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. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** The Wistar Institute

2. **Reporting Period (start and end date of grant award period):** 01/01/2011 – 6/30/2012

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Russel E. Kaufman, M.D.

4. **Grant Contact Person's Telephone Number:** 215-898-3926

5. **Grant SAP Number:** 4100054879

6. **Project Number and Title of Research Project:** 2 - SECTM1 is a Novel Mediator of Melanoma Tumorigenesis and Progression

7. **Start and End Date of Research Project:** 01/01/2011 – 6/30/2012

8. **Name of Principal Investigator for the Research Project:** Russel E. Kaufman, M.D.

9. **Research Project Expenses**.

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

   $ 458,383.27

   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
Ge	Visiting Scientist	100%	934.59
Kaufman	Principal Investigator	10%	27,442.13
Lopez-Coral	Research Assistant	100%	26,361.70
Wang	Staff Scientist	100%	89,736.96
Xiao	Research Assistant	100%	41,220.00
Ahn	Part-time Lab Assistant	100%	2,091.92
Azuma	Part-time Lab Assistant	100%	1,742.75

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
Olsen	Part-time Lab Assistant	100%

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_____ No____x_____

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

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_____ No_____x_____

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:
None	☐NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)		$	$

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:

We will apply for an NIH RO1 grant in 2013 after developing additional preliminary data.

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

Our data suggest that SECTM1 has the ability to promote tumor growth and metastasis through both the activation of internal signaling pathways and through the effect of tumor associated macrophages on tumor growth. We will need to develop an animal model, and then determine if interrupting SECTM1 effects can prevent or slow the growth and spread of cancers.

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	1			
Female	1	1		
Unknown				
Total	**2**	**1**		

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic	2	1		
Unknown				
Total	**2**	**1**		

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

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

    Yes____X_____ No_____

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

    Yingbin Ge, MD, Department of Physiology, Nanjing Medical University, China

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.

    These studies have helped create a perspective on the tumor microenvironment that will be the center of a new program on tumor microenvironment in our cancer center.

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_____ No____X_____

If yes, please describe the collaborations:

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 period that the project was funded (i.e., from project start date through end date). 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.

Several lines of evidence suggest that SECTM1 plays a role in the biology of human melanoma and likely other cancers. We have developed experimental evidence that it can directly promote cell growth and migration, and indirectly work through the immune system to activate T lymphocytes and monocytes with a secondary effect of producing macrophages, that can promote tumor growth and migration. These preliminary data have formed the basis for these studies and new data reported here support our existing hypotheses.

The SECTM1 gene encodes a 27kD type I integral membrane protein that is cleaved to produce a 20kD secreted form (sSECTM1). The extracellular domain contains an N-linked glycosylation motif and two immunoglobulin-like regions. The 81 amino acid cytoplasmic domain (C-terminus) contains serines that are phosphorylated (unpublished), a single tyrosine, and a proline-rich region that contains a highly conserved Src homology 3 (SH3) domain-binding motif, which frequently play critical roles in cell signaling.

SECTM1 has been shown to bind to the receptor CD7, which is expressed on T and NK cells, and activation of the receptor by antibodies costimulates T cell proliferation. Our data reveal that SECTM1 binding to CD7 leads to activation of T cells and production of interferon-γ (IFN-γ) and IL-2 in human cells. This is particularly interesting because we have shown that expression of SECTM1 is induced by IFN-γ in antigen-presenting cells, thymic epithelium and fibroblast cells, producing a possible positive feedback loop wherein SECTM1 production leads to more SECTM1 production. SECTM1 also activates regulatory T cells.

SECTM1 is expressed normally in a number of cell types, including neutrophils, dendritic cells, activated monocytes, CD4 T cells and many epithelial cell types. SECTM1 is also highly expressed in breast, ovarian, and prostate cancers, and in some myeloid leukemia cells. To further characterize the expression pattern of SECTM1, we examined a variety of malignant tissues and demonstrated that SECTM1 is highly expressed in most melanoma cell lines and melanoma tissue samples, but not melanocytes. Furthermore, we found that forced overexpression of SECTM1 in melanoma cell lines increases melanoma cell migration and invasion in those lines. The mechanism of this is still unknown, but our recent data indicated that SECTM1 activates NF-κB signaling pathway, a multifunctional transcriptional factor, that promotes tumor migration and invasion. The analysis of the direct effect of SECTM1 expression

in tumors is not the focus of the studies reported here.
Because SECTM1 can bind to receptors on several types of immune cells, we have hypothesized that it may have a significant role in controlling immune functions. Monoclonal antibodies against CD7 can induce signaling in T and NK cells via CD7. In our preliminary studies, we observed increased numbers of melanoma associated macrophages (MAM) in melanoma lesions that highly express SECTM1, so we have reasoned that this may directly or indirectly be due to interactions of SECTM1 with immune cells. This is the basis for the studies described in this report.

