymatic complex, suggesting that the multi-subunit dynein complex might not be involved in constitutive ERK activation in RKO human CRC cells (Fig. 3). Western blot analyses from the sucrose density gradient fractionation of human CRC cell lysates demonstrated that km23-1 co-sedimented with Ras, p-ERK, and ERK in a lower density complex, compared to the higher density complex containing components of the multisubunit dynein complex (ie, DIC, $p150^{glued}$), but not km23-1 (Fig. 4). Our results demonstrate for the first time that km23-1's role in TGFß-mediated Ras/ERK signaling in untransformed epithelial cells (UECs), as well as in regulating constitutive MEK/ERK activation in KRAS- and BRAF-mutant human CRC cells, is independent of this DLC's role in the dynein transport complex.

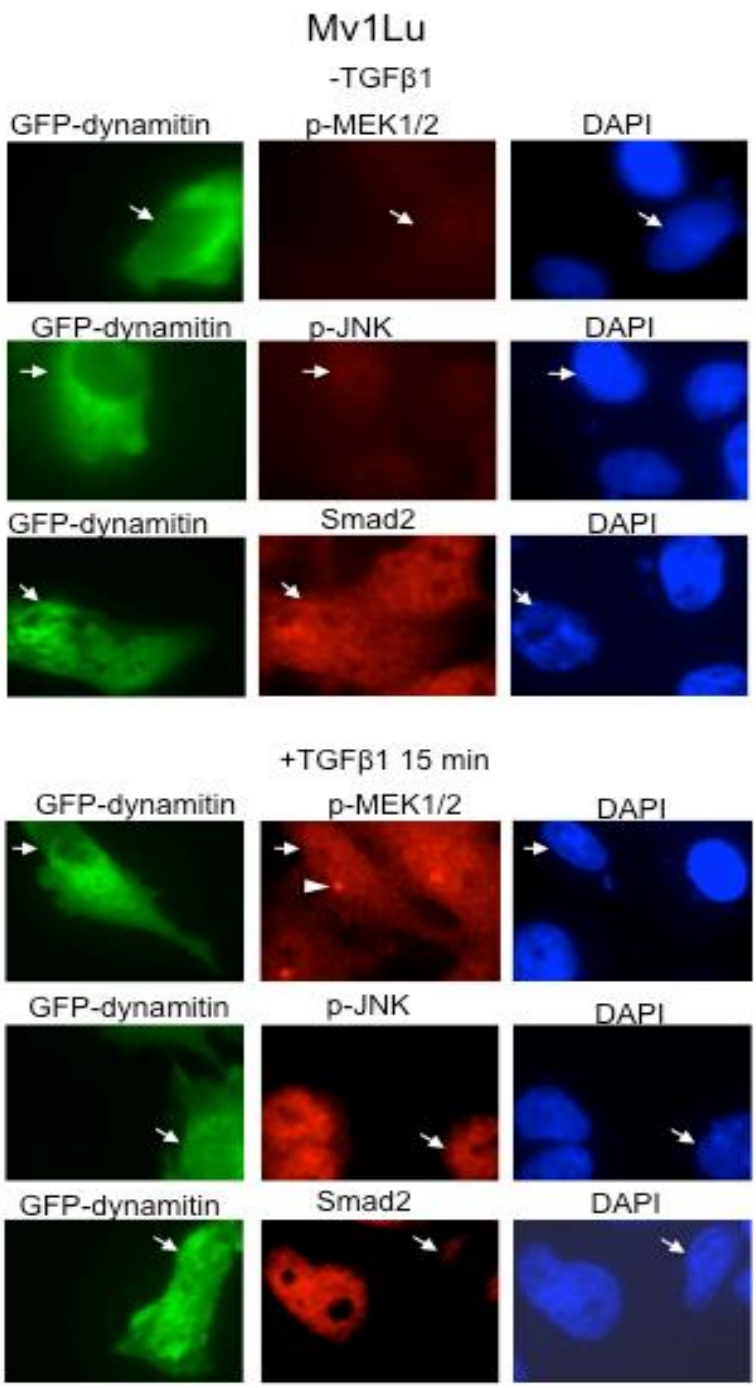

Fig. 1. The dynein motor is required for TGFβ-mediated Smad2 nuclear translocation by TGFß, but not for activation of MEK and JNK, in TGFß-sensitive UECs. Mv1Lu cells were transiently transfected with green fluorescent protein (GFP)-dynamitin to disrupt the dynein complex. 24 h after transfection, cells were incubated in serum-free medium before addition of TGFβ (5ng/ml) for 15 min. Cells were fixed, and endogenous p-MEK1/2, p-JNK, or Smad2 were detected using either a rabbit p-MEK1/2 antibody (Ab), rabbit p-JNK Ab, or a mouse Smad2 Ab, followed by secondary reagents Alexa 594 goat anti-rabbit IgG or Alexa 594 goat anti-rabbit IgG (*red*). *DAPI* staining permitted visualization of nuclei of individual cells (*blue*). GFP was used as a marker to designate cells transfected with dynamitin (*green*). The cells were analyzed with an Olympus IX81 microscope 1000X with appropriate filter sets. Arrows indicate that GFP-dynamitin positive cells. Arrowhead indicates puncta staining of p-MEK1/2 in TGFß treated cells.

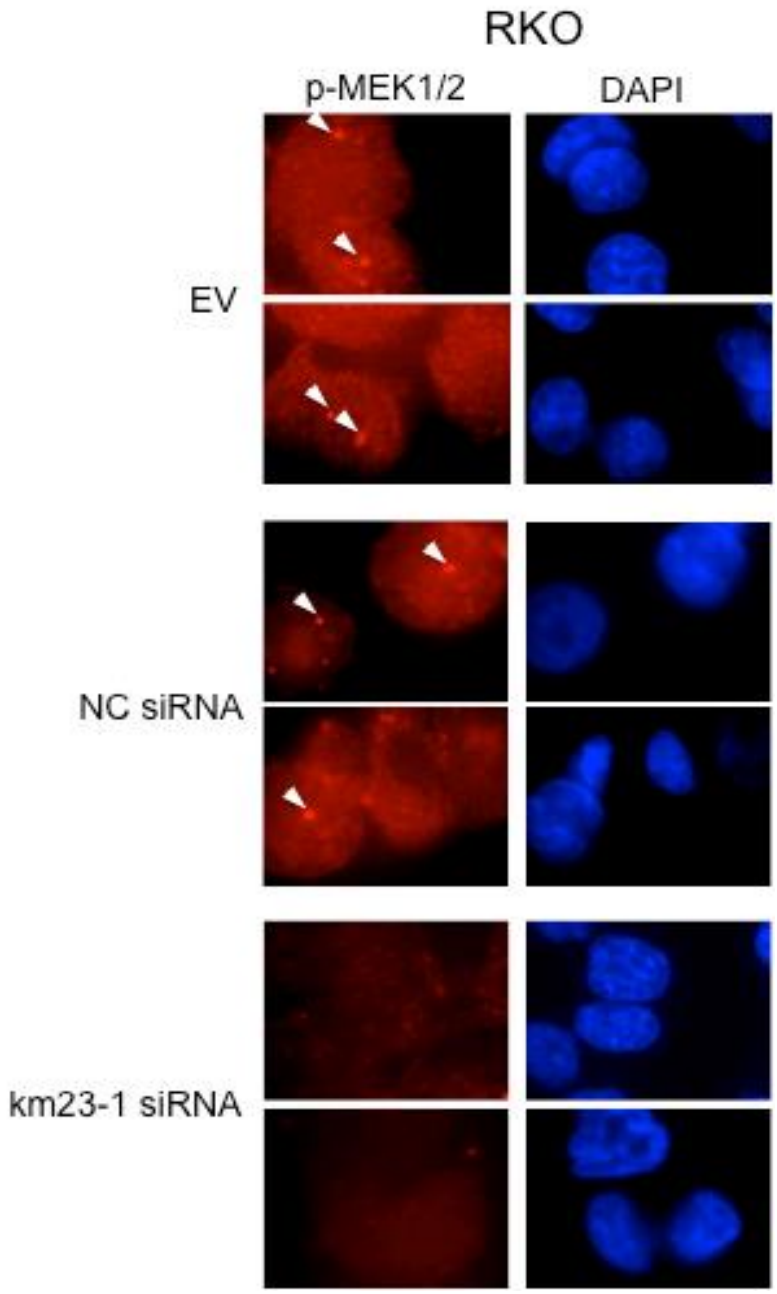

Fig. 2. Depletion of km23-1 decreases p-MEK1/2 in RKO human CRC cells. RKO cells stably expressing either empty vector (EV), non target control (NC siRNA), or km23-1 siRNA (km23-1 siRNA) were fixed, and endogenous p-MEK1/2 was detected using a rabbit p-MEK1/2 Ab and Alexa 594 goat anti-rabbit IgG (*red*). *DAPI* staining permitted visualization of nuclei of individual cells (*blue*). The cells were analyzed by an Olympus IX81 microscope at a magnification of 1000X with appropriate filter sets. Arrowheads designate punctate staining of p-MEK1/2 in control RKO cells but not in km23-1 siRNA cells.

