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:** Drexel University

2. **Reporting Period (start and end date of grant award period):** 1/1/2009-12/31/2010

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Anne Martella

4. **Grant Contact Person's Telephone Number:** (215) 895-6471

5. **Grant SAP Number:** 4100047631

6. **Project Number and Title of Research Project:** 5 - *Identification of Biomarkers and Therapeutic Targets in 3D Hypoxic Breast Cancer Mode*

7. **Start and End Date of Research Project:** 1/1/2009-12/31/2010

8. **Name of Principal Investigator for the Research Project:** Gregg Johannes, PhD

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:

   $ 187,500

   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
Johannes	Assistant Professor	5%	$ 3712
Reginato	Assistant Professor	5%	$ 6310
Jones	Ph.D. student	100%	$ 37439
Whelan	Ph.D. student	100%	$ 37659

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
None		

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_____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:
Regulation of the HIF1α mRNA by hypoxia	XNIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify: _____)		$200,000	$not funded

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:

I plan on resubmitting my grant on investigating the regulation of the HIF1α mRNA during hypoxia to the NIH.

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

To continue investigating the role of the loss of the HIF1α mRNA in regulating gene expression and tumor development.

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			1	
Unknown				
Total			**1**	

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

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

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:

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 funding for this project allowed for the continued collaboration between the Drs. Johannes and Reginato in study of the role of hypoxia and the HIF transcription factors in regulating gene expression and in the development of DCIS.

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 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.

Specific Aims: The overall goal of this proposal is to validate and test whether 5 key hypoxia-induced genes that we have identified by microarray analysis are critical for hypoxia-mediated cell survival and the changes in tissue architecture that are observed in our 3D hypoxic model of DCIS. We will also test whether targeting these genes may reverse the hypoxic survival phenotype and block hypoxia induced chemoresistance. Additionally, we will determine if these targets can serve as biomarkers for hypoxic breast cancer.
Aim 1: Validate the expression and function of 5 key hypoxia-regulated genes in 3D acini.
Aim 2: Verify whether the genes identified in hypoxic 3D acini are also altered in hypoxic DCIS tumors.
Aim 3: Determine whether altering hypoxic gene expression changes the sensitivity to chemotherapeutic agents in normal and oncogene expressing 3D acini.

Aim 1: Validate the expression and function of 5 key hypoxia-regulated genes in 3D acini. Based on our microarray analysis of genes up-regulated during hypoxia in 3D conditions, we chose five candidate genes to initially validate and test. The genes selected have met the following criteria: 1. RNA was upregulated at least four-fold in microarray analysis under hypoxic conditions in 3D acini; 2. all are known to activate signaling pathways implicated by our studies (Akt, Mek/Erk pathway); and 3. proteins are all drugable targets. These genes include: Lysyl Oxidase (LOX), Integrin α5 (ITGA5), Map Kinase Interacting Serine/Threonine Kinase 2 (MNK2), Endothelin 2 (ET2), and Angiopoietin-like 4 (ANGPL4).

Summary of completed work (Aim1): We have completed this aim. We validated the induction of the MNK2, LOX, ANGPL4, and ET2 mRNAs and found that the targets had different induction kinetics. In addition, we found that MNK2 was a HIF1 and HIF2 target, LOX and ANGPL4 were HIF1 specific targets, and ET2 was a HIF2 specific target. This work showed for the first time that MNK2 and ET2 were induced by hypoxia and that ET2 was a HIF2 specific target. We found that inhibition of these proteins by pharmacological agents or knockdown of the mRNA by RNA interference did not prevent the hypoxia mediated suppression of Bim expression. Bim suppression is required for the formation of DCIS structures when MCF10A cells are grown in 3D hypoxic culture. We could not test the impact of ET2 because we could not get reproducible and consistent knockdown of this mRNA. Since none of the tested targets showed any biological effects on the hypoxia mediated suppression of Bim, the continued analysis of these targets in Aim 2 and Aim 3 was of limited value. In light of this, we changed Aim 2 to focus on the expression and regulation HIF1 and HIF2 in response to acute and prolonged hypoxia (see Aim 2, below)

Lysyl Oxidase (LOX), Map Kinase Interacting Serine/Threonine Kinase 2 (MNK2), Endothelin 2 (ET2), and Angiopoietin-like 4 (ANGPL4) have different induction kinetics. Since, we have previously shown that these mRNAs are upregulated by hypoxia in 3D, suspension, and attached cultures we have utilized the attached culture system for the subsequent work. To further characterize the expression pattern of the target genes we analyzed the induction of each of the mRNAs after 6, 12, 24, and 48 hr of hypoxic exposure. As shown in Figure 1, all target genes were induced by 6 hr of hypoxic exposure but demonstrated different kinetics for maximal induction. The MNK2 (MNK) and ANGPL4 mRNAs were fully induced by 6 hr, the LOX mRNA required 24 hr, and the ET2 mRNA

required 48 hr of hypoxic exposure for maximum induction. The Adrenomedullin (ADM) mRNA was used as a positive control because it is a known HIF1 target and the Hypoxia Inducible Factor 2α (HIF2α) mRNA was used as a negative control. As expected, the ADM mRNA was fully induced by 6 hr of hypoxic exposure and the HIF2α mRNA was unaffected by hypoxia. This work shows the novel finding that ET2 and MNK2 mRNAs are hypoxia inducible.

ET2 is a HIF2 target. We next determined if the induction of these mRNAs was dependent on HIF1 or HIF2 by using RNA interference. siRNAs targeting HIF1α or HIF2α were transfected into MCF10A and the impact on target mRNA induction was evaluated by qRT-PCR under normoxic and hypoxic conditions. Figure 2 shows that the siRNA targeting HIF1α (siHIF1a) knocked down the expression of the HIF1α mRNA by >5-fold while only marginally affected HIF2α expression. Similarly, the siRNA targeting HIF2α (siHIF2a) knocked down expression of the HIF2α mRNA by >3-fold without affecting the HIF1α mRNA. This demonstrates that the siRNAs targeting HIF1α and HIF2α are efficient and specific for the targeted mRNA. Using these siRNAs the impact of HIF1 and HIF2 on the hypoxic induction of the target mRNAs was determined. Figure 3 shows that the hypoxic induction of the ANGPL4 (Figure 3A) and ADM (Figure 3B) mRNAs was significantly reduced in the HIF1α (siHIF1) and HIF2α (siHIF2) knockdown cells. Consistent with this, the knockdown of both α-subunits had an additive effect. This data indicates that the hypoxic induction of ANGPL4 and ADM mRNAs can be mediated by either HIF1 or HIF2. This ability of HIF1 and HIF2 to activate the transcription of common genes demonstrates their redundant function. Figure 4 shows that the hypoxic induction of the LOX (Figure 4A) and MNK2 (Figure 4B) mRNAs is significantly reduced in the HIF1α knockdown cells but not in the HIF2α knockdown cells. This indicates that the hypoxic induction of these two mRNAs is preferentially regulated by HIF1. This is the first report that the MNK2 mRNA is induced by hypoxia in a HIF1 specific manner. Figure 5 shows that the hypoxic induction of the ET2 mRNA is significantly reduced in the HIF2α knockdown cells but not in HIF1α knockdown cells. This is the first report that demonstrates that ET2 is a HIF2 specific target. This may explain why the ET2 mRNA requires prolonged hypoxic exposure for maximal induction (Figure 1), because HIF2α remains induced during prolonged hypoxia while HIF1α is only transiently induced (see Aim 2).

The promoter of the ET2 gene is stimulated by hypoxic treatment. Since it has not been previously reported that ET2 is hypoxia inducible, we analyzed the promoter region for hypoxic response elements. Two overlapping promoter regions of the ET2 gene (-654 to +60, and -459 to +60) were cloned into a promoterless firefly luciferase reporter vector (PGL4, Promega Inc.). The reporter constructs were then transfected into MCF10A cells and the impact of hypoxia on luciferase expression was evaluated. As seen in Figure 6, both constructs showed significant activation when cells were exposed to hypoxia for 48 hr. This indicates that this region of the ET2 promoter contains a hypoxic response element (HRE), although no canonical HRE is observed in the sequence. Considering that ET2 is a HIF2 specific target, the analysis of the ET2 promoter will provide important novel information regarding the cis-acting elements that distinguish HIF1 promoters from HIF2 promoters. This will be the subject of a future grant proposal.

MNK2 induction promotes eIF2α phosphorylation. MNK2 is highly homologous to another MAP-kinase interacting kinase, MNK1. Both of these kinases can phosphorylate the translation initiation factor 4E (eIF4E). To further investigate if the increased levels of MNK2 results in increased levels of eIF4E phosphorylation we performed immuno-blot analysis on extracts from hypoxic and normoxic MCF10A cells grown in 3D cultures. As shown in Figure 7, hypoxia induces the phosphorylation of eIF4E when 3D MCF10A cells are exposed to hypoxia. This is consistent with the increase in MNK2 expression.

To verify that MNK2 is responsible for this phosphorylation we used RNA interference to specifically knockdown the expression of MNK2 and evaluated the impact on hypoxia mediated eIF4E phosphorylation. The knockdown of MNK2 was achieved using small hairpin RNAs (shRNAs) that target the MNK2 mRNA. Lentivirus shRNA expression vectors were purchased commercially (Mission shRNA, Sigma) and used to generate lentiviruses. The shRNA expressing lentiviruses were used to transduce MCF10A cells to generate stable cell lines that express the shRNAs targeting the MNK2 mRNA. In addition, a virus expressing a shRNA targeting a scrambled sequence was used as a control. We tested 5 different MNK2 shRNAs. Figure 8A shows that 4 out of 5 shRNAs against MNK2 knocked down expression of the MNK2 mRNA by >70%. In addition, we examined the levels of the MNK2 protein and identified 3 shRNAs that significantly reduce the levels of the MNK2 protein (Figure 8B). This loss of MNK2 blocked the hypoxia mediated phosphorylation of eIF4E (Figure 8B). We also monitored the effect of the MNK2 shRNA on the MNK1 mRNA to ensure that the knockdown of MNK2 was specific. As shown in Figure 9, the MNK2 shRNA #98 knocked down the expression of the MNK2 mRNA by over 80% but only had a marginal effect on MNK1 mRNA levels. Together this data strongly indicates that the induction of MNK2 by hypoxia results in increased phosphorylation of eIF4E. We have selected the MNK2 shRNA #98 for use in biological assays to test whether MNK2 is required for hypoxic inhibition of anoikis and Bim suppression.