We hypothesize that induction of SECTM1 could lead to regulatory T cell activation, differentiation of monocytes to MAMs, and conversion of melanomas to a more advanced stage. Since a small percentage of melanoma patients appear to be improved with interferon therapy, understanding the role of SECTM1 in this group appears to be particularly important. Based on these observations, we proposed the following aims to understand the role of SECTM1 in immune cell activation, melanoma tumorigenicity and progression.

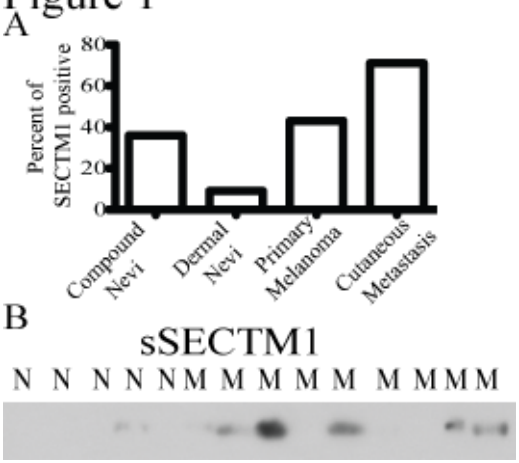
Specific Aim 1. To determine the significance of SECTM1 on MAMs in the melanoma microenvironment, we will:
a. Determine the mechanism for the interaction of SECTM1 with MAMs.
b. Determine the effect of SECTM1 on MAMs
c. Determine the role of SECTM1 in promoting interactions between MAMs and melanoma cells by using a 3D skin reconstruct model.

Specific Aim 2. To determine the effect of SECTM1 induced macrophage in the tumor microenvironment, we will:
a. Determine the biological effect of SECTM1 induced macrophages on melanoma progression and invasion.
b. Determine whether expression of SECTM1 in melanoma can recruit MAMs into tumors *in vivo* using a B16 mouse melanoma model, and if it leads to melanoma progression.

Results:
Specific Aim 1. To determine the significance of SECTM1 on MAMs in the melanoma microenvironment.

a. SECTM1 is highly expressed in melanoma cell lines and tissues lesions. Real-time PCR and Western blot analysis of human melanocytes and melanoma cells confirmed that SECTM1 is expressed at high levels in melanomas. Immunohistochemistry analysis of tumor tissue microarrays containing 172 primary melanomas, 49 melanocytic nevi, and 35 cutaneous metastases, demonstrated that SECTM1 was increased in primary melanoma and to a greater degree in metastatic lesions compared to nevi (Fig. 1A). We also found soluble SECTM1 to be highly expressed in serum samples from melanoma patients (M) compared to those from healthy donors (N) (Fig. 1B). Increased expression of SECTM1 in melanoma patients, especially in serum, suggests that SECTM1 might be a potential prognostic marker for melanoma.

Figure 1



b. SECTM1 promotes inflammatory cell infiltration in melanoma lesions by increasing monocyte differentiation to MAMs. By tissue staining for SECTM1, we observed a considerable increase in SECTM1 expression at sites where melanoma tumors had been infiltrated by immune cells. 64.5% of the 172 melanoma samples with inflammatory infiltration showed significant SECTM1 signals (Fig.2A). Supporting this, clinically normal dermal nevi with little or no inflammatory cell infiltration revealed particularly low SECTM1 expression (92% negative), while compound nevi which have high levels of inflammatory cell infiltration express SECTM1 (38%) (Fig.2C). This expression pattern, together with our finding that LPS and interferon-γ induce expression of SECTM1 in monocytes, suggest that SECTM1 secreted by macrophages or tumor cells may have either direct or indirect effects on melanoma progression. We tested the ability of SECTM1 to induce the differentiation of monocytes to macrophages. Monocytes from healthy donors were cultured with melanoma cell conditioned medium with or without SECTM1 for seven days. SECTM1 significantly increased the expression of macrophage markers, including CD68 and a later macrophage marker (Mφ marker) by flow cytometry analysis. Furthermore, SECTM1 also increased expression of SECTM1 in macrophages (Fig. 2C), indicating a positive feedback on SECTM1 expression.