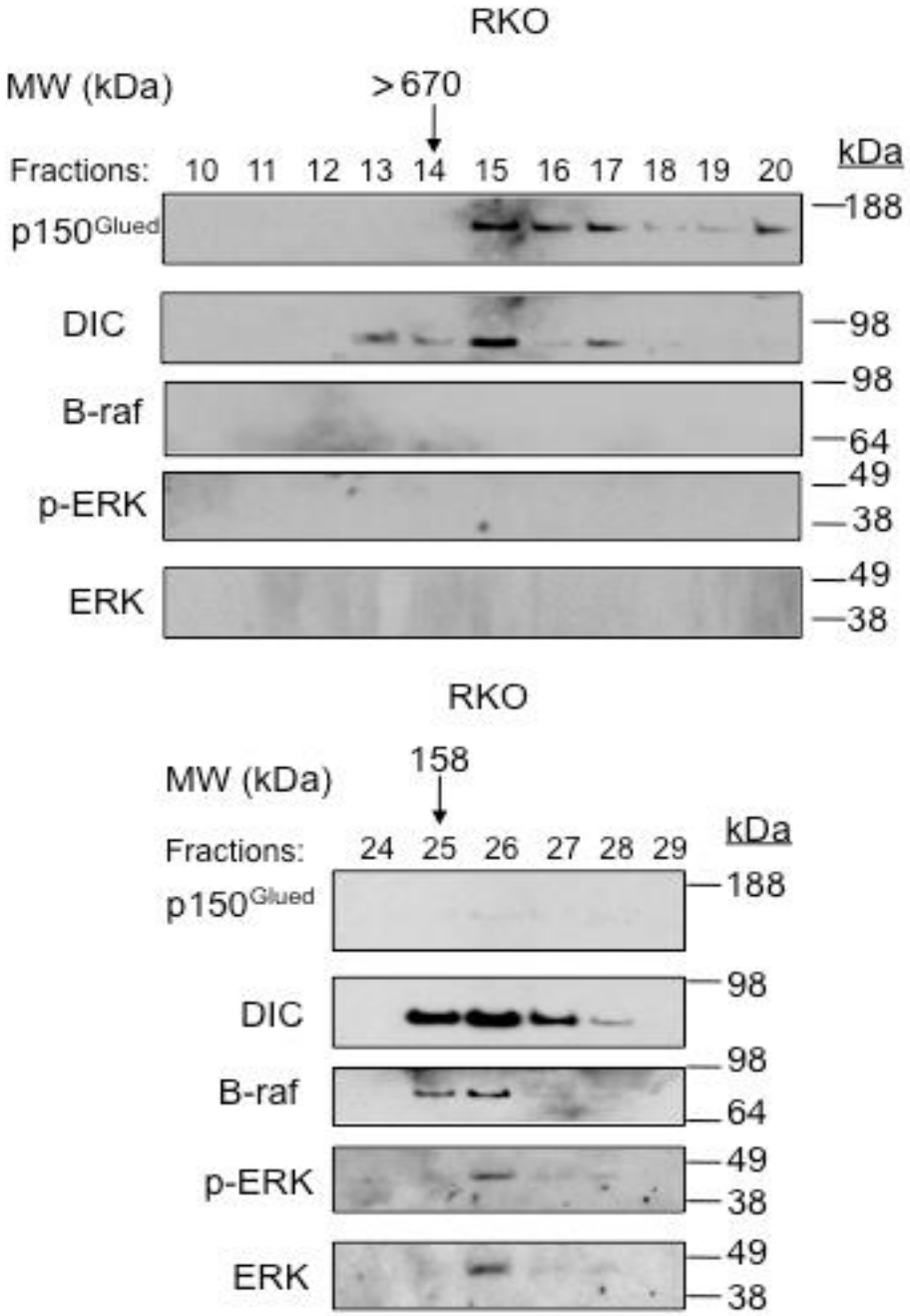

Fig. 3. BRAF and phospho-ERK did not co-fractionate with the high molecular weight (MW) dynein complex (fractions 13-17; top) in human CRC cells. RKO cells were washed and scraped into lysis buffer as described in Tang et al (*Mol Biol Cell* 13; 4484, 2002). After centrifugation at 100,000 x g for 10 min, clarified whole cell lysates of 500 µl (containing 1-2 mg of protein) were loaded onto a fast protein liquid chromatography (FPLC)/Superdex 200 10/300 GL column (Amersham Biosciences, Piscataway), equilibrated in lysis buffer without Triton x-100 and glycerol. Fractions were subjected to WB analyses with the indicated Ab's. As expected, free DIC that was not associated with the larger dynein complex, was also present in the smaller MW fractions (25-28).

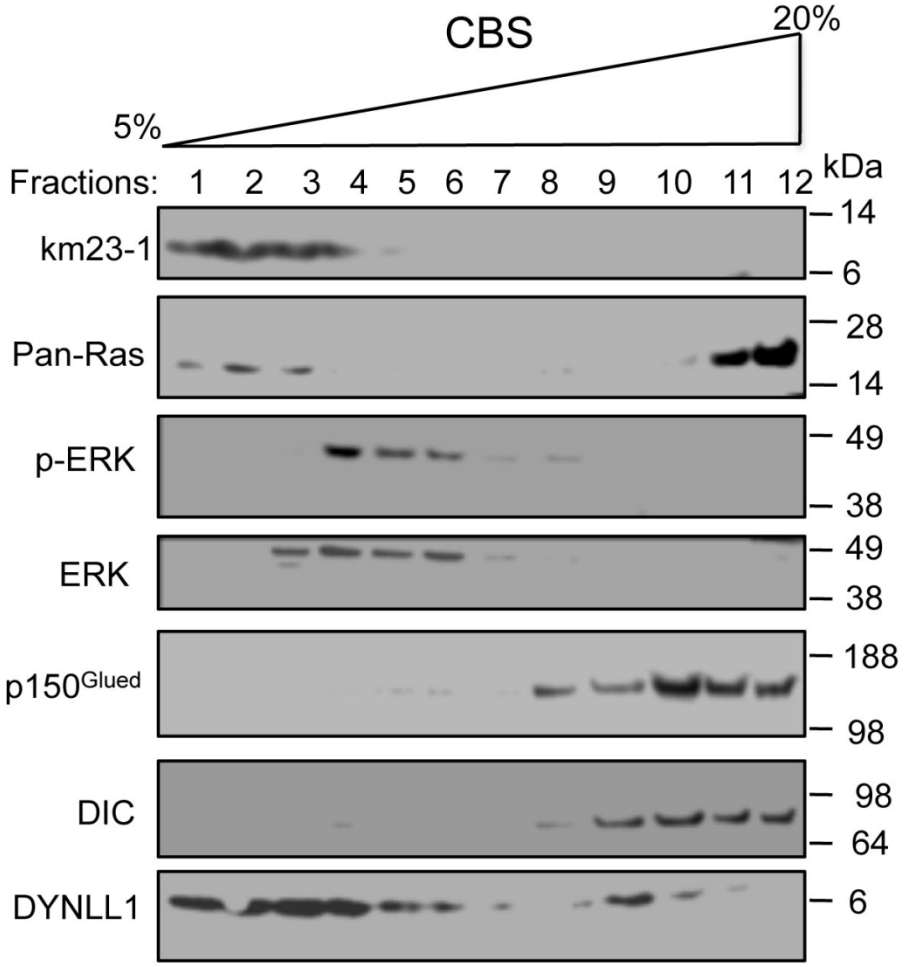

Fig. 4. km23-1 and phospho-ERK did not co-sediment with the p150Glued/DIC-containing dynein complex in CBS human CRC cells. Lysates of KRAS-mutant (12D) CBS human CRC cells were sedimented in a 5%–20% linear sucrose gradient. Each fraction was analyzed by WB analyses with the indicated Abs. Similar results were obtained with KRAS-mutant (13D) HCT116 CRC cells.

