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TITLE: The Contribution of Hypoxia-Inducible Factor (HIF)-1a to Normal Mammary Gland Development and Mammary Tumorigenesis

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13. ABSTRACT (Maximum 200 Words)

During pregnancy the mammary epithelium and it supporting vasculature rapidly expand to prepare for lactation. To investigate the role of oxygenation and metabolism in these processes, the oxygen-responsive component of the hypoxia-inducible factor (HIF)-1 complex, *HIF-1* ⟨, was deleted in the murine mammary gland using the Cre/*loxP* system. Although vascular density was similar, loss of HIF-1⟨ impaired mammary differentiation and lipid metabolism, culminating in lactation failure (**Objective#1**). HIF-1⟨ over-expression has been reported in breast tumors. Therefore, we next deleted the von Hippel Lindau (*VHL*) gene, which results in constitutive over-expression of HIF-1⟨, in order to determine if HIF-1⟨ over-expression directly contributes to mammary tumorigenesis (**Objective#2**). These studies have demonstrated that VHL is an important mediator of normal alveolar outgrowth and survival during pregnancy and lactation. However, neither deletion of VHL, nor the corresponding increased expression of HIF-1⟨ in the mammary epithelium, is sufficient to induce breast tumorigenesis. Observation of wild type or VHL deleted mice crossed to the MMTV-neu breast model is in progress, but no tumors have been observed to date in mice of either genotype. In conclusion, mammary gland development and epithelial cell function depend on the tightly regulated balance of HIF-1⟨ and VHL function.

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Introduction to the Hypoxic Response and the role of HIF-1α

In response to hypoxia, tissues attempt to restore homeostasis by regulating cellular metabolism and by inducing angiogenesis (reviewed in (Semenza, 2000)]). Both of these processes are primarily regulated by a heterodimeric transcription factor complex known as the Hypoxia Inducible Factor-1, or HIF-1 (Semenza, 2000). The HIF-1 heterodimer includes HIF-1α, a basic helix-loop-helix (bHLH) protein induced and stabilized by hypoxia, and the aryl hydrocarbon receptor nuclear translocator (ARNT) protein (also termed HIF-1β), which is expressed constitutively and heterodimerizes with multiple bHLH partners.

Under normoxic conditions, HIF-1α protein is rapidly degraded through targeted ubiquitination mediated by direct binding of its oxygen dependent domain (ODD) to the β subunit of von Hippel Lindau (VHL) tumor suppressor protein (reviewed in (Kondo and Kaelin, 2001)). In response to hypoxia, HIF-1α protein accumulates, due to decreased interaction with VHL (Krek, 2000). An increase in HIF-1α protein is first detectable at partial pressures of oxygen equivalent to 6% O_2, and becomes maximal between 0.5-1.0% O_2 (Stroka et al., 2001).

In a hypoxic environment, HIF-1 activates the hypoxic response elements (HREs) of target gene regulatory sequences (Huang et al., 1998; Salceda and Caro, 1997), resulting in the transcription of genes implicated in the control of metabolism and angiogenesis, as well as apoptosis and cellular stress (reviewed in (Giordano and Johnson, 2001)). Some of the direct targets include erythropoietin, the angiogenic factor vascular endothelial growth factor (VEGF), glucose transporters and multiple glycolytic enzymes. The connection between the hypoxic response and angiogenesis is also clear from the study of patients with VHL disease, an autosomal, dominantly inherited cancer syndrome. These patients often develop renal clear cell carcinomas

that are massively hypervascular, with highly elevated levels of VEGF expression due to constitutive HIF-1 activity (Kondo and Kaelin, 2001).

REPORT BODY-OBJECTIVE 1

Objective 1: To determine the function of HIF-1α during normal mammary development

As previously reported in the year 2 summary, all tasks were completed and the following key observations made regarding HIF-1α function in the developing murine mammary gland:

Key Research Accomplishments-Objective #1:

- We have confirmed that HIF-1α protein is expressed in purified mammary epithelial cells and is induced by hypoxia.
- We have confirmed that the hypoxic response is conserved in mammary epithelial cells.
- We have demonstrated a role for HIF-1α in secretory differentiation of alveoli.
- We have demonstrated that loss of HIF-1α blocks milk production and impairs milk secretion.
- We have demonstrated that loss of HIF-1α impacts milk nutrition.
- We have demonstrated that the angiogenesis that occurs during pregnancy during normal mammary gland development is HIF-1α-independent. There were no observed differences in microvessel patterning or density in HIF-1α null glands.
- We have determined that there are no differences in the rates of proliferation or apoptosis in HIF-1α null mammary tissue.
- We have discovered the first mouse model to separate mammary epithelial cell proliferation from differentiation.
-We have determined that loss of HIF-1α does not impact VEGF transcription *in vivo*.