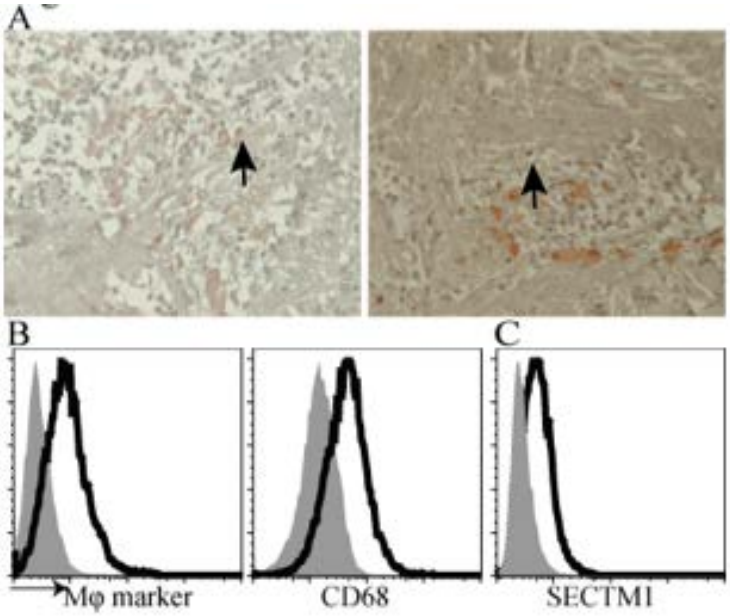


Figure 2. Expression of CD7 is expressed in monocytes/macrophages. (A) Immunohistochemistry analysis of CD7 expression in melanoma lesions. Arrow indicates CD7 positive macrophages. (B) & (C) Immunofluorescense staining of CD68 (upper panel) or CD163 (lower panel) with CD7 in melanoma lesions. (D) Real-time PCR was used to analyze expression of CD7 in monocytes, GM-CSF, M-CSF macrophages and C8161-Mϕ and 1205Lu-Mϕ. (E) Western blot analysis expression of CD7 in monocytes, GM-CSF, M-CSF macrophages and C8161-Mϕ and 1205Lu-Mϕ. (F) Monocytes were stained with the anti-human CD7 monoclonal antibody, 3A1, following by FITC-conjugated anti-mouse IgG staining. Expression of CD7 was analyzed by FACS analysis. Filled: Isotype control, black line: anti-CD7.

SECTM1 induces macrophage differentiation through direct and indirect mechanisms.
Our initial approach was to characterize the effect of SECTM1 on cells that express its receptor, CD7. CD7 is a cell-surface molecule, expressed on T lymphocytes and NK cells, which can act as a costimulatory receptor for T cell proliferation when liganded with CD7 antibodies. SECTM1 has been demonstrated to bind with high affinity to CD7. However, the function of this molecule, CD7, in human immune cells and role in human T cell function has been unclear. In order to determine the direct effect of SECTM1 on human T lymphocytes, we exposed these cells to human rSECTM1, and demonstrated that SECTM1 strongly costimulates CD4 and CD8 T cell proliferation and induces IFN-gamma production, likely via a CD7- dependent mechanism. In addition, (Fig. 3) SECTM1 synergizes with suboptimal anti-CD28 to strongly augment T cell functions. We found a robust induction of IL-2 production when SECTM1 and anti-CD28 signals were present with TCR ligation. Furthermore, addition of SECTM1 into a mixed lymphocyte reaction (MLR) significantly enhanced proliferation of alloantigen-activated T cells, whereas blockade of SECTM1 with a specific monoclonal antibody inhibited T cell proliferation in a two-way MLR assay. Simultaneously blocking the effect of SECTM1, along with CTLA-4/Fc, diminishes two-way MLR. Most significantly, SECTM1 induces interferon gamma production by T lymphocytes, and it is a potent activator of monocyte differentiation to

macrophage, providing a potential explanation for macrophage accumulation in melanoma tumors that highly express SECTM1 (Fig. 3).