2A. Depletion of km23-1 is associated with reduced Ezrin scaffolding and actin-regulatory protein functions at the leading edge of invading human CRC cells (Jin et al, 2013c). Ezrin expression levels are elevated in human CRC cells, compared to normal tissues (Nowak et al, 2010). Further, previous work has shown that disruption of Ezrin or F-actin abolished growth factor activation of Ras by removing Ezrin binding sites for Ras and for the Ras guanine nucleotide exchange factor (GEF) Sos (Sperka et al, 2011). Therefore, formation of an Ezrin-dependent complex is necessary to stimulate Sos and Ras. In addition to Ezrin's role in facilitating signaling through several growth factors (GFs) and adhesion molecules (Li et al, 2008; Neisch & Fehon, 2011), it provides a critical scaffold linkage between the plasma membrane and the actin cytoskeleton, thereby enhancing cell motility, invasion, and metastasis in many human cancers, including human CRC cells (Hayashi et al, 2011; Meng et al, 2010). We have shown that km23-1KD can reduce Ezrin expression in invading human CRC cells, thereby controlling at least some of Ezrin's functions (Fig. 2A-1, top). We hypothesized that km23-1 KD will be able to block this scaffolded complex at the leading edge, thereby inhibiting cell motility, invasion, and metastasis. We hypothesize, further, that the mechanism involves a requirement for km23-1 in the recruitment of actin regulatory protein complexes, which affect Ezrin and its binding protein (EBP50), as well as Ras/ERK signaling. Thus, after km23-1 depletion, the actin regulatory proteins will not be recruited to the leading edge of human CRC cells during active cell invasion, which will, in turn, affect recruitment of Ezrin-EBP50 and formation of signaling complexes needed for motility and invasion. Our data in Fig. 2A-1 (bottom) are consistent with this hypothesis; analysis of Ezrin expression in invading HCT116 cells in a 3-dimensional Matrigel environment revealed reduced Ezrin staining in the invaded cells after km23-1KD**.** Thus, km23-1 is required for Ezrin localization in invading human CRC cells.

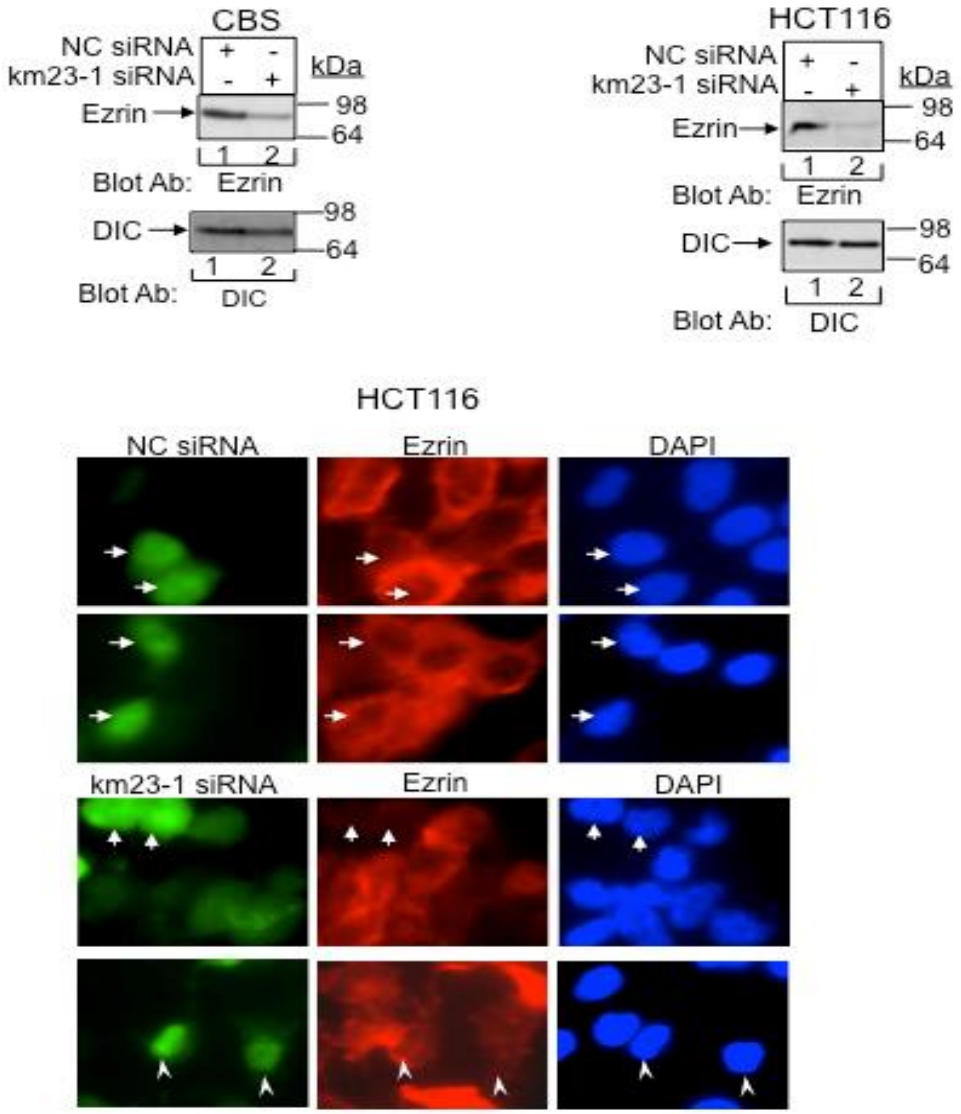

Fig. 2A-1. Depletion of km23-1 reduces Ezrin expression in invading human CRC cells. Top Left: Cell lysates from CBS cells deleted of km23-1 were subjected to Western blot analysis of Ezrin expression. Top Right: HCT116 cells were infected with either pilenti-NC siRNA-GFP or the pilenti-km23-1 siRNA-GFP set. 24 h after infection, Western blotting was performed using the indicated antibodies. Top panels confirm knockdown of endogenous Ezrin. Bottom panels show DIC protein expression as a loading control. Bottom: HCT116 stable pools that migrated through to the lower membrane in the Matrigel invasion assay were stained with an Ezrin Ab, followed by cy3-conjugated goat anti-rabbit IgG (red). DAPI staining permitted visualization of nuclei of individual cells (blue). GFP was used as a marker to designate cells transduced with siRNA (green). The cells were analyzed by an Olympus IX81 microscope at a magnification of 1000× with the appropriate filter sets. Arrowheads designate GFP expressing cells with reduced Ezrin expression.

2B. Decreased tumor progression and ERK activity by a novel anti-cell motility target for human colon carcinoma cells (Jin et al, 2013c).