Knockdown of the MNK2 or ANGPL4 mRNA does not prevent the hypoxic block in Bim expression. We have previously shown that Bim induction is responsible for the induction of apoptosis (Anoikis) and loss of cells in the lumen of 3D MCF10A acini. We have also shown that hypoxia suppresses Bim expression in an Erk dependent manner. This block in anoikis leads to the DCIS phenotype observed in our hypoxic 3D model system [1]. This induction of anoikis can be mimicked by placing cells in suspension and this phenomenon is blocked when cells are grown under hypoxic conditions, similar to what is observed in our 3D model system [1]. We used suspension cultures and Bim expression to evaluate the role of the individual target genes on the formation of the DCIS-like structures under hypoxic conditions. If the targeted mRNA is important for the DCIS phenotype then the knockdown of the mRNA should block the suppression of Bim when knockdown cells are placed in suspension under hypoxic conditions.

To examine the role of MNK2, we knocked down expression of MNK2 using shRNA#98 and evaluated the impact of hypoxia on Bim expression in cells grown in suspension for 48 hr. As shown in Figure 10, knockdown of MNK2 had no effect on the loss of Bim expression under hypoxic conditions. We did identical experiments to evaluate the impact of ANGPL4 on Bim expression or Erk activation during hypoxia. As shown in Figure 11, the shRNA

reduced ANGPL4 mRNA levels by >10-fold (Figure 11A). However this knockdown had no effect on Bim suppression under hypoxic conditions (Figure 11B). We conclude from these experiments that neither MNK2 nor ANGPL4 is involved with the inhibition of apoptosis that allows for the formation of the DCIS phenotype when MCF10A cells are grown in 3D culture under hypoxic conditions.

Inhibition of LOX does not prevent the hypoxic block in Bim expression. To evaluate the role of LOX in the hypoxic block of anoikis mediate by Bim suppression, we used the irreversible LOX inhibitor, β-aminopropionitrile (BAPN). As seen in Figure 12, BAPN treatment had no effect on the loss of Bim expression in MCF10A cells grown under hypoxic conditions in suspension (Figure 12A) or in 3D culture (Figure 12B). Thus we conclude that the induction of LOX by hypoxia had no effect on the inhibition of Bim mediated anoikis that results in the DCIS phenotype observed when 3D MCF10A cultures are exposed to hypoxia.

Knockdown of the ITGA5 mRNA does not prevent the hypoxic block in Bim expression. To test if ITGA5 is involved with the Bim mediated block of anoikis, MCF10A cells were transfected with siRNA targeting this mRNA. As shown in Figure 13, the knockdown of ITGA5 had no effect on the loss of Bim expression in MCF10A cells grown in suspension under hypoxic conditions. This indicates that ITGA5 induction by hypoxia is not involved with the block in anoikis that result in the formation of the DCIS phenotype that occurs when MCF10A cells are grown under hypoxia.

Aim 2: Verify whether the genes identified in hypoxic 3D acini are also altered in hypoxic DCIS tumors. Since none of the tested hypoxic targets showed any biological effect on the hypoxia mediated suppression of Bim (Aim 1), the continued analysis of these targets in Aim 2 and Aim 3 was of limited value. In light of this, we shifted to focus on the expression and regulation HIF1 and HIF2 in response to acute and prolonged hypoxia.

Analysis of the differential regulation of HIF1α and HIF2α in MCF10A cells exposed to hypoxia. Changes in gene expression in the hypoxic cells are primarily mediated by two transcription factors, HIF1 and HIF2 [2, 3]. The abundance and activity of these transcription factors are regulated by their α-subunit (HIF1α and HIF2α) which is stabilized under hypoxia [4-7]. HIF1 is considered to be the dominant transcription factor in most cell types [8-10]. Interestingly, it has been reported that HIF1 can inhibit the activity of HIF2 although the mechanism remains unclear [11]. Although HIF1 and HIF2 target many of the same genes, several studies have identified genes that appear to be preferentially activated by HIF1 or HIF2 [12-18]. Thus a growing body of evidence indicates that HIF1 and HIF2 have overlapping but non-redundant functions.

Summary of completed work. We found that HIF1α is transiently induced while HIF2α is continuously induced by hypoxic treatment. Furthermore, we show that the loss of the HIF1α protein and mRNA in response to hypoxia correlates with a reduction in the stability of the HIF1α mRNA. Moreover, this data shows that the induction of an endogenous HIF1α antisense RNA (aHIF) correlates with the reduction in the stability of the HIF1α mRNA during hypoxia. This data strongly suggests that HIF1 and HIF2 have overlapping as well as

unique roles in regulating gene expression during hypoxia and suggests that inhibition of both transcription factors may be required to prevent and/or treat breast cancer.

The HIF1α protein and mRNA are reduced in response to prolonged hypoxic treatment. Figure 14A shows that the HIF1α protein is maximally induced by 2 hr of hypoxic exposure after which it rapidly declines. Figure 14B shows that the HIF2α protein is also induced by 2 hr of hypoxic treatment but remains constant during prolonged hypoxic treatment. Thus, the HIF1α protein is transiently induced by acute hypoxia while the HIF2α protein remains induced during prolonged hypoxia. qRT-PCR analysis of the HIF1α and HIF2α mRNAs (Figure 14C) shows that the loss of the HIF1α protein correlates with a >2.5-fold reduction of the HIF1α mRNA. This suggests that the loss of the HIF1α mRNA is responsible for the loss of the HIF1α protein. In contrast the HIF2α mRNA does not significantly change in response to hypoxia.

The loss of HIF1α mRNA is not affected by HIF1α or HIF2α knockdown. To investigate if the reduction of the HIF1α mRNA is modulated by the HIF1 or HIF2 transcription complex, RNA interference was used. Figure 15 shows that the siRNA targeting HIF1α (siHIF1a) or HIF2α (siHIF2a) reduced the targeted mRNA by 80% (Figure 15A) and 70% (Figure 15B), respectively. Neither knockdown blocked the hypoxia induced reduction of the HIF1α mRNA (Figure 15A) as hypoxia reduced the expression of HIF1α mRNA by 3-fold in both cases. This indicates that the hypoxia induced loss of the HIF1α mRNA is independent of HIF1 or HIF2. However, it is also possible that HIF1 and HIF2 have redundant functions in regulating the HIF1α mRNA and so the simultaneous knockdown of both α-subunits might be required.

Hypoxia reduces the half-life of the HIF1α mRNA by 3-fold. We hypothesized that the loss of the HIF1α mRNA in response to hypoxia is mediated by a reduction in the half-life of the HIF1α mRNA. To examine the half-life of the HIF1α mRNA in response to hypoxia, the transcriptional inhibitor Actinomycin D (Act D) was added to normoxic cells and cells that have been exposed to hypoxia for 4 hr. Cells were then maintained in their appropriate environments and RNA was harvested after 2, 4, 6 and 8 hr of Act D treatment. As shown in Figure 16A, hypoxia reduced the half-life of the HIF1α mRNA from 30 hr under normoxic conditions to 9.5 hr under hypoxic conditions. This statistically significant 3-fold reduction ($P<0.005$) in half-life is sufficient to account for the loss of the HIF1α mRNA that is observed under hypoxic conditions. The half-life of the HIF2α mRNA was unaffected by hypoxic treatment demonstrating that the reduced stability of the HIF1α mRNA is a specific effect of hypoxia (Figure 16B).

The aHIF RNA is induced by hypoxia. The induction of an endogenous HIF1α anti-sense RNA (aHIF) has been implicated in the regulation of the HIF1α mRNA during hypoxia [19] [20]. qRT-PCR analysis shows that the aHIF RNA is induced 5-fold in response to 24 hr of hypoxia (Figure 17A). To determine whether HIF1 or HIF2 plays a role in the induction of the aHIF RNA, we monitored aHIF RNA expression in the presence of siRNAs targeting HIF1α or HIF2α. The HIF1α and HIF2α siRNAs knocked down expression of their target mRNA by 80 and 70%, respectively (Figure 17B). Knockdown of HIF1α reduced the induction of the aHIF RNA by approximately 2-fold while knockdown of HIF2α did not

(Figure 17A). This indicates for the first time that the aHIF RNA is primarily a HIF1 target. Since the HIF1α knockdown does not block the hypoxia induced reduction of the HIF1α mRNA (Figure 15A), it is likely that the 2-fold increase in the aHIF RNA in the HIF1α knockdown cells is sufficient to reduce the level of the HIF1α mRNA. Alternatively, hypoxia may promote the interaction of aHIF with the HIF1α mRNA and this interaction may not be solely dependent on the abundance of the aHIF RNA. It is also possible that other mechanisms are involved with regulating the HIF1α mRNA under hypoxia.