What does this work mean?

In order to provide a foundation for understanding the role of HIF-1 in mammary tumorigenesis, the function of HIF-1α was investigated during normal mammary gland development under objective #1 (Seagroves et al. 2003). Because the majority of human tumors, including breast tumors, contain hypoxic areas, which are more resistant to radiation and chemotherapy (Brown et al. 1998), an enhanced understanding of the molecular mechanisms of HIF-1α function could potentially result in development of new compounds that control mammary epithelial cell fate and/or specifically target hypoxic breast tumor cells.

REPORT BODY-OBJECTIVE 2

Revised Objective #2 (approved in 2003): To determine if over-expression of HIF-1α in the mammary gland, achieved through conditional deletion of VHL, results in development of mammary tumors.

Background Relevant to New Objective #2:

HIF-1α has been demonstrated to be up-regulated in a variety of human solid tumors, in particular breast tumors that exhibit high rates of proliferation (Bos et al., 2001; Zhong et al., 1999). Zhong et al. reported that HIF-1α protein was over-expressed in breast tumors, as well as bordering "normal" areas adjacent to tumors, but not in normal breast tissue (Zhong et al., 1999). These observations in breast tumors are consistent with our previous findings previous that HIF-1α functions as a positive regulator of tumor growth (Ryan et al., 2000; Seagroves and Johnson, 2002). In a subsequent study, the level of HIF-1α expression in breast tumors was correlated with other prognostic factors. Specifically, in ductal carcinoma *in situ* (DCIS) lesions, relatively high

levels of HIF-1α expression were associated with increased proliferation as well as increased expression of VEGF and the estrogen receptor (Bos et al., 2001). On the other hand, HIF-1α expression did not correlate with p53 expression, supporting our own laboratory's observations that p53 expression is independent of the effects of loss of HIF-1α on cell growth, metabolism or tumorigenesis (Ryan et al., 2000).

Because over-expression of HIF-1α can be achieved by deletion of the VHL protein, conditional deletion of VHL is a powerful method to determine if HIF-1α overexpression contributes to or is a result of tumorigenesis. The use of the VHL conditional deletion model has permitted our lab to directly test whether or not over-expression of HIF-1α contributes to tumorigenesis, or results from tumor adaptation to hypoxia. Finally, because a majority of mammary tumor models require multiple pregnancies to stimulate development of mammary tumors, using this approach, we have been able to determine that VHL plays an important role in normal mammary gland development by observing the effects of its deletion on the *first round* of pregnancy, lactation and involution.

Revised Statement of Work-Objective #2

Months 13-31 (October 2002-April 2004)

Task 1-Bred mice to generate test mice (genotype= VHL double-*floxed*, VHL *DF*; Cre-positive) and control females (genotype= VHL *DF*; Cre-negative), using either MMTV-Cre (line D) or WAP-Cre transgenic founders.

Task 2-Perform Western blotting for VHL protein on whole cell extracts prepared from normal mammary gland tissues isolated from wild type mice to determine the expression pattern of VHL.

Task 3-For each Cre transgenic line, biopsy the inguinal pairs of mammary glands from sacrificed test and control mice over the course of mammary development. Two hours prior to biopsy of both inguinal glands, inject all mice with 0.1ml bromodeoxyuridine (BrdU)

Task 4-Compare paraffin-embedded sections from test and control mice over the course of development for the first round of pregnancy for the following characteristics:

-proliferation rates by immunostaining for incorporated BrdU

-percentage of cells undergoing apoptosis, by TUNEL technique

-microvessel density by CD31 staining followed by Chalkley counting

-expression of HIF-1α protein, VEGF, PGK-1, Glut-1

Task 5-Begin to constitutively breed another cohort of female mice to attempt to induce mammary tumors by leaving a male with the female and weaning each litter at day 20 of gestation

Months 25-36 (October 2003-September 2004):

Task 6-Continue observing the constitutively bred female mice for the appearance of tumors.