We have previously described a novel modulator of the actin cytoskeleton that also regulates Ras and mitogen-activated protein kinase activities in TGFβ-sensitive epithelial cells. Here we examined the functional role of this signaling regulatory protein (km23-1) in mediating the migration, invasion, and tumor growth of human colorectal carcinoma (CRC) cells. We show that small interfering RNA (siRNA) depletion of km23-1 in human CRC cells inhibited constitutive extracellular signal-regulated kinase (ERK) activation (Fig. 2B-1), as well as pro-invasive ERK effector functions that include phosphorylation of Elk-1, constitutive regulation of c-Fos-DNA binding, TGFβ1 promoter transactivation, and TGFβ1 secretion (Fig. 2B-2). In addition, knockdown of km23-1 reduced the paracrine effects of CRC cell-secreted factors in conditioned medium and in fibroblast co-cultures (Fig. 2B-3). Moreover, km23-1 depletion in human CRC cells reduced cell migration and invasion (Fig. 2B-4), as well as expression of the ERK-regulated, metastasis-associated scaffold protein Ezrin (Fig. 2A-1 above). Finally, km23-1 inhibition significantly suppressed tumor formation in vivo (Fig. 2B-5). Thus, our results implicate km23-1 as a novel anti-metastasis target for human colon carcinoma cells, capable of decreasing tumor growth and invasion via a mechanism involving suppression of various pro-migratory features of CRC. These include a reduction in ERK signaling, diminished TGFβ1 production, decreased expression of the plasma membrane-cytoskeletal linker Ezrin, as well as attenuation of the paracrine effects of colon carcinoma-secreted factors on fibroblast migration and mitogenesis. As such, km23-1 inhibitors may represent a viable therapeutic strategy for interfering with colon cancer progression and invasion.

Fig. 2B- 1

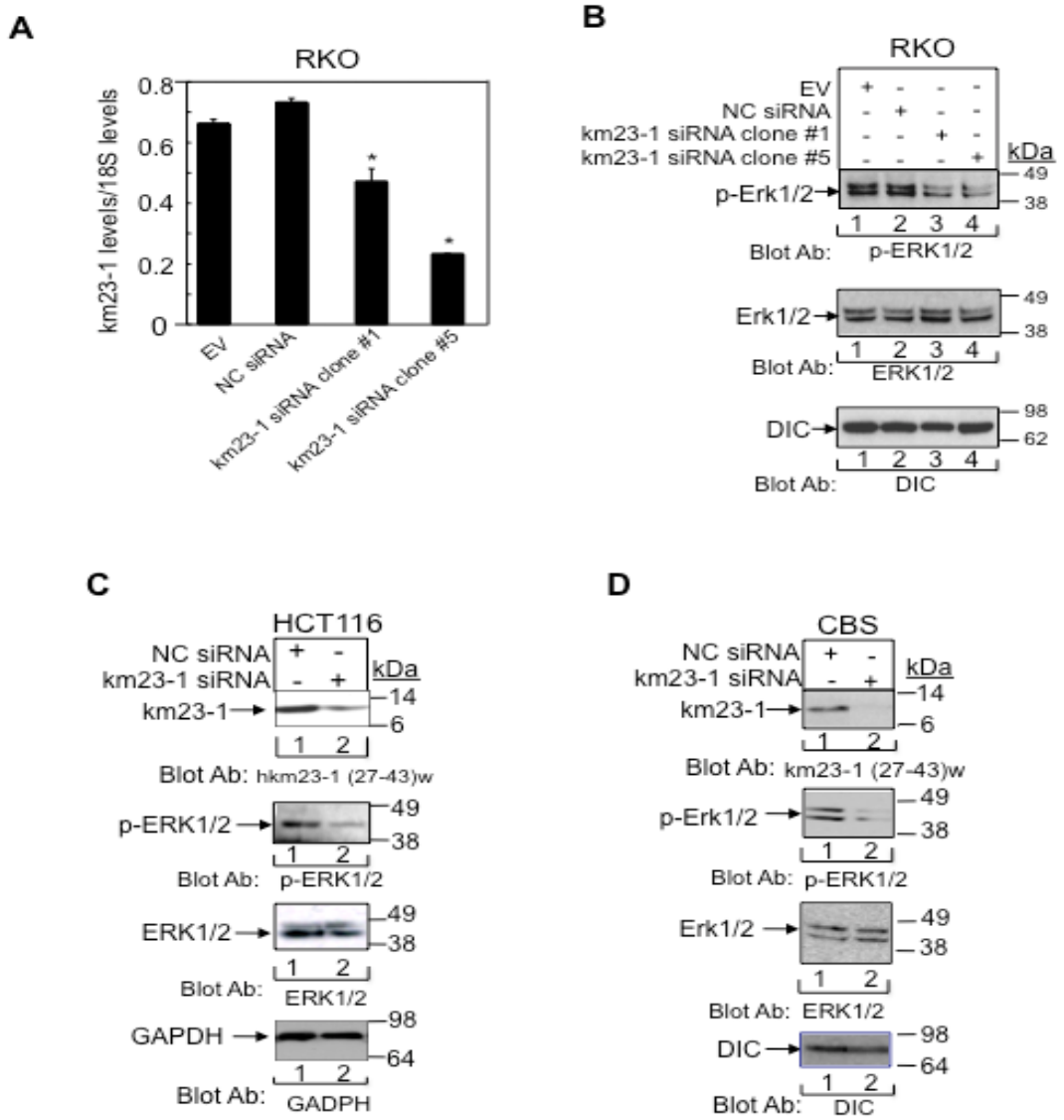

Fig. 2B-1. Depletion of km23-1 blocks constitutive ERK activation in human CRC cells. A. EV, NC siRNA, and km23-1 siRNA stable transfectants (clones 1 and 5) were used to isolate RNA. RT-PCR was performed and products were analyzed. The data plotted are the mean ± SE of three independent experiments. *$p<0.01$ compared to the NC siRNA. B: Western blotting of phospho- and total protein expression levels for ERK1/2 in RKO human CRC cells. Bottom, DIC protein was assessed as a loading control. Data are representative of three independent experiments. C: Western blotting of phospho- and total protein expression levels for ERK1/2 in HCT116 cells stably transduced with lentiviral particles. Top, confirms knockdown of endogenous km23-1. Bottom, GAPDH protein was assessed as a loading control. Data are representative of three independent experiments. D: CBS cells were infected with either pilenti-NC siRNA-GFP or pilenti-km23-1 siRNA-GFP pools. 24 h after infection, Western blotting was performed using the indicated antibodies. Top, confirms knockdown of endogenous km23-1. Bottom, DIC protein was assessed as a loading control. Three independent experiments were performed and representative figures are shown.