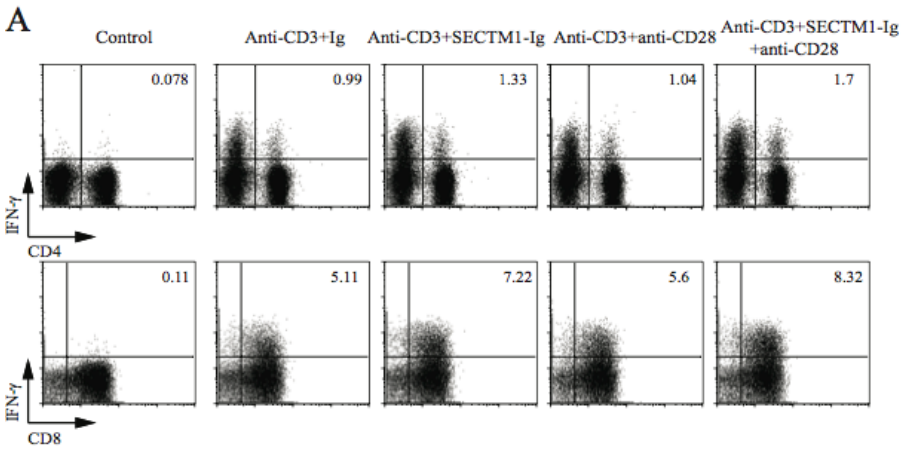


Figure 3. SECTM1 costimulates cytokine production in T cells. Purified T cells were stimulated with immobilized SECTM1-Ig (2 microg/ml), with or without soluble anti-CD28 mAb (2 microg/ml) in the presence of anti-CD3 mAb (0.5 microg/ml), and intracellular staining was performed to measure expression of IFN-gamma (A). For IFN-gamma expression, cells were stimulated for 6 h. Results shown are representative from four donors of two independent experiments.

These results indicate that SECTM1 is a broadly expressed, IFN-gamma-inducible molecule, which functions as a potent costimulatory ligand for T cell activation and is synergistic with anti-CD28. Induction of T cells by SECTM1 produces IL2 and interferon gamma, which could activate monocytes. This was published in *J. Leukoc. Biol. Volume 91: 449-459; 2012.*

Two important questions confronted us: How do factors elaborated from tumors recruit and differentiate macrophage and does SECTM1 promote macrophage recruitment and differentiation. To further study this, we first attempted to determine if conditioned media from melanoma cell lines could affect monocyte differentiation to macrophage, and if so, to characterize the macrophage since there are two general classes of macrophage. These results were published in *Pigment Cell Melanoma Res. 25; 493–505, 2012 under the title: Melanoma-derived conditioned media efficiently induce the differentiation of monocytes to macrophages that display a highly invasive gene signature.*

Specifically, we developed a novel method to produce conditioned medium from melanoma cell lines, some of which expressed SECTM1. Although it had been known that the presence of tumor-associated macrophages (TAMs) in melanomas is correlated with a poor clinical prognosis, there was limited information on the characteristics and biological activities of human TAMs in melanomas. In this study, we developed an in vitro method to differentiate human monocytes to macrophages using modified melanoma-conditioned medium (MCM) that had many of the waste products removed through filtration. We demonstrated that factors from Melanoma Conditioned Media (MCM)-induced macrophages (MCMI-M/) express both M1-Macrophage and M2-Macrophage markers and inhibit melanoma-specific T-cell proliferation.

Furthermore, microarray analyses reveal that the majority of genes up-regulated in MCMI-M/ are associated with tumor invasion. The most strikingly up-regulated genes are CCL2 and MMP-9. Consistent with this, blockade of both CCL-2 and MMPs diminish MCMI-M/-induced melanoma invasion. Finally, we demonstrated that both MCMI-M/ and *in vivo* TAMs express the pro-invasive, melanoma-associated gene, *glycoprotein non-metastatic melanoma protein B*.