Task 7-Record date of onset when tumor first palpable, continue to measure tumor growth by caliper

bi-weekly until tumors reach a pre-determined size, likely to be 1cm x 1cm.

Task 8-Upon tumors growing to the pre-determined size, sacrifice mice, biopsy mammary tumors, fix, prepare for sectioning and archive.

Task 9-Compare time to onset, histology, tumor grade, pathology between test and control mice

Task 10-Determine if areas of pathology correspond to over-expression of HIF-1α protein

Task 11-Determine tumor microvessel density, rates of proliferation and apoptosis

Task 12-Compare expression of HIF-1α target genes in tumors

Task 13-Determine expression levels of HIF-1α and correlate to Cre expression in tumors by double

immunohistochemical staining

Task 14-Determine statistical relevance of results

Task 15-*Alternative approach if no tumors are observed:*

*If no tumors are observed as a result of VHL deletion by month 25, I will begin to cross the VHL *DF*, Cre-positive mice to the MMTV-neu mammary tumor model available from Charles River Laboratories. Approximately 50% of MMTV-neu-positive female mice develop mammary tumors by 6 months of age, approaching 90% penetrance by 1 year of age.

RESULTS

Summary: New Objective #2 (Months 13-24):

As reported in the year 2 summary, we have completed histological analysis of the effects of deletion of VHL upon normal mammary gland development using each Cre transgenic line during the first round of pregnancy and lactation. We have also continued to multiply bred VHL test and control mice as outlined in Task 6 and have analyzed >5 females for each Cre transgenic line for up to 8 rounds of breeding and 24 months of age. The important conclusion from these studies is that no tumors or hyperplastic alveolar nodules (HANs) were observed in either cohort in which VHL was deleted in the mammary epithelium. Therefore, deletion of VHL, nor over-expression of HIF-1α protein, is sufficient to result in mammary tumorigenesis. It is expected that publication of these results will occur in mid-2005. As mentioned in the year 2 summary, as it became apparent that deletion of VHL was not sufficient to produce mammary tumors, we began breeding the VHL *DF*, Cre-positive females to MMTV-neu mammary tumor transgenic mice obtained from Dr. Bill Muller

to create the following genotypes for observation (VHL *DF*; Cre-negative; neu-positive; VHL *DF*; Cre-positive; neu-negative; and VHL *DF*; Cre-positive; neu-positive).

As indicated in the reportable outcomes section of this report, I have begun to search for tenure-track faculty positions and have received two offers that are based on the results of my Department of Defense funded work on HIF-1α and VHL in the mammary gland, one from the University of Minnesota-Minneapolis and one from the University of Tennessee Health Science Center in Memphis, TN. The work described in Tasks 6-13 will be continued in my own independent research lab, including use of the neu mammary tumor transgenic model to determine how the hypoxic response impacts breast tumorigenesis.

Results by Task:

Task 1-Bred mice to generate test mice (genotype= VHL double-*floxed*, VHL *DF*; Cre-positive) and control females (genotype= VHL *DF*; Cre-negative), using either MMTV-Cre (line D) or WAP-Cre transgenic founders.

As reported in the year 2 summary, this task was completed in December 2002. Both VHL *DF* MMTV-Cre (line D)-positive progeny and VHL *DF* WAP-Cre-positive progeny are viable and fertile, as expected.

Task 2-Perform Western blotting for VHL protein on whole cell extracts prepared from normal mammary gland tissues isolated from wild type mice to determine the expression pattern of VHL.

As reported in the year 2 summary, VHL protein expression increased as the number of epithelial cells increased during mammary gland development, suggesting that VHL is predominantly expressed in the mammary epithelium. There was a dramatic increase in expression

from day 6 to day 10 of pregnancy that was maintained throughout lactation, and that decreased during involution.