Fig. 2B-2

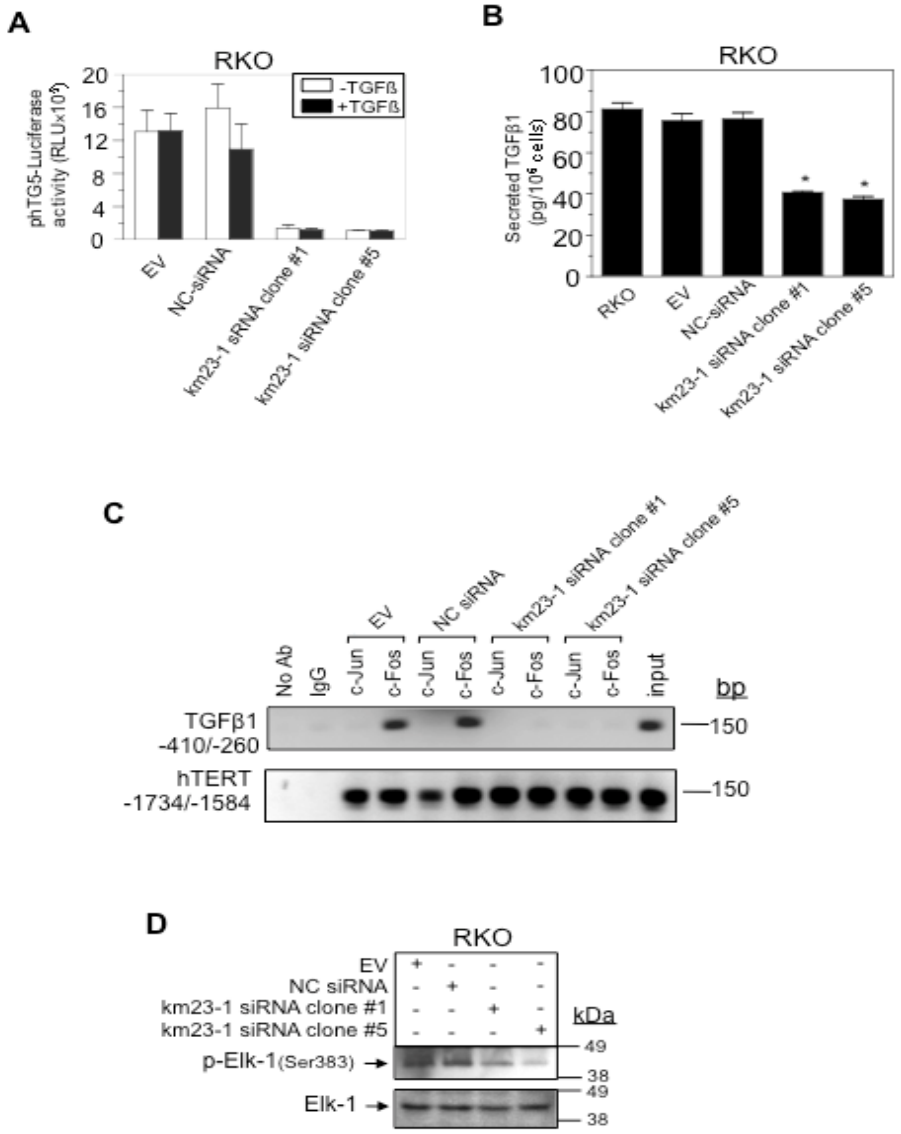

Fig. 2B-2. Depletion of km23-1 in RKO human CRC cells inhibits transactivation of the proximal AP-1 site in the TGFβ1 promoter, TGFβ1 secretion, c- Fos-DNA binding to the TGFß1 promoter, and Elk-1 activation.

A: RKO stable transfectants were transiently transfected with phTG5-Lux and luciferase assays were performed. Data are representative of three independent experiments. **B:** TGFβ1 concentrations in CM from RKO stable transfectants were measured by ELISAs. *$p<0.01$ compared to the NC siRNA. **C:** ChIP assays were performed using either the human TGFβ1 promoter region from −410 to −260 (upper panel) or the hTERT promoter region from −1734 to −1584 (lower panel) with the indicated antibodies. Input: Equal samples of chromatin DNA without prior immunoprecipitation. **D:** Western blotting of phospho- and total protein expression levels for Elk-1 in RKO human CRC cells. Three independent experiments were performed and representative figures are shown.

Fig. 2B-3

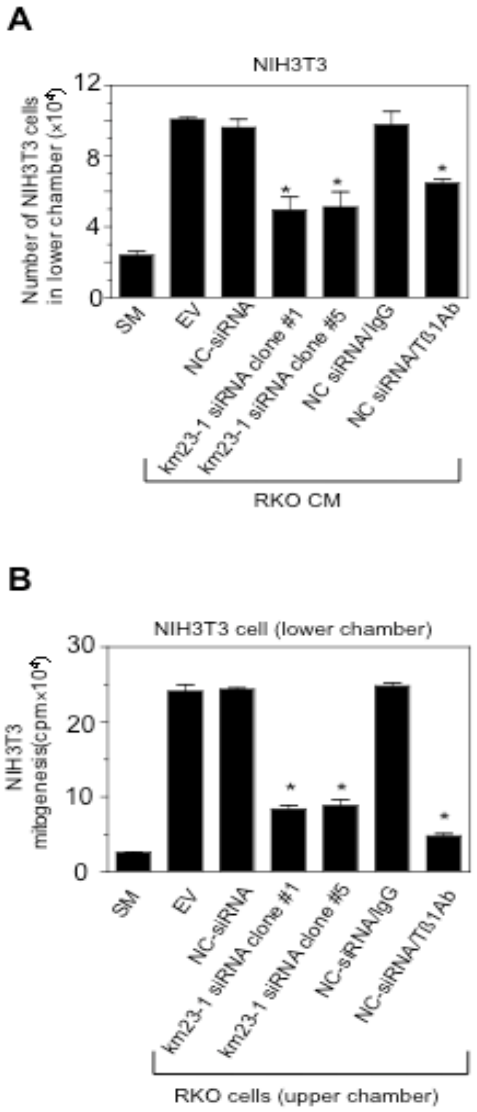

Fig. 2B-3. km23-1 regulates the paracrine effects of active TGFβ1 on NIH3T3 cell migration and mitogenesis.

A: NIH3T3 fibroblasts were plated onto polycarbonate membrane filter inserts (8.0 µm pore size) in 6-well Transwells and CM from RKO cells and their siRNA stable transfectants were added to the NIH3T3 cells. Cells migrating into the lower chambers were counted. SM: supplemented McCoy's (SM) medium; EV: CM from RKO-EV cells; NC siRNA: CM from NC siRNA-RKO cells; km23-1-siRNA clone #1: CM from km23-1-siRNA-RKO clone #1; km23-1-siRNA clone #5: CM from km23-1-siRNA-RKO clone #5; NC siRNA/Tβ1Ab: CM from NC-siRNA cells that had been incubated with a neutralizing anti-TGFβ1 antibody (30 µg/ml); Ctrl/IgG: CM from Ctrl with IgG. Data plotted are the mean ±SE of triplicate wells from a representative experiment (n = 3). *$p<0.01$ compared to either NC-siRNA or NC-siRNA/IgG. **B:** NIH3T3 cells were plated in the lower chamber and made quiescent. RKO cells and their siRNA stable transfectants were then plated onto polyester membrane filter inserts (0.4 µm pore size) in 12-well Transwells. Mitogenesis assays were performed to assess the effect on NIH3T3 cell mitogenesis after co-culture with RKO stable transfectants. Data plotted are the mean ±SE of triplicate wells from a representative experiment (n = 3). *$p<0.01$ compared to the NC-siRNA or NC-siRNA/IgG.

Fig. 2B-4

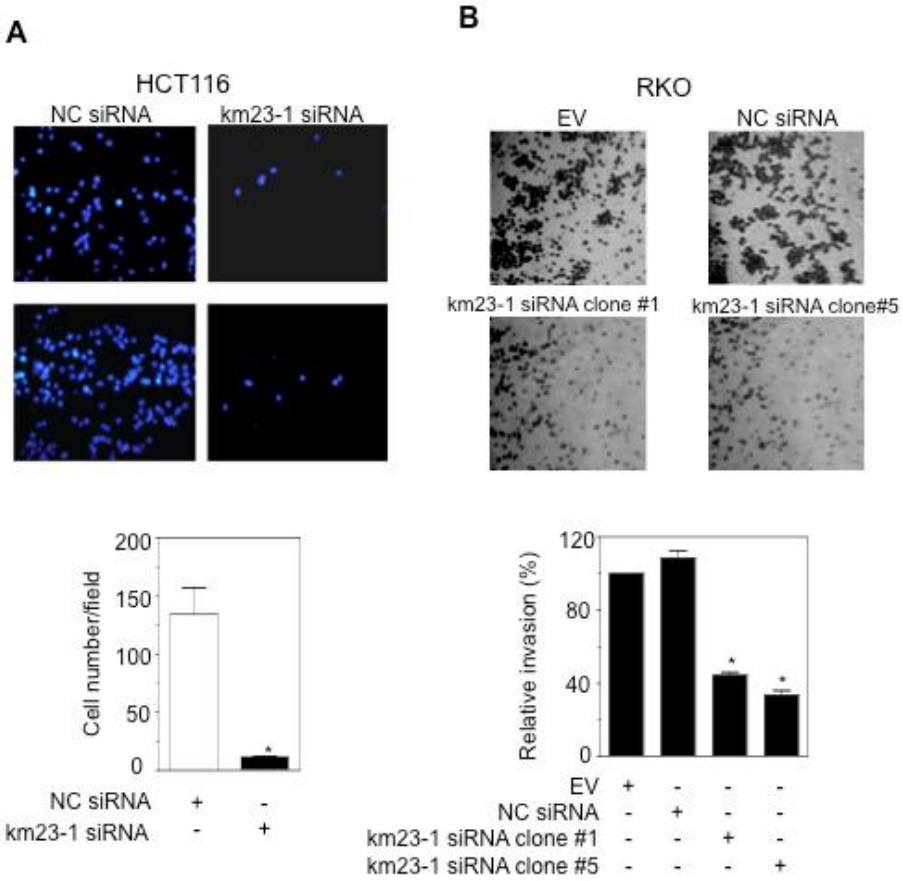

Fig. 2B-4. Depletion of km23-1 inhibits cell migration and invasion of human CRC cells. **A:** Transwell migration assays were performed. Briefly, HCT116 cells stably transduced with lentiviral vectors expressing either piLenti-NC siRNA-GFP or piLenti-km23-1 siRNA-GFP were seeded into the upper wells of the Costar Transwell System (8-µm pore size polycarbonate membrane, 6.5-mm diameter), and the cells on the lower surface of the well after 24 h were fixed in methanol and stained with DAPI. Top, representative images of the lower surface of the membrane are shown (100× magnification). Bottom, the number of migrating cells of both NC siRNA- and km23-1-siRNA-HCT116 stable transfectants were counted under a fluorescence microscope and statistically analyzed. *$p<0.01$ compared to NC siRNA. **B:** Matrigel invasion assays were performed with the indicated RKO stable cells clones (clones #1, 5) for 24 h using EGF (20 ng/ml) as the stimulus. Invaded cells were stained with 0.2% crystal violet. Top, representative images of the lower membrane surface from Matrigel are shown (100× magnification). Bottom, the number of invading cells for both NC siRNA and km23-1-siRNA HCT116 stable transfectants were counted under a light microscope and statistically analyzed. *$p<0.01$ compared to EV.