The HIF1α mRNA redistributes to the cytoplasm in response to hypoxia. It has been reported that the aHIF RNA is predominately nuclear while the HIF1α mRNA is evenly distributed between the nucleus and the cytoplasm [20]. To evaluate if the aHIF RNA or the HIF1α mRNA redistributes from the nucleus to the cytoplasm in response to hypoxia, the amount of the HIF1α mRNA and the aHIF RNA in nuclear and cytoplasmic RNA fractions was determined. Figure 18 shows that the aHIF RNA is 10% cytoplasmic under both normoxic and hypoxic conditions indicating that it does not redistribute during hypoxia. However, the HIF1α mRNA is 20% cytoplasmic under normoxic conditions and 70% cytoplasmic during hypoxia As expected the cyclophilin A mRNA is 80% cytoplasmic under both conditions verifying the efficient fractionation of the nuclear and cytoplasmic RNA. . This novel finding indicates that the HIF1α mRNA redistributes from the nucleus to the cytoplasm in response to hypoxia. The molecular mechanism responsible for this redistribution and its importance in regulating HIF1α expression remains unknown and is the subject of a grant (RO1, NIH) that will be resubmitted in the near future.

References:

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3. Semenza, G.L., et al., *Hypoxia-inducible nuclear factors bind to an enhancer element located 3' to the human erythropoietin gene.* Proc Natl Acad Sci U S A, 1991. **88**(13): p. 5680-4.
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6. Kallio, P.J., et al., *Regulation of the hypoxia-inducible transcription factor 1alpha by the ubiquitin-proteasome pathway.* J Biol Chem, 1999. **274**(10): p. 6519-25.
7. Jiang, B.H., et al., *Hypoxia-inducible factor 1 levels vary exponentially over a physiologically relevant range of O2 tension.* Am J Physiol, 1996. **271**(4 Pt 1): p. C1172-80.
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Figures follow:

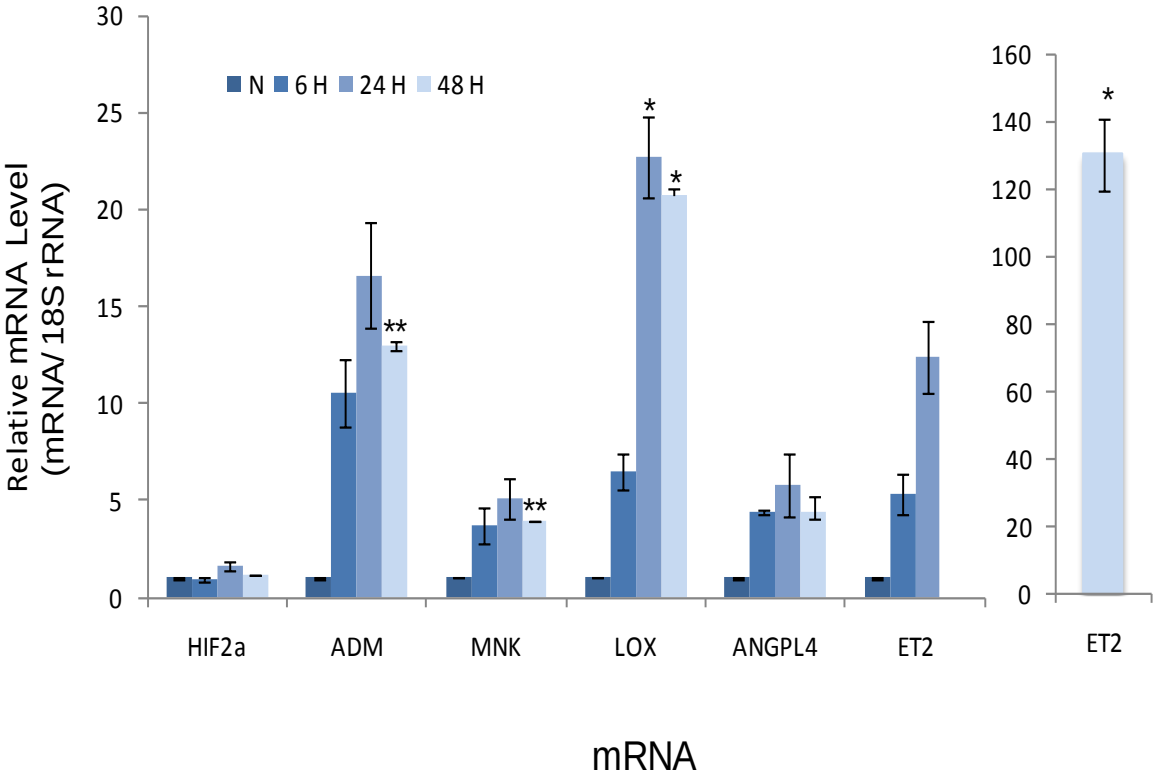

Figure 1: Induction of the hypoxia inducible target mRNAs. MCF10A cells were exposed to hypoxia (1.0% O_2) for 0, 6, 24, and 48 hr and the amounts of the HIF2a, ADM, MNK2, LOX, ANGPL4, and ET2 was determined by qRT-PCR. All mRNAs were normalized to 18S rRNA and the level found under normoxic conditions was set to 1. Standard error mean bars are shown. * $p \leq 0.05$, ** $p \leq 0.005$. For all studies, qRT-PCR analysis was performed using gene specific primer-probe set (ABI) and the Stratagene MX3000P real-time machine.

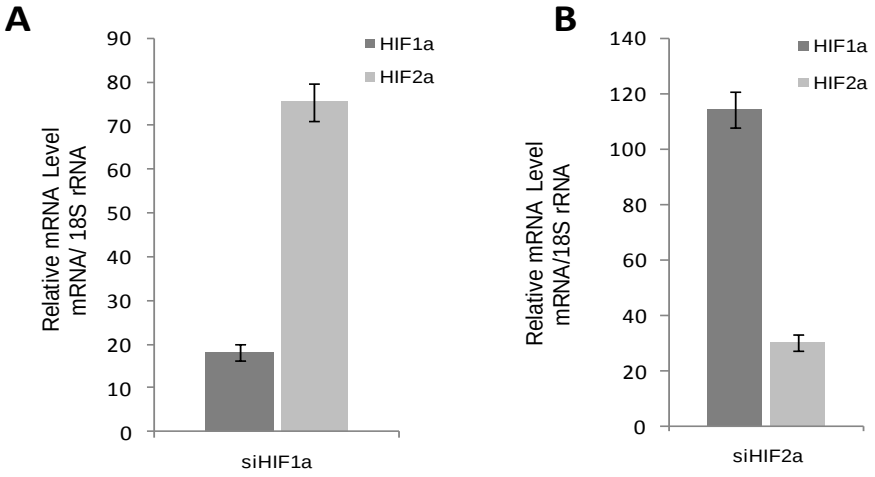

Figure 2. siRNAs directed against HIF1a or HIF2a efficiently knockdowns the expression of the targeted mRNA. MCF10A cells were transfected with siRNAs targeting HIF1a (siHIF1a), HIF2a (siHIF2a), or scramble sequence. 48 hr after transfection, RNA was harvested and the amount of the HIF1a mRNA, HIF2a mRNA, and 18S rRNA was determined by qRT-PCR. The ratio of HIF1α mRNA /18S rRNA and HIF2α mRNA/18S rRNA found in cells transfected with the control siRNA was set to 100% (data not shown). The Relative mRNA level was determined by normalizing the amount of HIF1a (A) or HIF2a (B) mRNA in the knockdown cells to the level found in the cells transfected with the control siRNA. Standard deviation bars are shown.

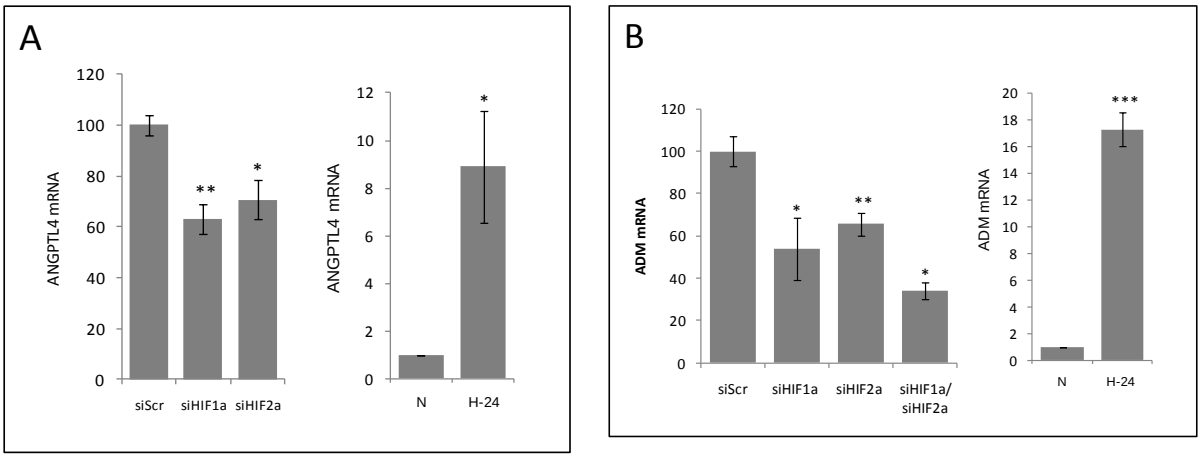

Figure 3. ANGPL4 and ADM are HIF1 and HIF2 targets. MCF10A cells were transfected with siRNAs targeting HIF1a (siHIF1a), HIF2a (siHIF2a), or a control siRNA (siScr) and placed under hypoxic conditions for 24 hr. The amount of the ANGL4 and ADM mRNAs was determined by qRT-PCR and normalized to the amount of the 18S rRNA. The normalized level of ANGPL4 (A) and ADM mRNA (B) in hypoxic cells transfected with the control siRNA was set to 100%. The graph to the right shows the induction of the mRNA in response to 24 hr of hypoxic treatment the level of the mRNA/18S rRNA under normoxic conditions was set to 1. Standard error mean bars are shown. * p-value ≤0.05, **p-value ≤0.005, ***p-value ≤ 0.005