Our study provided a framework for understanding the mechanisms of cross-talk between TAMs and melanoma cells within the tumor microenvironment. Soluble factors produced by melanoma cells can induce differentiation of monocytes to macrophages. Macrophages have been classified as activated macrophages (M1-M/) and 'alternatively activated macrophages' (M2-M/), largely based on factors they produce. M1-M/ are induced by proinflammatory factors, produce a lower level of IL-10 and high levels of IL-12, IL-6, and TNF-alpha and have antitumor activity. Conversely, M2-M/ produce high levels of IL-10, TGFbeta, CCL1, and CCL-22 and a lower level of IL-12 and promote tumor growth and metastasis. Most TAMs characterized to date demonstrate an M2-M/ phenotype. However, current evidence suggests that TAMs are a mixed population bearing both M1 and M2 phenotypes. Our data supports this model.

An additional valuable observation come from these studies. Specific identification of TAMs in melanoma is difficult because the expression of the most commonly used macrophage marker, CD68, is also expressed in melanoma cells. In an earlier study, we found by gene microarray analysis that seven members of the metallothionein (MTs) family were upregulated in melanoma-conditioned medium induced macrophages (MCIM-Mϕ). MTs have been implicated in zinc metabolism and inflammation. We confirmed that expression of metallothionein is induced in M-CSF differentiated macrophages (M-CSF/Mϕ) and MCIM-Mϕ at both the mRNA and protein levels using real-time PCR, immunofluorescence, and western blot analysis. Furthermore, we demonstrated the presence of metallothionein in melanoma tissues *in vivo* and that metallothionein was co-localized with TAMs markers, CD68 and CD163. Finally, we demonstrated the induction of the zinc importer gene Zip8 both in M-CSF/Mϕ and MCIM-Mϕ. These experiments identify metallothionein as a novel marker for TAMs and suggests that metallothionein might play important roles in macrophage adaptation and function in the tumor microenvironment. These results were published in *Frontiers of Biology August 2012, Volume 7, Issue 4, pp 359-367, Induction of metallothionein expression during monocyte to melanoma-associated macrophage differentiation.*

Although it appears that macrophages can be differentiated and attracted to tumors by a range of factors, including M-CSF, other factors, such as VEGF, CCL2, IL-6, LIF, and GM-CSF appear to have the ability to differentiate monocytes to macrophage. Based on our observation that monocytes express CD7, the ligand for SECTM1, we needed to determine if SECTM1 also had a direct effect on monocytes and macrophages.

Although interferon gamma from T cell activation provided one way for macrophage activation, we wanted to determine if SECTM1 could activate monocytes directly. Previous studies had indicated that CD7 was not expressed on monocytes by flow cytometry analysis. However, we then examined monocytes for CD7 RNA production and, surprisingly found significant levels in resting monocytes and lower levels in macrophage. Also, by careful cell staining of monocytes with our CD7 antibody, we were able to show low levels of CD7 protein expression. The

observation that monocytes, the precursor to macrophages, express CD7 at a very low level raised the possibility that monocytes could also be a target for SECTM1 directly. Through gene expression and microarray data, we demonstrated that CD7, an "old" T and NK cell costimulatory receptor, is expressed in monocytes, while expression of CD7 is down-regulated in melanoma-conditioned medium induced macrophages (MCMI-Mϕ). In this study, we demonstrated that CD7 is highly expressed in human monocytes at the level of RNA, and a significant decreased expression of CD7 was found in M2 macrophages and tumor-conditioned medium induced macrophages in comparison to monocytes. Expression was detected, but only at lower levels in M1 macrophages. CD7 is also expressed in tumor infiltrating macrophages *in vivo*. In addition we found that CD7 ligand, SECTM1 is highly expressed in melanoma cell lines in comparison to normal melanocytes, and is present in melanoma and breast cancer tissues *in vivo*. We exposed freshly harvested human monocytes to recombinant SECTM1 and demonstrated that SECTM1 binds to CD7 to increase monocyte migration, in part by activation of MAPK and PI3K pathways.

First we performed a transwell assay to determine the effect of SECTM1 on monocyte migration. As shown in Figure 4A, a soluble recombinant SECTM1 protein was able to function as a strong chemo-attractant for monocytes in comparison with M-CSF. This effect of SECTM1 on monocytes is totally blocked by anti-CD7 monoclonal antibody 3A1. We then examined the chemo-attracting effect of conditioned media produced from a melanoma cell line, WM35 that expresses SECTM1 at a high level. Again, strong chemo-attraction was demonstrated, and the 3A1 antibody also could block this effect.