Task 3-For each Cre transgenic line, biopsy the inguinal pairs of mammary glands from sacrificed test and control mice over the course of mammary development. Two hours prior to biopsy of both inguinal glands, inject all mice with 0.1ml bromodeoxyuridine (BrdU)

As reported in the year 2 summary, I have analyzed mammary gland development at day 15 of pregnancy (15-P), day 18 of pregnancy (18-P) and day 10 lactation (10-L) for MMTV-Cre line D and WAP-Cre lines (n>4 Cre-positive; n>4 Cre-positive/timepoint). In VHL null mammary tissue regardless of the Cre transgene used, there is a decrease in cell number at day 15 of pregnancy, and a corresponding 30% decrease in BrdU incorporation. Furthermore, at day 15 of pregnancy, there is a block in differentiation since all of the alveoli in these VHL null glands are collapsed and do not accumulated lipid or proteinaceous milk precursors. By day 18 of pregnancy, the VHL null alveoli are only partially differentiated compared to wild type. Interestingly, even with these changes in alveolar architecture, the VHL null dams are able to nurse their pups and pup growth is normal at first lactation in contrast to the HIF-1α null mice, which failed to successfully nurse at first lactation.

As previously reported in the year 2 summary, the glands of VHL null females biopsied during the second round of lactation were filled with fewer alveoli per field and were less well-differentiated than during the first lactation, however pup growth and weight gain was unaffected. During months 25-36, we have continued to multiply breed the mice to attempt to induce tumors. We have observed several (n>4) females of each genotype up to 24 months of age and up to 8 rounds of lactation. Interestingly, instead of tumor formation as expected, we have observed that in

response to deletion of VHL, fewer alveoli occupied the mammary fat pad, and that these alveoli became less differentiated with each successive round of lactation.

For example, **Figure 1** shows representative images of H&E-stained sections from VHL wild type or null mammary glands at either the first round of lactation (*A-B*), the third round of lactation (*C-D*), or the fifth round of lactation (*E*). As expected, in wild type mice, the mammary gland is fully differentiated and no gross changes in histology are noted between the first lactation and any subsequent period of nursing. In contrast, in the VHL null tissue, there is a striking decrease in mammary epithelial cell number, differentiation and intra-alveolar organization. The majority of the alveoli present in the VHL null gland at the third lactation are collapsed and devoid of milk (panel D), and pups nursing these glands weigh 50% less than those nursing wild type dams by day 10 of lactation. In addition, only 30% of the mammary gland is filled with epithelium by the third round of lactation.

Dams are unable to support pups for even one day by the fourth round of lactation. By the fifth round of lactation, only 10% of the mammary gland is filled with epithelium, and these alveoli appear de-differentiated (panel E). Interestingly, red blood cells appear to be intermingled with the alveoli (panel E) suggesting that there has been a disruption of the basement membrane, which normally seals the epithelium from the supporting stromal compartment. Furthermore, deletion of VHL in the mammary epithelium has impacted the vasculature in the stroma since the mammary epithelial-associated vessels are hyper-dilated and there is a general increase in microvessel density per field of H&E sections compared to wild type glands. Based on these observations, it is likely that over-expression and diffusion into the stroma of one of HIF-1α's target genes, secreted VEGF, is responsible for this disruption of the vasculature.

It is important to note that these results are in contrast to what we expected in response to deletion of VHL. We had predicted that deletion of VHL, which functions as a tumor suppressor in the kidney epithelium, would produce mammary epithelial hyperplasias or tumors. However, we have actually observed that fewer and less well-differentiated alveoli are present in the gland with each round of lactation. These phenotypes suggest that either VHL plays an important role in differentiation of adult tissues such as the mammary gland, which would be a novel finding, and/or that VHL is somehow implicated in mammary epithelial progenitor cell renewal. Therefore, the complete function of VHL in tissue homeostasis is not clear and requires further investigation.

Task 4-Compare paraffin-embedded sections from test and control mice over the course of development for the first round of pregnancy .

Sections have been archived from VHL wild type and null pregnant mice injected with BrdU, and BrdU immunostaining has been performed. Cell counting to determine percentage of proliferating cells at day 15 of pregnancy has revealed a 30% decrease in cell proliferation in the VHL null gland. No significant difference in the percentage of cells undergoing apoptosis was observed between genotypes following anti-caspase-3 or TUNEL immunostaining during pregnancy or lactation. As previously reported in the year 2 summary, we confirmed that, upon deletion of VHL, HIF-1α is indeed over-expressed in mammary epithelial cells, and that the HIF-1 target gene Glut-1 is also over-expressed. In addition, although CD31 immunostaining has yet to be performed, it is evident that loss of VHL results in increased angiogenesis and blood vessel dilation, as expected based on finding in VHL clinical patients. Several attempts to stain the sections with anti-VEGF antibodies have been unsuccessful, either resulting in no signal or in background level staining.