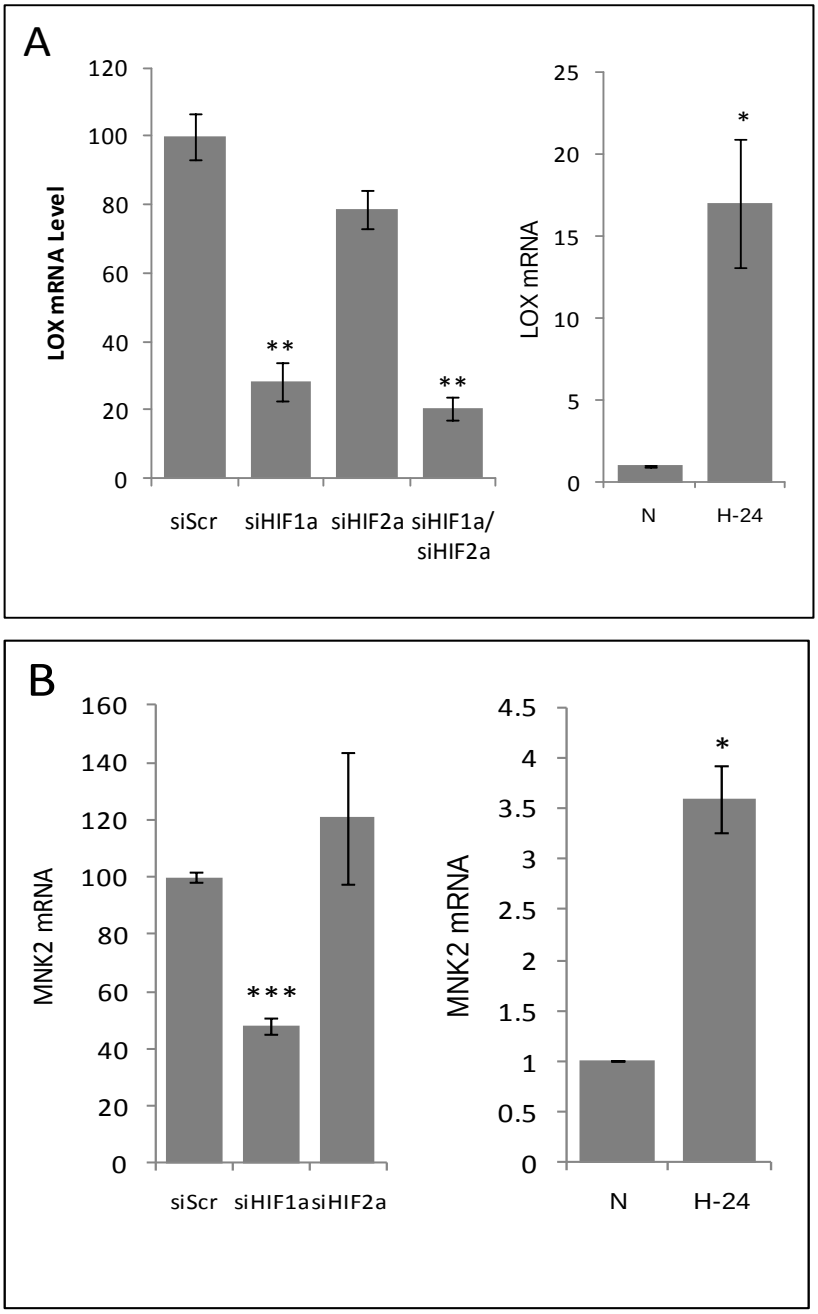

Figure 4 LOX and MNK2 are HIF1 targets. MCF10A cells were transfected with siRNAs targeting HIF1a (siHIF1a), HIF2a (siHIF2a), both (siHIF1a/siHIF2a), or a control siRNA (siScr) and placed under hypoxic conditions for 24 hr. The amount of the LOX and MNK2 mRNAs was determined by qRT-PCR and normalized to the amount of the 18S rRNA. The normalized levels of the LOX (A) and MNK2 mRNAs (B) in hypoxic cells transfected with the control siRNA were set to 100%. The graph to the right shows the induction of the mRNA in response to 24 hr of hypoxic treatment (the level of the mRNA/18S rRNA under normoxic conditions was set to 1). Standard error mean bars are shown. * p-value ≤0.05, **p-value ≤0.005, ***p-value ≤ 0.005.

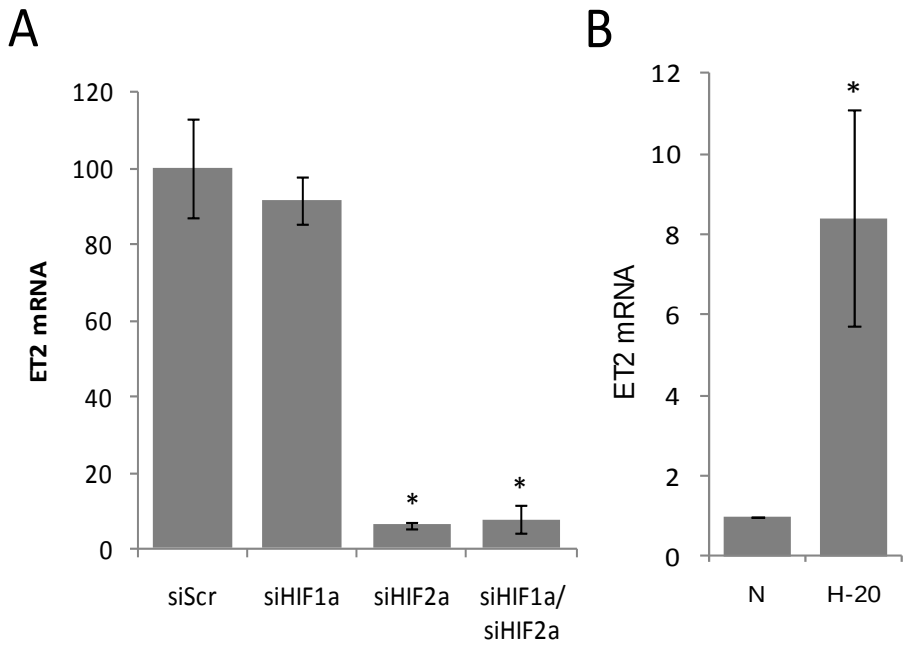

Figure 5. ET2 is a HIF2 target. A) MCF10A cells were transfected with siRNAs targeting HIF1a (siHIF1a), HIF2a (siHIF2a), both (siHIF1a/siHIF2a), or a control siRNA (siScr) and placed under hypoxic conditions for 24 hr. The amount of the ET2 mRNA was determined by qRT-PCR and normalized to the amount of the 18S rRNA. The normalized level of ET2 mRNA in hypoxic cells transfected with the control siRNA was set to 100%. B) The induction of the mRNA in response to 20 hr of hypoxic treatment. The level of the mRNA/18S rRNA under normoxic conditions was set to 1. Standard error mean bars are shown. * p-value ≤0.05, **p-value ≤0.005, ***p-value ≤ 0.005.

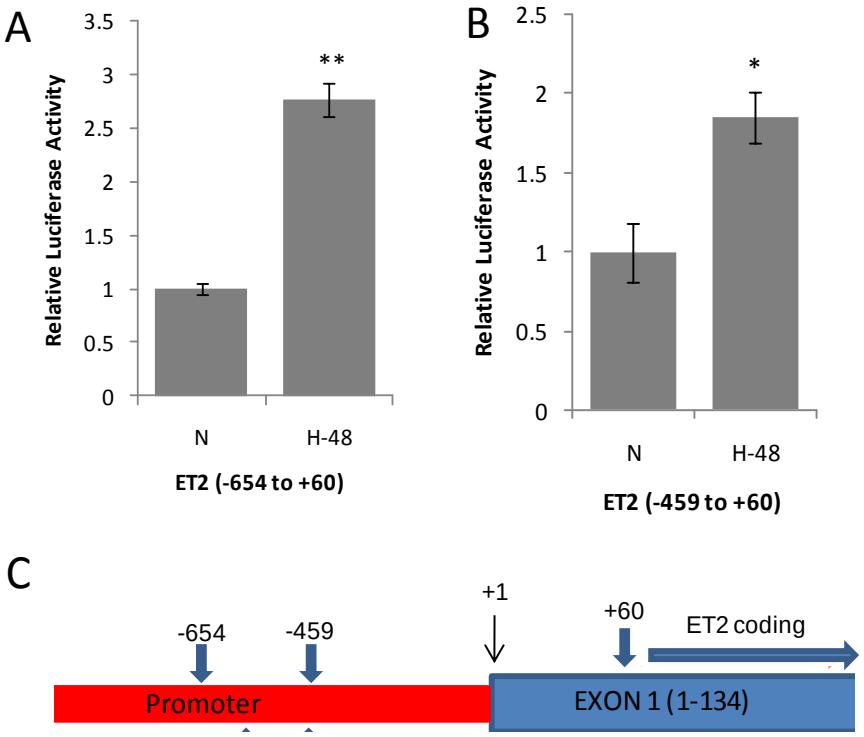

Figure 6. ET2 promoter activity is stimulated by hypoxia. MCF10A cells were transfected with ET2 promoter reporter constructs that contained (A) 650 bp (-654 to +60) or (B) -459 bp (-459 to +60) of the ET2 promoter region. The cells were incubated for 24 hrs and then either maintained under normoxic (N) conditions or placed under hypoxic (H-48) conditions. Cells were harvested and the amount of firefly luciferase (Fluc) activity in the extract was determined using the Dual Luciferase Assay Kit (Promega). All constructs were transfected with a Rennila luciferase control reporter that was used for normalization. The level of normalized Fluc found in normoxic samples was set to 1. Standard error mean bars are shown. *$p \leq 0.05$, **$p \leq 0.005$. C) Schematic representation of the promoter region and first exon of the Endothelin 2 gene. The transcription start site (+1) and the beginning of the ET2 open reading frame is denoted. The location of the forward and reverse primers used to generate the ET2 fragments for cloning into the PGL4.10 (Luc2) plasmid are denoted by arrows.