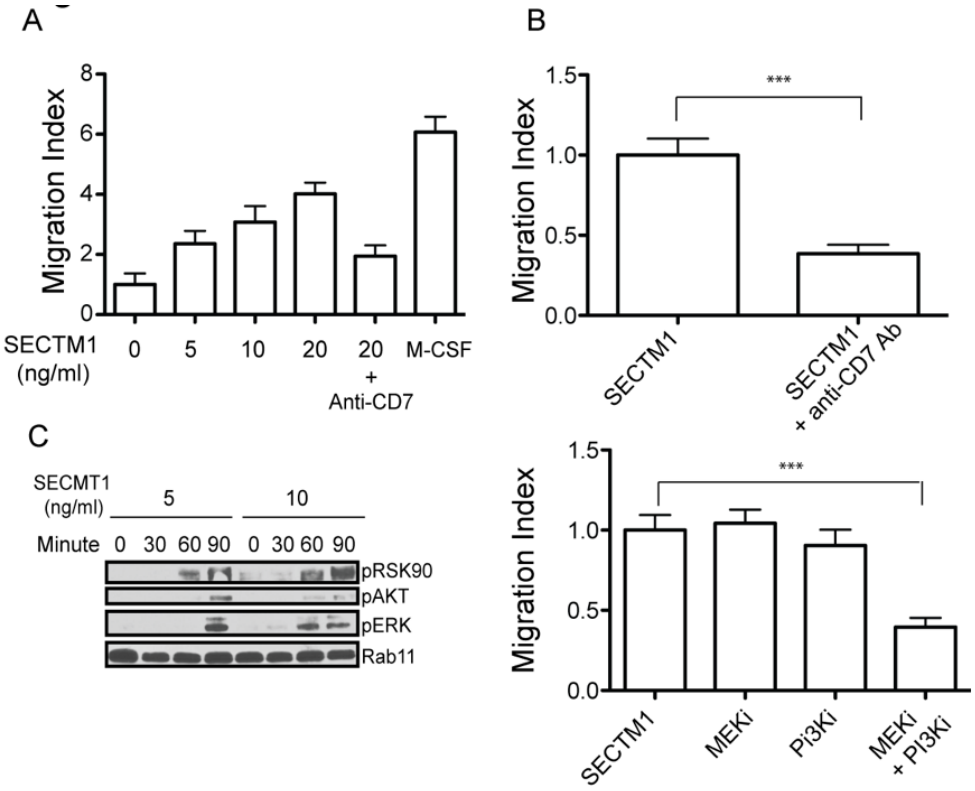


Figure 4. SECTM1 increases monocyte migration through activation of the PI3K and MAPK pathways
(A) SECTM1 increases monocytes migration by a transwell assay. Monocytes were added to upper chamber, and increased concentrations of recombinant SECTM1 protein was added into the lower chamber. M-CSF was used as a positive control and anti-CD7 monoclonal Ab (10 μg/ml) was used for blocking migration. Migration was evaluated after 24h of incubation by counting stained migrating cells on the lower side of the filter. (B) Monocytes from healthy donors were added to the upper chamber of the transwell, and conditioned medium from SECTM1 overexpression WM35 cells were used as the chemoattractant. Anti-CD7 antibodies (10 μg/ml) were used to block SECTM1 induced monocyte migration. Result is reported as migration index (fold increase over control). Data represent the mean ± SD of four independent experiments. ** $P<0.01$. (C) Monocytes were seeded in serum free medium for 2 hours and then stimulated with SECTM1 for indicated time. Cells were lysed for Western Blot analysis. (D) Migration assay was conducted with PI3K inhibitor (LY3940002) and MEK inhibitor (GSK1120212).

We then determined that SECTM1 induced monocyte migration is dependent on the activation of MAPK and PI3K pathways. It has been shown that ligation of CD7 with antibody results in activation of the PI3K pathway in T cells. We investigated the downstream signaling pathway by recombinant human SECTM1 by analyzing whether binding CD7 with SECTM1 also had similar effects on monocyte signal transduction. Surprisingly, SECTM1 not only strongly activates phospho-AKT, but also activates phospho-ERK and its downstream signaling molecule phospho-RSK90 (Fig. 4C). Furthermore, by blocking both pathways, we were able to inhibit SECTM1 induced monocyte migration, while no significant effect was seen when used alone. (Fig. 4D). Our data suggested that SECTM1 is a potent chemo-attractant for monocyte via CD7, and dependent on activation of the MAPK and PI3K pathways.