Fig. 2B-5

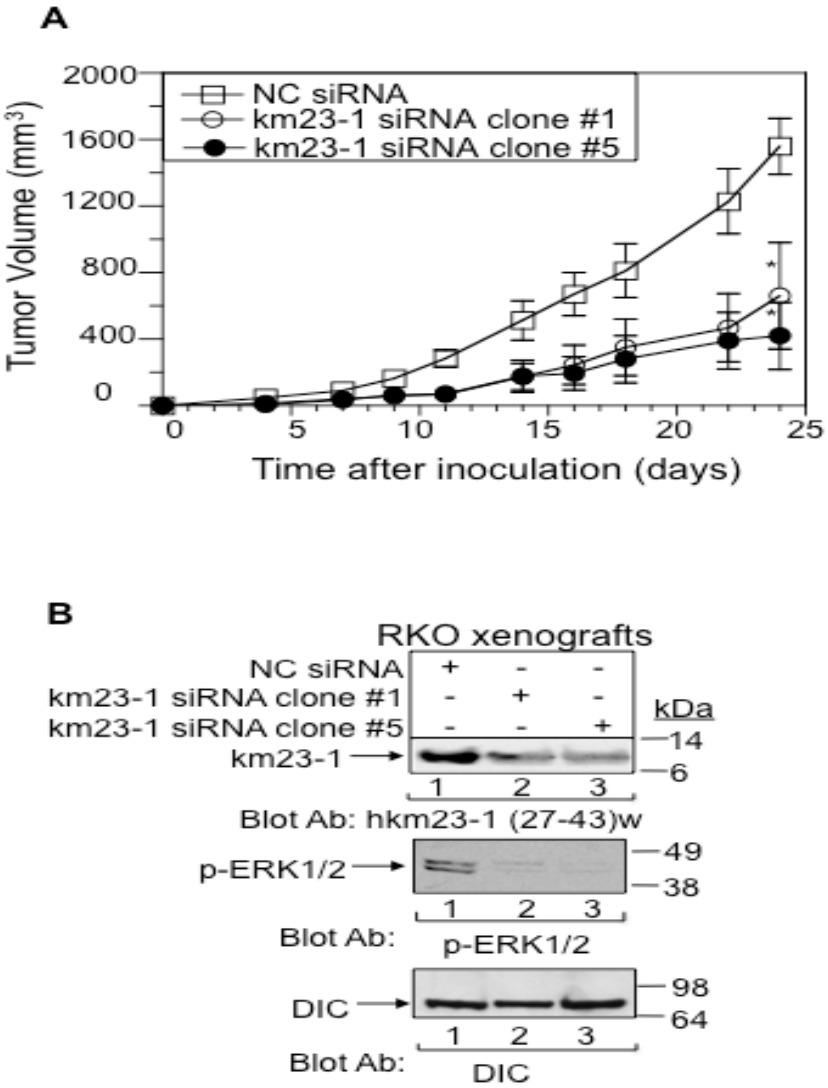

Fig. 2B-5. km23-1-siRNA blocks tumor growth of RKO cells *in vivo*.
A: NC-siRNA RKO and km23-1siRNA RKO clone #1 and #5 cells (5×106) were inoculated subcutaneously behind the right anterior forelimb of nude mice (n = 5). Tumor volumes were calculated as (length×width2)/2. *p<0.05 compared to NC, ANOVA. **B:** At the termination of the study, ERK activation and km23-1 knockdown in RKO xenografts were confirmed by Western blotting using the indicated antibodies. DIC, loading control.

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Jin Q, Gao G and Mulder KM. Requirement of a dynein light chain in TGFbeta/Smad3 signaling. *J Cell Physiol* 221: 707-715, 2009.

Jin Q, Gao G and Mulder KM. Requirement of a dynein light chain in transforming growth factor β signaling in zebrafish ovarian follicle cells. *Mol Cell Endocrinol* 348: 233-240, 2012a.

Jin, Q., Zhong, Y. and **Mulder, K. M.** Requirement for protein kinase A in the phosphorylation of the TGFβ receptor-interacting protein km23-1 as a component of TGFβ downstream effects. Exp Cell Res. 319: 897-907, 2013a.

Jin Q, Gao, G., and **Mulder, K. M**. A dynein motor attachment complex regulates TGFß/Smad3 signaling. Int J Biol Sci. 9 (6): 531-540, 2013b.

Jin, Q., Liu, G., Domeier, P. P., Ding, W., and **Mulder, K. M**. Decreased tumor progression and invasion by a novel anti-cell motility target for human colorectal carcinoma cells. Plos one 8(6): e66439, 2013c.

Jin, Q. and **Mulder, K.M.** Dynein-independent role of km23-1 in the regulation of ERK signaling in KRAS- and BRAF-mutant human CRC cells. In Prep, Int J Oncol, 2014.

Jin Q, Pulipati NR, Zhou W, Staub CM, Liotta LA and Mulder KM. Role of km23-1 in RhoA/actin-based cell migration. *Biochem Biophys Res Commun* 428: 333-338, 2012c.

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Yue J and Mulder KM. Requirement of Ras/MAPK pathway activation by transforming growth factor beta for transforming growth factor beta 1 production in a Smad-dependent pathway. *J Biol Chem* 275: 30765-30773, 2000.

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Zhang Y, Franco M, Ducret A and Mignot T. A bacterial Ras-like small GTP-binding protein and its cognate GAP establish a dynamic spatial polarity axis to control directed motility. *PLoS Biol* 8: e1000430, 2010.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
___X__ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?

_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication, listed in the table, in a PDF version 5.0.5 format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:
Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer
If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. Decreased tumor progression and ERK activity by a novel anti-cell motility target for human colon carcinoma cells	Jin, Q., Liu, G., Domeier, P. P., Ding, W., and **Mulder, K.M.**	PLOS One	March 2013	Submitted Accepted X Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes____x_____ No_____

If yes, please describe your plans:

We plan to submit publications pertaining to the research described in section 17. For example, one manuscript in preparation is:

Jin, Q. and **Mulder, K.M.** Dynein-independent role of km23-1 in the regulation of ERK signaling in KRAS- and BRAF-mutant human CRC cells. In Prep, Int J Oncol, 2014.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

This research project will have significant impact on the understanding of specific events that drive the growth and invasion of human colon cancer. It will also assist with the future development of screens to identify km23-1 inhibitors for the treatment of colon cancer.