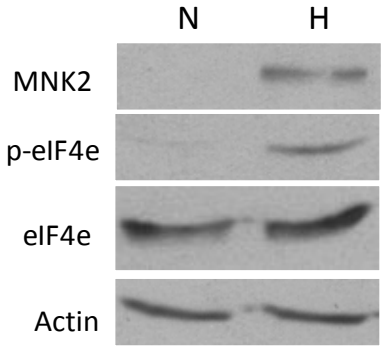

Figure 7: Hypoxia induces MNK2 expression and eIF4E phosphorylation in MCF10A 3D acini. MCF10A cells were grown in matrigel (3D) under normoxic conditions until day 6 then maintained in normoxic conditions (N) or placed at 1% O_2 (H) for 48 hours. Total protein was isolated and the amount of MNK2, total (eIF4E) and phosphorylated eIF4E (p-eIF4E), and Actin was determined by immuno-blot analysis. Actin was used as a loading control.

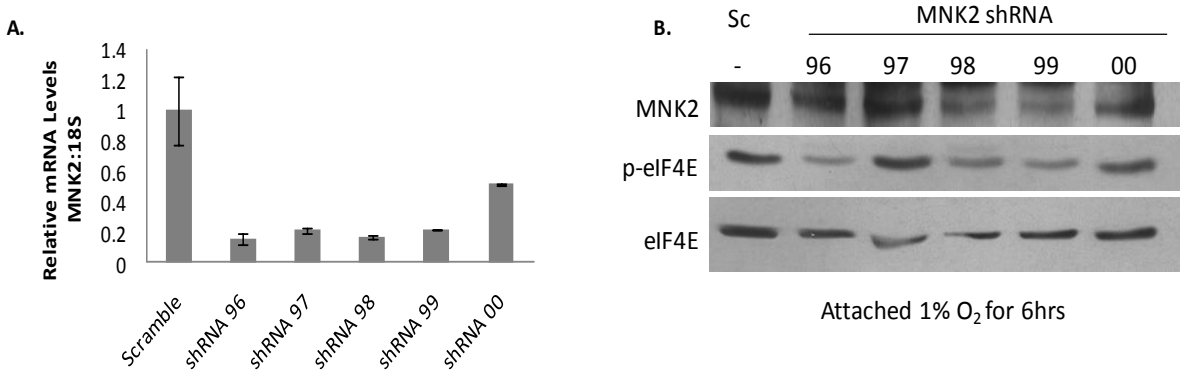

Figure 8: Knockdown of MNK2 blocks hypoxia induced phosphorylation of eIF4E. MCF10A cells expressing shRNA constructs targeting MNK2 (#'s 96-00) or scrambled sequence (Sc) were grown in monolayer culture and placed in hypoxia (1% O_2) for 6 hours at which point cells were lysed and total mRNA (A) and protein (B) were isolated. A) The amount of the MNK2 mRNA was determined by qRT-PCR. 18S ribosomal RNA was used as a normalization control. The normalized level of each mRNA under normoxic conditions was set to 1. B) Equal amounts of protein (30 ug) were subjected to immuno-blot analysis with antibodies targeting MNK2, total eIF4e, and phospho-eIF4E (p-eIF4E).

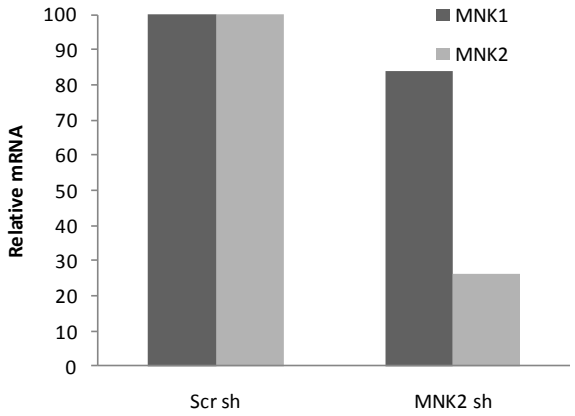

Figure 9: shRNA directed against MNK2 does not target MNK1. Cells were transduced with a lentivirus expressing a shRNA targeting the MNK2 mRNA (MNK2 sh) or a scrambled (Scr sh) sequence. 24 hr after transduction RNA was harvested and the amount of MNK1 and MNK2 mRNA present in the cells was determined by qRT-PCR. All data were normalized to the amount of 18S rRNA mRNA. The normalized amount of each RNA in the control cells were set to 100 (relative mRNA).

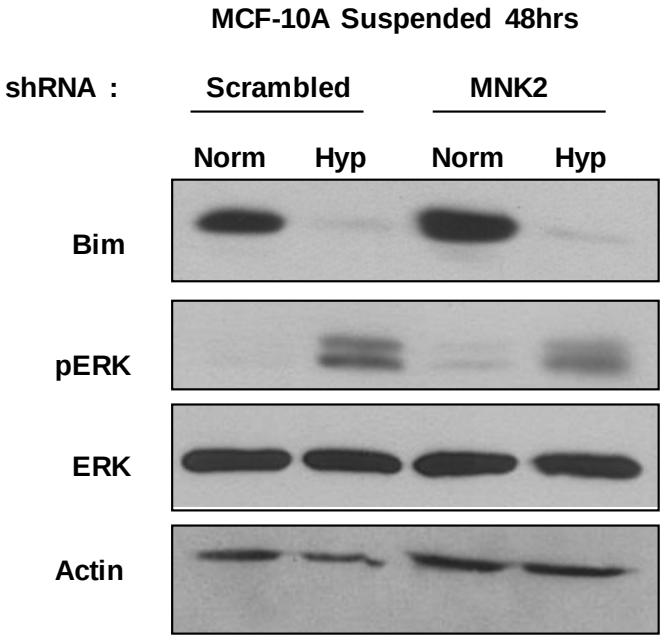

Figure 10. MNK2 induction by hypoxia is not required for the hypoxia-mediated Bim suppression or Erk activation. MCF-10A cells were infected with a lentivirus encoding a scrambled shRNA (scrambled) or one targeting MNK2 (sh #98, MNK2). 48 hours after infection, cells were placed in suspension under normoxic or hypoxic conditions. Immuno-blot analysis was used to determine levels of Bim, phospho-Erk (pERK), and total Erk. Actin was used as a loading control.

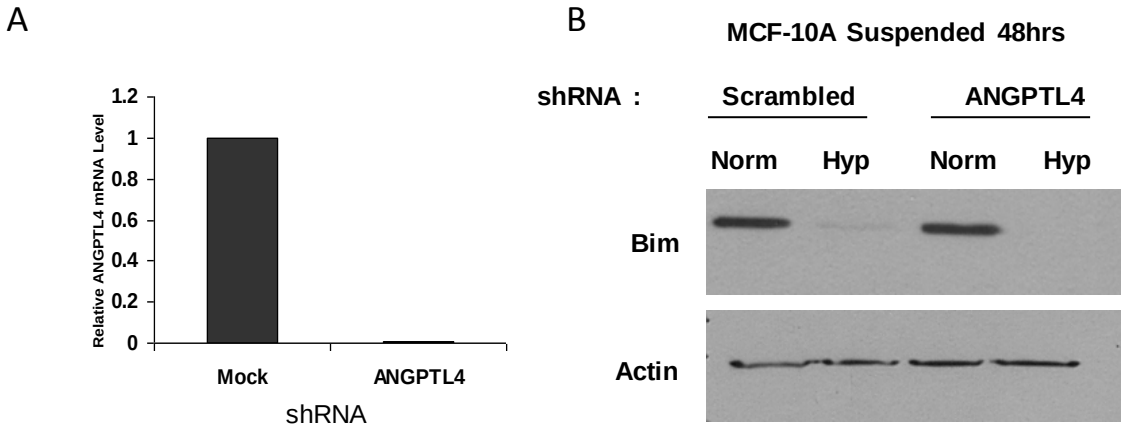

Figure 11. Angiopoietin-like 4 (ANGPL4) expression is not required for hypoxia-mediated Bim suppression. A) MCF-10A cells were infected with a lentivirus encoding a scrambled shRNA (mock) or one targeting ANGPTL4. 48 hours after infection total mRNA was harvested. The amount of ANGPL4 normalized to cyclophilin A was determined by qRT-PCR. The amount of normalized ANGPL4 mRNA found under normoxic conditions was set to 1. B) 48 hours after infection with the ANGPL4 shRNA encoding lentivirus, MCF-10A cells were placed in suspension under normoxic or hypoxic conditions for 48 hours. Immuno-blot analysis was used to determine levels of Bim. Actin was used as a loading control.