Finally, we found that SECTM1 is strongly induced by interferon-alpha and proteosome inhibitor velcade, two drugs which are used clinically for melanoma therapy. Taken together, our data support a possibly important role for SECTM1 and CD7 in the tumor microenvironment, and specifically on the attraction of monocytes to tumor, where they are exposed to a wide range of factors that induce their differentiation to macrophage. This work currently is being prepared for submission for publication.

In summary, we demonstrate that CD7 is expressed on monocytes, TAMs, M2 macrophages and TCIM-Mϕ, but not in M1 macrophages, and broad expression of SECTM1, which is regulated by IFN-γ, may play a significant role in the maturation and recruitment of macrophages to the tumor microenvironment, where macrophages can then release factors that promote growth, migration, and metastasis. We believe these observations are relevant to not only melanoma but also many other cancers where SECTM1 is highly expressed.

c. Determine the role of SECTM1 in promoting interactions between MAMs and melanoma cells using a 3D skin reconstruct model. One of our goals was to translate and examine the effect of SECTM1 in a tissue model. Soluble SECTM1 (sSECTM1) from SECTM1/WM3899 culture medium enhances differentiation of monocytes. Furthermore, stimulation of monocytes by sSECTM1 results in a significant increase in the expression of SECTM1, creating a positive feedback effect and thus amplifying the effect of SECTM1 on macrophages, as well as potentially on melanoma cells. Restating this, SECTM1 production by either tumor or macrophages increases SECTM1 production by the other cell and itself. Current models studying melanoma and MAMs in 2D culture systems may not fully reflect the physiological conditions of the melanoma microenvironment. The 3D skin reconstruct model, developed in the laboratory of our close collaborator Dr. Meenhard Herlyn, consists of epidermal keratinocytes, melanoma cells and fibroblasts. Importantly, the skin reconstruct can be implanted into (NOD/SCID/IL2rγnull, NSG) mice, providing a useful model for further analysis of melanoma biology *in vivo*. Our preliminary experiments demonstrated that human macrophages can survive in 3D culture conditions, making it possible to include macrophages in 3D skin reconstructs, thus facilitating the study of the interaction of MAMs with melanoma cells.

In order to validate the biological significance of this study, we needed to produce the 3D culture system with one component overexpressing SECTM1 and for the macrophage to survive. We initially cultured monocytes in melanoma conditioned medium with or without SECTM1 for three and seven days as we described earlier. However following manipulation of macrophages, we were unable to get the macrophage to survive in the 3D culture system and unable to complete this set of experiments.

A second set of tissue related experiments was designed to determine if high levels of SECTM1 in melanoma can recruit MAMs into tumors *in vivo* using a B16 mouse melanoma model, and if it leads to melanoma progression. Although we were able to set up controls with B16 mouse melanoma cells, SECTM1 transfected cells underwent apoptosis before inoculation into the mice and we were unable to overcome this problem using these cells. We are uncertain if the high level of expression induced intracellular signaling events since high levels activate intracellular NFkappaB expression. We intend to continue to pursue these studies since they are essential to understanding the true biological effects of high levels of SECTM1 by tumors.

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

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

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 or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) 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. K12/Sectm1, an interferon-y regulated molecule, synergizes with CD28 to costimulate human T cell proliferation	Tao Wang, Catherine Huang, Alfonso Lopez-Coral, Kimberly A. Slentz-Kesler, Min Xiao, E. John Wherry, and Russel E. Kaufman	J Leukoc Biol	October 2011	☐Submitted ☐Accepted ☒ Published
2. Melanoma-Derived Conditioned Media Efficiently Induce the Differentiation Of Monocytes To Macrophages That Display A Highly Invasive Gene Signature	Tao Wang, Yingbin Ge, Min Xiao, Alfonso Lopez-Coral, Rikka Azuma, Rajasekharan Somasundaram, Gao Zhang, Zhi Wei, Xiaowei Xu, Frank J. Rauscher III, Meenhard Herlyn and Russel E. Kaufman	Pigment Cell Melanoma Res	March 2012	☐Submitted ☐Accepted ☒ Published
3. Induction of metallothionein expression during monocyte to melanoma-associated macrophage differentiation	Yingbin Ge, Rikka Azuma, Bethsebah Gekonge, Alfonso Lopez-Coral, Min Xiao, Gao Zhang, Xiaowei Xu, Luis J. Montaner, Zhi Wei, Meenhard Herlyn, Tao Wang, Russel E. Kaufman	Front. Biol	May 2012	☐Submitted ☒ Accepted ☐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 are preparing the description of monocyte expression of CD7 and the response of monocytes to recombinant SECTM1 for submission for publication within 30 days.