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

The major discoveries attributable to this completed research project are our findings that small interfering RNA (siRNA) depletion of km23-1 in human CRC cells inhibited constitutive extracellular signal-regulated kinase (ERK) activation, as well as pro-invasive ERK effector functions that include phosphorylation of Elk-1, constitutive regulation of c-Fos-DNA binding, TGFβ1 promoter transactivation, and TGFβ1 secretion. In addition, knockdown of km23-1 reduced the paracrine effects of CRC cell-secreted factors in conditioned medium and in fibroblast co-cultures. Moreover, km23-1 depletion in human CRC cells reduced cell migration and invasion, as well as expression of the ERK-regulated, metastasis-associated scaffold protein Ezrin. Finally, km23-1 inhibition significantly suppressed tumor formation in vivo. Thus, our results implicate km23-1 as a novel anti-metastasis target for human colon carcinoma cells, capable of decreasing tumor growth and invasion via a mechanism involving suppression of various pro-migratory features of CRC. These include a reduction in ERK signaling, diminished TGFβ1 production, decreased expression of the plasma membrane-cytoskeletal linker Ezrin, as well as attenuation of the paracrine effects of colon carcinoma-secreted factors on fibroblast migration and

mitogenesis. As such, km23-1 inhibitors may represent a viable therapeutic strategy for interfering with colon cancer progression and invasion.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.

NAME Mulder, Kathleen M.	POSITION TITLE Professor
eRA COMMONS NAME kmulder	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Muhlenberg College, Allentown, PA	B.S.	1979	Nat. Sciences/Math
State University of NY at Buffalo, NY	Ph.D.	1985	Pharmacology
Baylor College of Medicine, Houston, TX	Postdoc	1988	Molecular & Cell Biol; Cancer Biology

A. Personal Statement

We have been performing cancer research investigating altered mechanisms of TGF-beta production and signaling responsiveness in human cancer cells for 25 years. We have also identified the TGFβ receptor-interacting protein km23-1, which plays an important role in TGFβ signaling and in tumor growth and invasion of human carcinoma cells. As a critical scaffold, it couples TGFß receptor activation to stimulation of Ras and Rho family effector pathways. In human cancer cells, it regulates RhoA and actin effector pathways important for cell motility and invasion. Additional research involves addressing how these events are regulated by km23-1 in 3-dimensional and organotypic invasion models of human cancers, as well as how km23-1 can be targeted therapeutically to develop anti-metastatic agents. We have considerable expertise in the regulation of cell signaling and compartmentalization of signaling complexes. Since we have found that km23-1 functions in the assembly of critical scaffolded signaling complexes, it appears to be a key player in many important cellular processes that are deregulated in cancer. The overall goal of the Mulder Laboratory is the identification of novel signal transduction-based therapeutics for human cancers. Our research uses a multidisciplinary approach that spans cancer biology, cell biology, biochemistry, molecular biology, and genetics.

B. Positions and Honors

Positions and Employment

1980-1984 Graduate Research Assistant, State University of NY at Buffalo (SUNY), School of Medicine & Biomedical Sciences, Buffalo, NY

1985-1988 Research Associate, Dept. of Pharmacology, Baylor College of Medicine, Houston, TX

1988-1991 Assistant Professor (tenure-track), Dept. of Pharmacology, Baylor College of Medicine, Houston, TX

1989-1990 Consultant, Bristol-Myers Squibb, Wallingford, CT

1991-1993 Assistant Professor, Dept. Pharmacology, Penn State Univ., College of Medicine, Hershey, PA

1993-1998 Tenured Associate Professor, Dept. Pharmacology, Penn State College of Med, Hershey, PA

1998-2007	Professor, Dept. of Pharmacology, Penn State University College of Medicine, Hershey, PA
2000- pres	Member, Penn State Cancer Institute, Hershey Medical Center, Hershey, PA
2001- pres	Member, Experimental Therapeutics Program, Penn State Hershey Cancer Institute (PSHCI)
2005- pres	Member, Gastroenterology Disease Team, PSHCI, Hershey, PA
2007- pres	Professor, Dept. of Biochemistry & Molecular Biology, Penn State Hershey College of Medicine, Hershey, PA

Honors

1980-1983	NIH Predoctoral Training Grant recipient
1984	First Prize – Sigma XI Research Award Competition
1989-1994	NIH PHS First Independent Research Support and Transition Award: 5.2 percentile
1993-1998	NIH Research Career Development Award (NCI)

Peer Review

NCDDG Grant Review – 10/89; NC Biotech Center – 4/96;
NIH Program Project Site Visits-6/90, 3/94, 9/95, 9/03;
USAMRMC Breast Cancer Reviews – 11/95-8/99
Reviewer, National Science Foundation – 9/97
NIH R13 Grant Review – 12/90, 12/96
Reviewer, NCI Phased Application Awards, SBIR-STTR VA Merit Review Application – 3/92, 9/92, 8/95
Cancer Prognostics & Prediction, 10/02
Institutional Res. Grant Reviews – 3/93-pres.
Natl. Cancer Inst. of Canada Program Project Site Visit
Israel Science Foundation – 5/93-10/93
Ad Hoc, MEP Study Section, NIH – 6/96-3/97
Ad Hoc Reviewer, TCB Study Section, NIH, 2/04
Regular Member, MEP NIH Study Section– 6/97-7/01
Reviewer, MONC, NIH ZRG1, CSR, 6/05
ACS, Local Chapter – 10/94-9/97
Cancer Res UK, Ext Peer Rev CSFR03, London -- 2/09
NIH R03/RFA SS – 3/95
Editorial Board Member, *J. Biol. Chem*. – 2001-2007
Active Mem, SBIR/STTR Special SSS2-11/94-11/96
NCI-Nature Pathway Interaction Database Reviewer, Nature, Cambridge, MA, 2007
NIH Challenge Grants, CAMP (ONC1) – 6/09;
Invited Reviewer, RGC General Research Fund, CUHK external review, Dept. of Anatomical & Cellular Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong – 8/10-10/10
NIH Study Section, Drug Discovery & Pharmacology (DMP) – 2/11
Invited Reviewer, NIH Special Emphasis Panels: ZRG1-Oncology, Basic, Translational (OBT): 8/12; 4/13
Cancer Research UK, Research grant reviewer – 9/12

NIH Tumor Cell Biology Study Section, Oncology 1-Basic Translational Integrated Review Group, 8/13-11/13
NIH 2014/05 ZRG1 BMCT-C Special Emphasis Panel, 1-2/14

C. Selected Peer-Reviewed Publications (in chronological order)

1. Yue J, **Mulder KM**. Requirement of Ras/MAPK pathway activation by TGFβ for $TGF\beta_1$ production in a Smad-dependent pathway. J Biol Chem 275: 30765-73, 2000.
2. Tang Q, Staub CM, Gao G, Jin Q, Wang Z, Ding W, Aurigemma RE, **Mulder KM**. A novel TGFβ receptor-interacting protein that is also a light chain of the motor protein dynein. Mol Biol Cell 13: 4484-96, 2002. PMCID: PMC138648
3. Jin Q, Ding W, Staub CM, Gao G, Tang Q, **Mulder KM**. Requirement of km23 for TGFβ-mediated growth inhibition and induction of fibronectin expression. Cell Signal 17(11): 1363-72, 2005.
4. Liu G, Ding W, Liu X, **Mulder KM**. c-Fos is required for TGFβ1 production and the associated paracrine migratory effects of human colon carcinoma cells. Mol Carcinogen 45(8): 582-93, 2006.
5. Liu G, Ding W, Neiman J, **Mulder KM**. Requirement of Smad3 and CREB-1 in mediating transforming growth factor-beta (TGFbeta) induction of TGFbeta3 secretion. J Biol Chem 281(40): 29479-90, 2006.
6. Jin Q, Ding W, **Mulder KM**. Requirement for the dynein light chain km23-1 in a Smad2-dependent TGFβ signaling pathway. J Biol Chem 282(26):19122-32, 2007.
7. Jin, Q., Gao, G., and **Mulder, K. M.** Requirement of a dynein light chain in TGFβ/Smad3 signaling. J. Cell. Physiol. 221: 707–715, 2009. PMCID: PMC2881827.
8. Pulipati, N.R., Jin, Q., Liu, X., Sun, B., Zhao, Y., Pandey, M. K., Huber, J., Ding, W., **Mulder, K.M.** Overexpression of the dynein light chain km23-1 in human ovarian carcinoma cells inhibits tumor formation in vivo and causes mitotic delay at prometaphase/metaphase. Int J Cancer, 129(3):553-64, 2011. PMCID: PMC3103626.
9. Pandey, M.K., Liu, G., Cooper, T.K., and **Mulder, K.M.** Inhibition of c-Fos suppresses the growth of human colon carcinoma cells in athymic mice. Int J Cancer 130: 213-222, 2012.
10. Jin, Q, Gao, G, and **Mulder, K.M**. Requirement of a dynein light chain in transforming growth factor β signaling in zebrafish ovarian follicle cells. Mol Cell Endocrinol 348: 233-240, 2012.
11. Jin, Q., Ding, W., and **Mulder, K.M.** The TGFß receptor interacting protein km23-1/DYNLRB1 plays an adaptor role in TGFβ1 autoinduction via its association with Ras. J Biol Chem 287(31): 26453-63, 2012. PMCID: PMC3406728
12. Jin, Q., Pulipati, N. R. Zhou, W. Staub, C. M. Liotta, L. A. and **Mulder, K. M**. Role of km23-1 in RhoA/actin-based cell migration. Biochem Biophys Res Commun 428(3): 333-338, 2012.
13. Jin, Q., Zhong, Y. and **Mulder, K. M.** Requirement for protein kinase A in the phosphorylation of the TGFβ receptor-interacting protein km23-1 as a component of TGFβ downstream effects. Exp Cell Res. 319: 897-907, 2013.
14. Jin Q, Gao, G., and **Mulder, K. M**. A dynein motor attachment complex regulates TGFß/Smad3 signaling. Int J Biol Sci. 9 (6): 531-540, 2013.
15. Jin, Q., Liu, G., Domeier, P. P., Ding, W., and **Mulder, K. M**. Decreased tumor progression and invasion by a novel anti-cell motility target for human colorectal carcinoma cells. Plos one 8(6): e66439, 2013.