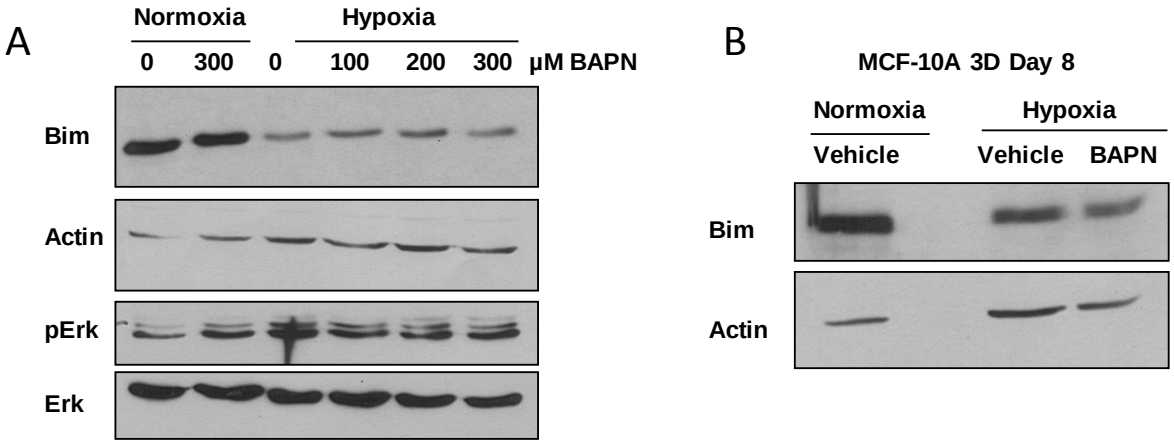

Figure 12. Inhibition of lysly oxidase (LOX) does not reverse hypoxic Bim suppression or Erk activation. A) MCF10A cells were treated with the indicated concentration of the LOX inhibitor β-aminopropionitrile (BAPN) and then placed in suspension for 48 hr under normoxic or hypoxic conditions. Immuno-blot analysis was used to determine the levels of Bim, phospho-Erk (pErk), and total Erk. Actin was used as a loading control. B) MCF10A cells were cultured in 3D until day 6 at which point cells were treated with vehicle (DMSO) or BAPN (300 uM) and then kept under normoxic conditions or placed in hypoxic conditions for 48 hours. Immuno-blot analysis was used to determine levels of Bim. Actin was used as a loading control.

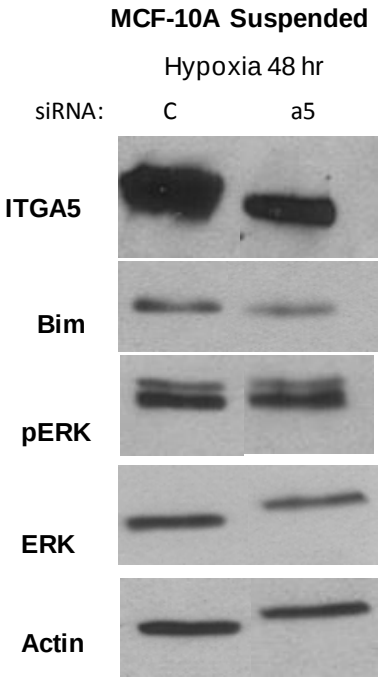

Figure 13. Integrin α is not required for the hypoxia-mediated Bim suppression and Erk activation. MCF-10A cells were mock transfected or transfected with integrin α5-targeting siRNA. 48 hours after transfection, cells were placed in suspension under hypoxic conditions. Immuno-blot analysis was used to determine the levels of integrinα5, Bim, phospho-Erk (pERK), and total Erk. Actin was used as a loading control. C = Mock transfected; a5 = ITGA5

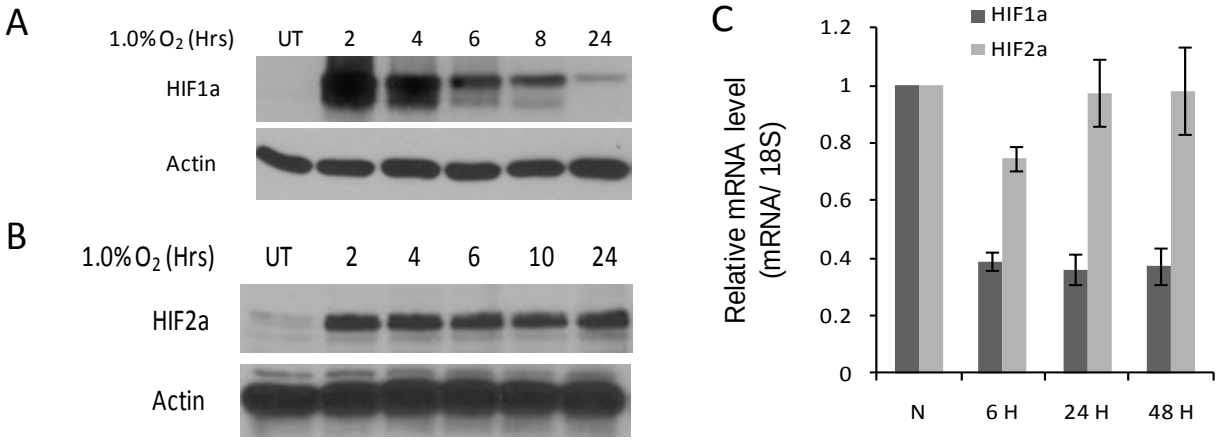

Figure 14. HIF1α is transiently induced by acute hypoxia. MCF10A cells were grown under normoxic conditions (UT) or hypoxic conditions for various times as indicated. A&B) The amount of HIF1α (A) or HIF2α (B) protein was detected by immuno-blot analysis using HIF1α (BD Bioscience) or HIF2α (Novus, Inc) specific antibodies. Actin (antibody from Sigma, Inc) was used as a loading control. C) RNA was isolated from normoxic MCF10A cells or cells that were grown in hypoxia (H) for 6, 24, or 48 hr and the levels of the HIF1α mRNA, HIF2α mRNA, and 18S rRNA was determined by qRT-PCR. All mRNA levels were normalized to 18S rRNA (Relative mRNA level) and the level found under normoxic conditions was set to 1. Standard error bars are shown.

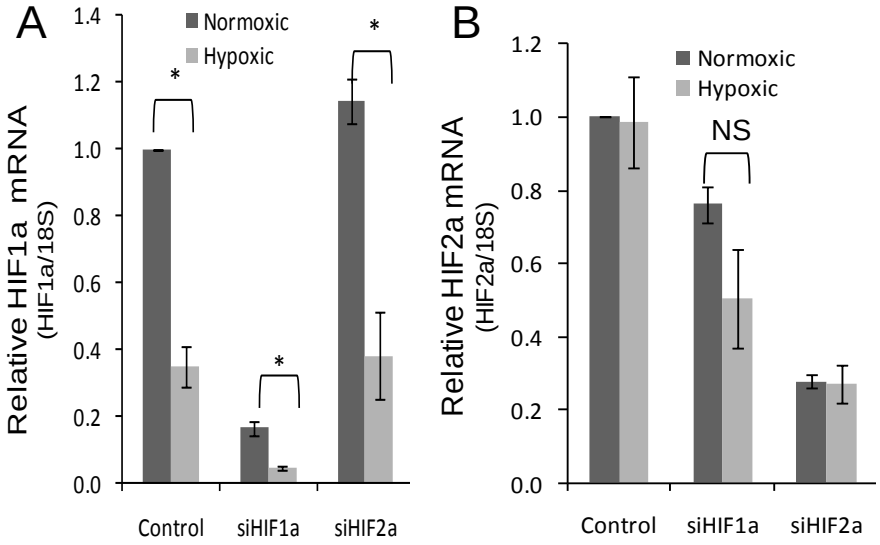

Figure 15. The hypoxic induced reduction of the HIF1α mRNA is independent of HIF1α or HIF2α expression. MCF10A cells were transfected with an siRNA targeting the HIF1α mRNA (siHIF1a), HIF2α mRNA (siHIF2a), or a scrambled sequence (control). The cells were placed under hypoxic or normoxic conditions for 24 hr and the amount of the HIF1α mRNA, HIF2α mRNA and 18S rRNA was determined by qRT-PCR after 24 hr of hypoxic conditions or normoxic cells. The ratio of HIF1α mRNA /18S rRNA (A) or HIF2α mRNA/18S rRNA (B) under normoxic conditions for the control siRNA was set to 1. Standard deviation bars are shown. * : P value <0.05, NS: not significant P value >0.2

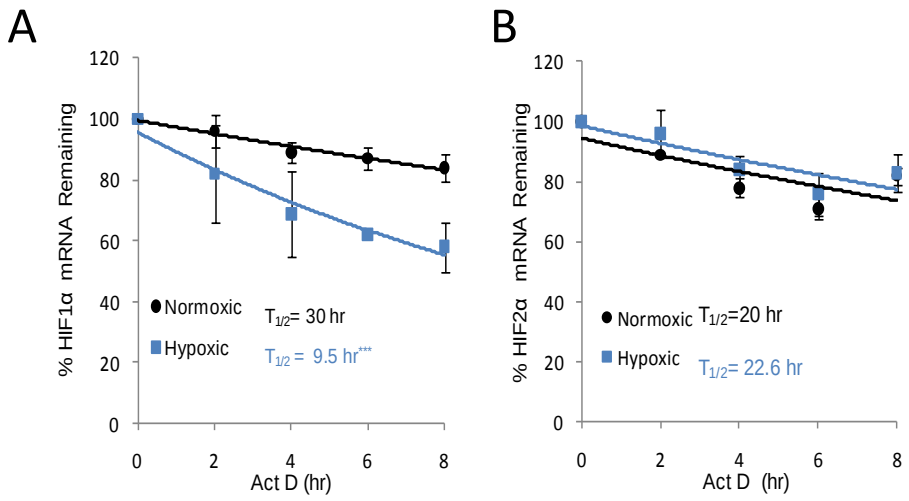

Figure 16. Hypoxia reduces the half-life of the HIF1α mRNA by 3.0-fold. MCF10A cells were grown under normoxic conditions or placed in hypoxia for 4 hr at which point Act D was added and the cells were maintained in the appropriate environment. RNA was harvested at 0, 2, 4, 6, and 8 hr after Act D addition. The amounts of the HIF1α (A) and HIF2α (B) mRNAs were determined by qRT-PCR. Cyclophilin A was used as a normalization control and the normalized value at 0 hr of Act D addition was set to 100%. A linear scale is used to more clearly show the decay of the long-lived HIF1α and HIF2α mRNAs. The half-life for each RNA was determined using the exponential trend line (shown). SEM bars are shown. ***P<0.005 (N vs. H half-life)