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.

We initially discovered SECTM1 and have been attempting to determine its biological significance. We now know that it is expressed at high levels in many tumors and through the work of others, it is now one of a group of 20 biomarkers for ovarian cancer. Our studies demonstrating the detection of SECTM1 in the serum of patients with advanced melanoma suggest it has a similar possible function as a biomarker to follow or detect melanoma.

Equally important, we now know that SECTM1 can activate the immune system through T cells and directly through monocytes and dendritic cells. The mode of activation and the ability of SECTM1 to attract macrophage to tumors suggests that it will have a very important role in tumor promotion, migration, and spread.

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.

SECTM1 could have a role to activate the immune system in certain immunodeficiency states, and blocking it could help block tumor promotion. Furthermore, it may be a useful biomarker for melanoma or ovarian cancer.

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. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
KAUFMAN, Russel E.	**President & CEO**
eRA Commons Username: **KAUFMAN**	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Ohio State University	B.S.	1968	Zoology/ Biochemistry
The Ohio State University, College of Medicine	M.D. Cum Laude	1973	Medicine

A.

Since 2002, I have served as president and CEO of The Wistar Institute and director of the Wistar Cancer Center. In addition to my administrative leadership of Wistar, I maintain an active research program at the Institute, investigating the genetics of blood diseases and cancer. During my tenure at Wistar, I have been actively recruiting top-notch scientists, developing new biomedical research centers and expanding scientific programs and facilities. I have held leadership positions in national academic organizations in internal medicine and on committees within the National Institutes of Health, the National Academy of Sciences and the American Association of Medical Colleges. I was named one of the first Searle Scholars, a prestigious program that supports top-flight academic research in biomedicine and chemistry. Other honors for my work include; the March of Dimes, Scholar of the Leukemia and Lymphoma Society of America and the Arthritis Foundation. Additionally I served on NCI's initial review group subcommittee A for cancer centers.

B. Positions:

1973-1976	Internal Medical Residency, Duke University Medical Center
1976-1980	Hematology-Oncology Fellowship, Duke University Medical Center
1977-1978	Chief Medical Residency, Duke University Medical Center
1977-1980	Associate in Medicine, Duke University Medical Center
1978-1980	Research Hematologist, National Institutes of Health
1980-1987	Assistant Professor of Medicine, Dept. of Medicine, Duke University Medical Center
1984-1995	Assistant Professor of Biochemistry, Dept. of Biochemistry, Duke University Medical Center
1987-1995	Associate Professor of Medicine, Dept. of Medicine, Duke University Medical Center
1989-1995	Director, Division of Hematology/Oncology - Duke University Medical Center
1995-2002	Associate Professor of Biochemistry, Dept. of Biochemistry, Duke University Medical Center

1995-2002	Professor of Medicine, Dept. of Medicine, Duke University Medical Center
1995-2002	Chief, Div. of Medical Oncology & Transplantation, Duke University Medical Center
1995-2002	Vice-Chair for Academic Programs, Dept. of Medicine, Duke University Medical Center
1999-2002	Vice Dean for Education and Academic Affairs, Duke University School of Medicine
2000-2002	Associate Vice Chancellor, Duke University School of Medicine
2002-2004	Director & CEO, The Wistar Institute
2002-2010	Director, The Wistar Institute Cancer Center
2004-	President & CEO, The Wistar Institute

Honors and Appointments:

1972	Alpha Omega Alpha, Ohio State University
1982-1985	Searle Scholar
1985-1990	Leukemia Society Scholar
1997-1999	Chair, ACS Scientific Advisory Committee, Cell Cycle and Growth Control
1999	Chair, Federated Council for Internal Medicine
2007-2009	Chair, National Grants Council, American Cancer Society
2009	Fellow, College of Physicians of Philadelphia
2009	Professional Achievement Award, Ohio State University Alumni Association
2009-2011	NCI IRG Subcommittee A for Cancer Centers