Task 5-Begin to constitutively breed another cohort of female mice to attempt to induce mammary tumors by leaving a male with the female and weaning each litters at day 20 of gestation.

No tumors or HANs have been observed in VHL null glands by either whole mount preparation or H&E staining in multiply-bred females up to 24 months of age and following eight rounds of breeding. Therefore, neither deletion of VHL, nor corresponding over-expression of HIF-1α protein, is sufficient to produce mammary tumorigenesis.

Task 15-*Alternative approach if no tumors are observed:*

*If no tumors are observed as a result of VHL deletion by month 25, I will begin to cross the VHL *DF*, Cre-positive mice to the MMTV-neu mammary tumor model available from Charles River Laboratories.

The goal of these experiments is to determine how deletion of VHL in the mammary epithelium, achieved through expression of either MMTV-Cre or WAP-Cre, and the corresponding over-expression of HIF-1α will impact mammary tumorigenesis in the well-characterized MMTV-neu mammary tumor transgenic model. The genotypes to be observed are: VHL *DF*, Cre-negative, neu-positive; VHL *DF*, Cre-positive, neu-negative; and VHL *DF*, Cre-positive, neu-positive). In the last report, we had anticipated that a majority of these tri-genic mice would have developed tumors by the time the fellowship period had ended in September 2004. However, breeding the mice to generate females that harbored both *floxed* alleles of VHL (DF), either the MMTV-Cre transgene or the WAP-Cre transgene, AND the MMTV-neu tumor transgene required 5 months of crosses and genotyping spanning October 2003 to March 2004. We then expanded these lines to generate females of each genotype above.

As of October 2004, we have maintained in our colony a cohort of 6-10 females of each genotype. We are currently palpating the females to determine tumor onset and burden per animal. However, none of the females of either genotype have developed a palpable tumor by the time of writing this report. Although the reported latency of the tumors in the MMTV-neu transgenic is about six months of age in a pure FVB background, it is possible that upon breeding the neu transgenic mice to the VHL and Cre mice, which are maintained in mixed genetic backgrounds, the tumor latency will have increased. Dr. Johnson, my mentor, has agreed to permit me to take these models with me upon securing a faculty position. Therefore, these experiments will be continued in my own independent research laboratory upon securing a position at either University of Minnesota or University of Tennessee at Memphis as discussed in the reportable outcome section.

Key Research Accomplishments-Objective #2:

- We have confirmed that VHL protein is expressed in the normal murine mammary gland and that expression increases during pregnancy, is maintained at lactation and decreases at involution.
- We have demonstrated that VHL regulates differentiation in the normal mouse mammary gland during pregnancy and lactation.
- We have confirmed that loss of VHL results in over-expression of HIF-1α protein in the mammary epithelium.
-We have demonstrated that loss of VHL results in mammary-associated vasculature hypervascularity and vessel dilation.
-We have demonstrated that the effects of deletion of VHL upon mammary epithelial cell architecture and function progressively worsen with each round of lactation.

-We have demonstrated that neither loss of VHL, nor over-expression of HIF-1α protein, is sufficient to result in hyperplastic changes in the mammary gland, or development of mammary tumors.

What do these results mean?

Based on these results, we have demonstrated that loss of VHL impairs cellular proliferation and differentiation of the normal murine mammary gland, a novel observation. Therefore, it is clear that both deletion or over-expression of HIF-1α is detrimental to normal mammary gland development, and that the hypoxic response must be tightly regulated in mammary epithelium. It is also now clear that loss of VHL function does not produce mammary tumors in aged, multiply-bred mice. How deletion of VHL, and therefore, over-expression of HIF-1α, impacts mammary tumorigenesis in the well-characterized tumor model MMTV-neu is in progress. The results of these studies, to be completed upon obtaining an independent faculty position, will reveal how manipulation of the hypoxic response pathway affects mammary tumor growth, pathology and metastasis to the lung.