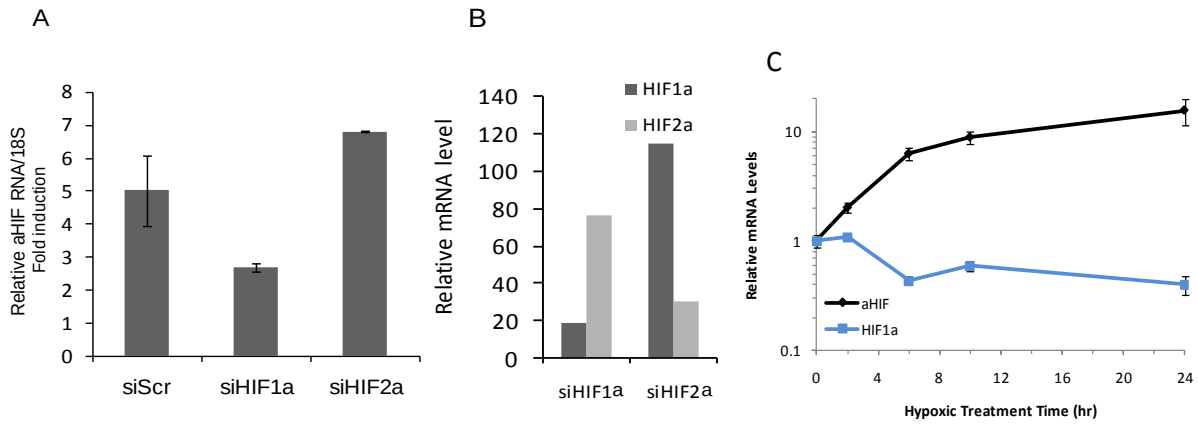

Figure 17. The aHIF RNA is induced by hypoxia. MCF10A cells were transfected with a siRNA targeting the HIF1α mRNA (siHIF1a), HIF2α mRNA (siHIF2a), or a scrambled sequence (siScr) and grown under normoxic conditions for 24 hr. Transfected cells were then placed under hypoxic conditions for 24 hr or maintained in normoxic conditions. Total RNA was isolated and the amount of the aHIF RNA (A), HIF1α mRNA (B), HIF2α mRNA (B), and 18S rRNA was determined by qRT-PCR. A) The ratio of aHIF RNA/18S rRNA under normoxic (N) conditions was set to 1. The fold induction of the aHIF RNA under hypoxia is shown. B) The ratio of HIF1α/18S rRNA or HIF2α/18S rRNA was determined for cells transfected with siHIF1a or siHIF2a and normalized to the value found in cells transfected with the scrambled siRNA (which was set to 100%). C. MCF-10A cells were exposed to hypoxia for the indicated times (hr) and the amount of the HIF1a mRNA and aHIF RNA was determined by qRT-PCR and normalized to the 18S rRNA. The amount of the normalized HIF1a mRNA and aHIF RNA found under normoxic conditions (0 hr was set to 1. Standard error mean bars are shown.

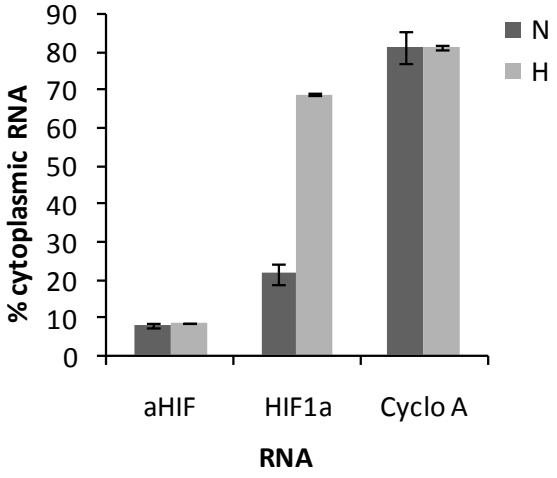

Figure 18. The HIF1α mRNA redistributes from the nucleus to the cytoplasm in response to hypoxic treatment. Nuclear and cytoplasmic RNA was fractionated from MCF10A cells grown under normoxic (N) or hypoxic (H) conditions for 24 hr. The amount of aHIF RNA, HIF1α mRNA, and Cyclophilin A (Cyclo A) mRNA in the nuclear and cytoplasmic RNA fractions was analyzed by qRT-PCR and the % cytoplasmic is shown.

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. None				☐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 plan on submitting a manuscripts based on these findings detailing the expression and regulation of the HIF transcription factors and the HIF2 specific induction of Endothelin 2

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.

None

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.

None

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
Gregg J. Johannes, Ph.D.	Assistant Professor
eRA COMMONS USER NAME (credential, e.g., agency login)	Department of Pathology and Laboratory Medicine

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of the Sciences in Philadelphia	B.S.	1987	Biochemistry
University of South Carolina	Ph.D.	1993	Biological Sciences
University of Colorado, HSC	Post-doc	1993	Molecular Biology
Stanford University	Post-doc	1996	Molecular Biology

Positions and Honors

Positions and Employment

1999-2002 Senior Scientist, Target Development Group
Message Pharmaceuticals, Malvern, PA

2003 – Present Assistant Professor, Dept. of Pathology,
Drexel University College of Medicine, Philadelphia, PA

2005 - Present Assistant (Adjunct) Professor, Dept. of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, PA

2006 - 2009 Program Director, Graduate Studies in Molecular Pathobiology
Drexel University College of Medicine, Philadelphia, PA

2007 – Present Technical Director, Molecular Diagnostic Laboratory, Department of Pathology, Drexel University College of Medicine, Philadelphia, PA

Other Experience and Professional Membership

2004- 2008 Member, American Association for Cancer Research
2005- Present Member, American Association for the Advancement of Science
2009- On-line Reviewer, Department of Defense CDMRP Prostate Cancer Research Program
2008- Present Ad-hoc Reviewer for RNA Journal

Honors and Awards

1995 National Research Service Award, NIH
1996 Small RNA Grant, University of Colorado, Boulder

Selected peer-reviewed publications

Most Relevant to current application

1. Waggoner, S.A., **G.J. Johannes**, and S.A. Liebhaber, 2009. Depletion of the poly(C) binding proteins alpha CP1 and 2 from K562 cells leads to p53-independent induction of cyclin dependent kinase inhibitor (CDKN1A) and G1 arrest. J Biol Chem 284:9039-9049. PMCID: PMC2666552
2. Thomas, JD and **Johannes, GJ** 2007 Identification of mRNAs that continue to associate with polysomes during hypoxia. Rna 13:1116-1131 PMCID: PMC1894931
3. Thomas JD, Dias LM, **Johannes GJ** 2008 Translational repression during chronic hypoxia is dependent on glucose levels. RNA 14:771-781 PMCID: PMC2271361
4. **Johannes, G.** and Sarnow, P. (1998) Cap-Independent Polysomal Association of Natural mRNAs Encoding c-Myc, BiP, and eIF4G Conferred by Internal Ribosome Entry Sites. RNA 4: 1500-1513 PMCID: PMC1369721

Additional recent publications of importance to the field (in chronological order)

1. Whelan, K.A., S.A. Caldwell, K.S. Shahriari, S.R. Jackson, L.D. Franchetti, G.J. Johannes, and M.J. Reginato, *Hypoxia suppression of Bim and Bmf blocks anoikis and luminal clearing during mammary morphogenesis.* Mol Biol Cell, 2010. **21**(22): p. 3829-37.
2. Crowe E, Sell C, Thomas JD, **Johannes GJ**, Torres C 2009 Activation of proteasome by insulin-like growth factor-I may enhance clearance of oxidized proteins in the brain. Mech Ageing Dev 130:793-800. PMCID: PMC2821674
3. Stoddard FR, 2nd, Brooks AD, Eskin BA, **Johannes GJ** 2008 Iodine alters gene expression in the MCF7 breast cancer cell line: evidence for an anti-estrogen effect of iodine. Int J Med Sci 5:189-196 PMCID: PMC2452979
4. Cascio, S, Bartella, V, Auriemma, A, **Johannes, GJ**, Russo, A, Giordano, A, and Surmacz ,E 2007 Mechanism of leptin expression in breast cancer cells: role of hypoxia-inducible factor-1alpha. Oncogene 2008. **27**(4): p. 540-7. PMID: 17653093
5. Dolloff, N.G, Shulby, S.S., Nelson A.V., Stearns, M.E., **Johannes, G. J.**, Thomas, J. D., Mecucci, O., and Fatatis, A. (2005) Bone-metastatic potential of human prostate cancer cells correlates with Akt/PKB activation by alpha platelet-derived growth factor receptor. Oncogene, 2005. 24(45): p. 6848-54. PMCID: PMC2712354
6. **Johannes, G**., Carter, M.S., Eisen, E.B., Brown, P.O., and Sarnow, P. (1999) Identification of Eukaryotic mRNAs that are Translated at Reduced Cap Binding Complex eIF4F Concentrations Using a cDNA Microarray. PNAS 96: 13118-13123 PMCID: PMC23910
7. Kosovsky, M. J. and **Johannes, G.** (1995) A Primer Extension Method for Quantitation of in Vitro Synthesized mRNA Transcripts Directed by Promoter Elements Containing Multiple Transcription Initiation Sites. Analytical Biochemistry 232: 258-261
8. **Johannes, G.** and Berger, F.G. (1993) Domains Within the Mammalian Ornithine Decarboxylase Messenger RNA have Evolved Independently and Episodically. Journal of Molecular Evolution 36: 555-567
9. **Johannes, G.** and Berger, F.G. (1992) Alterations in mRNA Translation as a Mechanism for the Modification of Enzyme Synthesis During Evolution. Journal of Biological Chemistry 267: 10108-10115