BIOGRAPHICAL SKETCH

NAME Qunyan Jin	POSITION TITLE Research Associate
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Shanghai Second Medical University	MD	08/1998	Experimental Medicine
Penn State College of Medicine (COM)	Postdoc	09/2001	TGFβ signaling
Penn State Hershey Cancer Institute/COM	Research Associate	06/2003	Cancer biology

A. Personal Statement

The goal of the proposed research is to investigate a novel anti-cell motility target for human pancreatic cancer cells. Specifically, I will focus on the mechanism of km23-1 regulation of cell migration and invasion in human pancreatic cancer. I have a broad background in cell biology, molecular biology, signaling pathways, motor protein, and cancer biology fields with specific training and expertise in key research areas for this application. As a postdoctoral fellow at Penn State College of Medicine, I discovered for the first time that siRNA blockade of km23-1 decreased cellular responses to TGFβ, including induction of fibronectin expression. Also, I found that forced expressed km23-1 induced JNK activation and c-Jun phosphorylation, which are downstream of Ras. Recently, my work has demonstrated that knockdown (KD) of km23-1 inhibits TGFβ-mediated activation of Ras, extracellular signal-regulated kinase (ERK), Jun N-terminal kinase (JNK), phosphorylation of c-Jun, and transactivation of the c-Jun promoter. More importantly, my findings demonstrate that km23-1 functions as a critical adaptor coupling TGFβ receptor activation to activation of Ras effector pathways downstream. In addition, my recent findings suggest that km23-1 plays an important role in RhoA/actin-based cell migration. Now, I am working on the effect of km23-1 KD on RhoA activation, cell migration, and invasion in human pancreatic cancer cells. In summary, I have a demonstrated record of successful and productive research in an area of high relevance. My experience has prepared me to be a valuable part of the proposed research project.

B. Positions and Honors

Positions and Employment

07/1998 - 01/2001 Assistant Researcher, Shanghai Institute of Endocrinology, Shanghai, P. R. China
09/2001 - 05/2003 Post-doctoral Fellow, Penn State College of Medicine, Hershey, PA 17033
06/2003 – present Research Associate, Penn State College of Medicine, Hershey, PA 17033

Other Experience and Professional Memberships

2003 -- Member, American Association for Cancer Research

2001 -- Member, American Society of Cell Biology

C. Selected Peer-reviewed Publications

1. **Jin, Q**., Liu, G., Domeier, P. P., Ding, W., and Mulder, K. M. (2013c). Decreased tumor progression and invasion by a novel anti-cell motility target for human colorectal carcinoma cells. Plos one 8(6): e66439.

2. **Jin Q**, Gao, G., and Mulder, K. M. (2013b). A dynein motor attachment complex regulates TGFß/Smad3 signaling. Int J Biol Sci. 9 (6): 531-540.

3. **Jin, Q.,** Zhong, Y. and Mulder, K. M. (2013a). Requirement for protein kinase A in the phosphorylation of the TGFβ receptor-interacting protein km23-1 as a component of TGFβ downstream effects. Exp Cell Res. 319: 897-907.

4. **Jin, Q.,** Pulipati, N. R. Zhou, W. Staub, C. M. Liotta, L. A. and Mulder, K. M. (2012c). Role of km23-1 in RhoA/actin-based cell migration. Biochem Biophys Res Commun 428: 333-338. PMCID: PMC3513371.

5. **Jin, Q.,** Ding, W., and Mulder, K. M. (2012b). The TGFβ receptor-interacting protein km23-1/DYNLRB1 plays an adaptor role in TGFβ1 autoinduction via its association with Ras. J Biol Chem. 287,26453-63. PMCID: PMC3406728

6. **Jin, Q.,** Gao, G., Mulder, K.M. (2012a). Requirement of a dynein light chain in transforming growth factor β signaling in zebrafish ovarian follicle cells. Mol Cell Endocrinol. 348, 233-240. PMCID: PMC3205241.

7. Pulipati, NR, **Jin, Q.,** Liu X, Sun, B., Pandey, M.K., Huber, J.P., Ding, W., Mulder, K, M. (2011). Overexpression of the dynein light chain km23-1 in human ovarian carcinoma cells inhibits tumor formation in vivo and causes mitotic delay at prometaphase/metaphase. Int J Cancer.129, 553-564. PMCID: PMC3103626.

8. **Jin, Q.,** Gao, G., Mulder, K.M. (2009). Requirement of a dynein light chain in TGFβ/Smad3 signaling. J Cell Physiol., 221, 707-715. PMCID: PMC2881827.

9. **Jin, Q**., Gao, G., and Mulder, K.M (2008). Involvement of km23 dynein light chains in TGFβ signaling and intracellular transport. In: *Transforming Growth Factor-Beta in Cancer Therapy, Vol. 1: Basic and clinical Biology*, 169-184, Humana Press, Totowa, NJ.

10. **Jin, Q**., Ding, W., and Mulder, K. M. (2007). Requirement for the dynein light chain km23-1 in a Smad2-dependent TGFβ signaling pathway. J Biol Chem. 282, 19122 –19132.

11. **Jin, Q.**, Ding, W., Staub,C. M., Gao, G., Tang, Q., Mulder, K. M. (2005). Requirement of km23 for TGFβ-mediated growth inhibition and induction of fibronectin expression. Cell Signal. 17, 1363-1372.

12. Tang, Q., Staub, C. M., Gao, G., **Jin, Q,** Ding, W., Wang, Z., Aurigemma, R. E., and Mulder, K. M. (2002). A novel TGFβ receptor-interacting protein that is also a light chain of the motor protein dynein. Mol Biol Cell. 13, 4484-4496. PMCID: PMC138648.

13. Luo, M., Chen, Y., Cai, D., Zong, Y., **Jin, Q**. (2000). Thyroid hormone regulates the expression of neuron nitric oxide synthase (nNOS) gene in cerebellum, olfactory bulb and hippocampus of rats. *Chinese Journal of Endocrinology and Metabolism* 16,172-174.

14. Zong, Y., Luo, M., Ding, W., **Jin, Q**., Chen, Y., Chen, G., Cai, D., Liu Y. Xie, C. Molecular cloning and prokaryotic expression of the type 1 Diabetes-related autoantigen, IA-2 gene. *Chinese Journal of Endocrinology and Metabalism.*

15. Cai, D., Chen, Y., Chen, G., **Jin, Q.,** Li, G., Luo, M. (2000). Goα and Gsα Gene expression in piriform Cortex of Brain in neonatal Rats with Hypothyroidism. *Acta Universitatis Medicinalis Secondae Shanghai*. 20, 193-195.