BIOGRAPHICAL SKETCH

NAME **Mauricio J. Reginato**	POSITION TITLE **Assistant Professor of Biochemistry and Molecular Biology**
eRA COMMONS USER NAME **reginato1**	

EDUCATION/TRAINING			
INSTITUTION AND LOCATION	DEGREE *(If applicable)*	YEAR(s)	FIELD OF STUDY
Penn State University	B.S.	1989	Biology/Physiology
University of Pennsylvania	Ph.D.	1998	Pharmacology
Harvard Medical School	Postdoctoral	2004	Cancer Cell Biology

POSITIONS AND EMPLOYMENT:

6/89-8/92 **Associate Scientist**, Department of Investigative Toxicology, SmithKline Beecham Pharmaceuticals, King of Prussia, PA

9/92-5/98 **Graduate Student**, Laboratory of Dr. Mitchell A. Lazar, Department of Pharmacology, University of Pennsylvania, Philadelphia, PA

5/98-7/04 **Postdoctoral Fellow**, Laboratory of Dr. Joan S. Brugge, Department of Cell Biology, Harvard Medical School, Boston, MA

8/04- Assistant Professor**, Co-Director Imaging Facility, Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, PA**

Other Experience and Professional Memberships

2006-present Grant Reviewer, New Jersey Commission on Cancer Research

2009 Ad-hoc Grant Reviewer, Ireland Health Research Board

2004-present Ad-hoc reviewer, Genes & Dev., Oncogene, Mol. Bio. Cell, J. Biol. Chem., Breast Cancer Research

2010 Grant Reviewer, Department of Defense Breast Cancer Research Program

2010 AACR 2011 Annual Meeting Program Committee: Member of the Cellular Stress Responses Subcommittee

Honors/Award

1991: Impact Award-Smithkline Beecham Pharmaceuticals
1992: Fontaine Fellowship Predoctoral Award-University of Pennsylvania
1992: Predoctoral Fellowsip Award-NIGMS
1995: Predoctoral Minority Award-Merck-Dupont Pharmaceuticals
1998: Saul Weingrad Best Dissertation Award: Pharmacological Sciences, University of Pennsylvania
1999: Susan G. Komen Breast Cancer Foundation Postdoctoral Fellowship
2010: Professional Enrichment and Growth Award, Drexel University College of Medicine
2010: Young Investigator Award, Drexel University College of Medicine

C. Selected Peer-reviewed Publications (Selected from 23 peer-reviewed publications)

Most relevant to the current application

1. Caldwell, S. A., Jackson, S. R., Shahriari, K. S., Lynch, T., Sethi, G., Walker, S., Vosseller, K., and **Reginato, M. J.** (2010) Nutrient Sensor O-GlcNAc Transferase Regulates Breast Cancer Tumorigenesis via Targeting of the Oncogenic Transcription Factor FoxM1. Oncogene, May 13; 29:2831-42. PMCID: 20190804
2. Whelan, K. A., Caldwell, S. A., Shahriari, K., Jones, L., Johannes, G. and **Reginato, M. J.** (2010) Hypoxia suppression of Bim and Bmf blocks anoikis and luminal clearing during mammary morphogenesis. Mol. Biol. Cell, 21: 3829-3837. PMID: 20861305
3. Haenssen, K. K., Caldwell, S. A., Shahriari, K., Jackson, R., Whelan, K., Klein-Szanto, A., and **Reginato, M. J.** (2010) ErbB2 requires integrin α5 for anoikis resistance via c-Src regulation of receptor activity in human mammary epithelial cells. J. of Cell Science, Apr 15; 123:1373-82. PMCID: 20332114
4. Palacios, C., **Reginato, M. J.** and López-Rivas A. (2010) Cellular $FLIP_L$ plays a survival role and regulates morphogenesis in breast epithelial cells. Biochim Biophys Acta. Oct 13. [Epub ahead of print]
5. Sodunke, T. R., Turner, K. K., Caldwell, S. A., McBride, K. W., **Reginato, M. J.** and Noh, N. (2007) Micropatterns of Matrigel for Three-Dimensional Epithelial Cultures. Biomaterials 28:4006-4016. PMCID: 17574663

Additional recent publications of importance (in chronological order)

1. Katz, D., **Reginato, M. J.,** and Lazar, M. A. (1995) Functional regulation of thyroid hormone receptor variant TR□2 by phosphorylation. Mol. Cell. Biol. 15, 2341-2348. PMCID: 7739517
2. **Reginato, M. J.,** Zhang, J., and Lazar M. A. (1996) DNA-independent and DNA-dependent mechanisms regulate the differential heterodimerization of the isoforms of the thyroid hormone receptor with retinoid X receptor. J. Biol. Chem. 271, 28199-28205 PMCID: 8910436
3. Adams, M., **Reginato, M. J.,** Shao, D., Lazar, M. A., and Chatterjee, K. (1997) Transcriptional activation by peroxisome proliferator-activated receptor □ is inhibited by phosphorylation at a consensus mitogen-activated protein kinase site. J. Biol. Chem. 272, 5128-5132. PMCID: 9030579
4. **Reginato, M. J.,** Krakow, S. L., Bailey, S. T., and Lazar, M. A. (1998) Prostaglandins promote and block adipogenesis through opposing effects on PPAR□. J. Biol. Chem. 273, 1855-1858. PMCID: 9442016
5. Shao, D., Rangwala, S. M., Bailey, S. T., Krakow, S. L., **Reginato, M. J.,** and Lazar, M. A. (1998) Interdomain communication regulating ligand binding by PPAR□□ Nature 396, 377-380. PMCID: 9845075
6. **Reginato, M. J.,** Bailey, S. T., Krakow, S. L., Minami, C., Ishii, S., Tanaka, H., and Lazar, M. A. (1998) A potent antidiabetic thiazolidinedione with unique PPAR□-activating properties. J. Biol. Chem. 273, 32679-32684. PMCID: 9830009
7. Debnath, J., Mills, K. R., Collins, N., **Reginato, M. J.,** Muthuswamy S., and Brugge, J. S. (2002) The role of apoptosis in creating and maintaining luminal space within normal and oncogene expressing mammary acini. Cell 111, 29-40. PMCID: 12372298
8. **Reginato, M. J.,** Mills, K. R., Paulus, J. K., Lynch, D. K., Sgroi, D.C., Debnath J., Muthuswamy S., and Brugge, J. S. (2003) Integrins and EGFR coordinately regulate the pro-apoptotic protein Bim to prevent anoikis. Nature Cell Biol. 5, 733-740. PMCID: 12844146

9. Mills. K. R., **Reginato, M. J.,** Debnath, J., Queenan, B., and Brugge, J. S. (2004) Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is required for induction of autophagy during lumen formation in vitro. Proc. Natl. Acad. Sci. USA, 101, 3438-3443. PMCID: 14993595
10. **Reginato, M. J.,** Mills, K. R., Becker, E. B., Lynch, D. K., Bonni, A., Muthuswamy S., and Brugge, J. S. (2005) Bim regulation of lumen formation in cultured mammary epithelial acini is targeted by oncogenes. Mol. Cell. Biol. 25, 4591-4601. PMCID: 15899862
11. Collins, N., **Reginato, M. J.,** Paulus, J. K., LeBear, J., and Brugge, J. S. (2005) G1/S cell cycle arrest provides anoikis resistance through Erk-mediated Bim suppression. Mol. Cell. Biol. 25, 5282-5291. PMCID: 15923641
12. **Reginato, M. J.** and Muthuswamy, S. K. Illuminating the Center: Mechanisms Regulating Lumen Formation and Maintenance in Mammary Morphogenesis. (2006) J. Mammary Gland Bio & Neoplasia, 11:205-11. PMCID: 17115263

Research Support.

Ongoing Research Support:

Project Title: **Role of nutrient sensor O-GlcNAc transferase in regulating cancer**
Sponsor: NIH/NCI, 1R01CA155413-01
Project Period: 2/11-1/16
P.I: Mauricio Reginato

Completed Research Support

Project Title: **Targeting Protein O-GlcNAc Modifications in Breast Cancer**
Sponsor: Department of Defense, Breast Cancer Research Program (BCRP), Synergistic Idea Award, BC074374
Project Period: 8/08-8/10
P.I: Mauricio Reginato

Project Title: **MNK1/2 is Required for Hypoxia-Mediated Cell Survival of Breast Cancer Cells**
Sponsor: Department of Defense, BCRP, Concept Award, BC086596
Project Period: 5/09-4/10
P.I: Mauricio Reginato

Project Title: **Identification of Biomarkers and Therapeutic Targets in 3D Hypoxic Breast Cancer Model**
Sponsor: Drexel Univ. Translational Foundation Grants
Project Period: 5/09-4/10
CO-P.I: Mauricio Reginato and Gregg Johannes

Project Title: **Protein O-GlcNAc Modifications are Required for Breast Cancer Cell Survival**
Sponsor: Department of Defense, BCRP, Concept Award, BC062762
Project Period: 7/07-8/08
P.I: Mauricio Reginato

Project Title: **Role of O-GlcNAc Modifications in Breast Cancer**
Sponsor: Drexel Univ. Translational Foundation Grants
Project Period: 11/06 -11/07
P.I: Mauricio Reginato