Reportable Outcomes since Year 1:

Manuscripts:

Seagroves, T.N., Hadsell, D., McManaman, J., Palmer, C., Liao, D., McNulty, W., Welm, B., Wagner, K.-U., Neville, M. and R.S. Johnson. 2003. HIF-1alpha is a critical regulator of secretory differentiation and activation, but not vascular expansion, in the mouse mammary gland. *Development* **130**: 1713-1724.

Abstracts:

* Gordon Research Conference on Mammary Gland Biology, May 2004

* Keystone Conference, Hypoxia, Steamboat Springs, CO, February 2004

* Oxygen and the Cell, Max Planck Institute, Berlin, Germany, September 2003.

* Keystone Conference, Angiogenesis, Banff, Canada, February 2002.

* Gordon Research Conference on Mammary Gland Biology, Barga, Italy, 2002.

Presentations:

* Invited plenary speaker, Gordon Research Conference, Barga, Italy, May 2004, "The Role of HIF-1α in Lactation and Lipid Secretion".

* Department of Defense, Era of Hope meeting, Orlando, FL, 2002.

Funding received based in part on work supported by this award:

NIH renewal of CA82515, awarded to Randall S. Johnson, to support the research costs associated with this project and other projects ongoing in the laboratory to investigate the hypoxic response in a variety of tissue types.

Employment Opportunities Received Based on Experience/Training Supported by This Award:

Interviews for Tenure-Track Faculty Positions (June 2004-present):

1) Nationally-advertised tenure-track faculty position at the University of Minnesota, Minneapolis, Cancer Center, Department of Genetics, Cell Biology and Development. *An offer is currently under consideration.*

2) Nationally-advertised tenure-track faculty position at the University of Massachusetts, Worcester, MA, Department of Cell Biology.

3) Nationally-advertised tenure-track faculty position in the Laboratory of Pathology, National Cancer Institute.

4) Nationally-advertised tenure-track faculty position in the Department of Physiology at the Medical School of the University of Maryland, Baltimore, MD.

5) Tenure-track faculty position at the University of Tennessee Health Science Center, Memphis, TN, Cancer Center appointment, Department of Pathology. *An offer is currently under consideration.*

References

Bos, R., Zhong, H., Hanrahan, C. F., Mommers, E., Semenza, G. L., Pinedo, H. M., Abeloff, M. D., Simons, J. W., van Diest, P. J., and van der Wall, E. (2001). Levels of hypoxia-inducible factor-1α during breast carcinogenesis. J Natl Cancer Instit *93*, 309-314.

Brown, J. M., and Giaccia, A. J. (1998). The unique physiology of solid tumors: opportunities (and problems) for cancer therapy. Cancer Research *58*, 1408-1416.

Giordano, F., and Johnson, R. (2001). Angiogenesis: the role of the microenvironment in flipping the switch. Curr Opin Genet Dev *11*, 35-40.

Huang, L. E., Gu, J., Schau, M., and Bunn, H. F. (1998). Regulation of hypoxia-inducible factor 1alpha is mediated by an O2-dependent degradation domain via the ubiquitin-proteasome pathway. Proceedings of the National Academy of Sciences of the United States of America *95*, 7987-7992.

Kondo, K., and Kaelin, W. G., Jr. (2001). The von Hippel-Lindau tumor suppressor gene. Exp Cell Res *264*, 117-125.

Krek, W. (2000). VHL takes HIF's breath away. Nature Cell Biology *2*, E121-123.

Ryan, H. E., Poloni, M., McNulty, W., Elson, D., Gassmann, M., Arbeit, J. M., and Johnson, R. S. (2000). Hypoxia-inducible factor-1 alpha is a positive factor in solid tumor growth. Cancer Research *60*, 4010-4015.

Salceda, S., and Caro, J. (1997). Hypoxia-inducible factor 1alpha (HIF-1alpha) protein is rapidly degraded by the ubiquitin-proteasome system under normoxic conditions. Its stabilization by hypoxia depends on redox-induced changes. Journal of Biological Chemistry *272*, 22642-22647.

Seagroves, T., and Johnson, R. S. (2002). Two HIFs may be better than one. Cancer Cell *1*, 211-213.

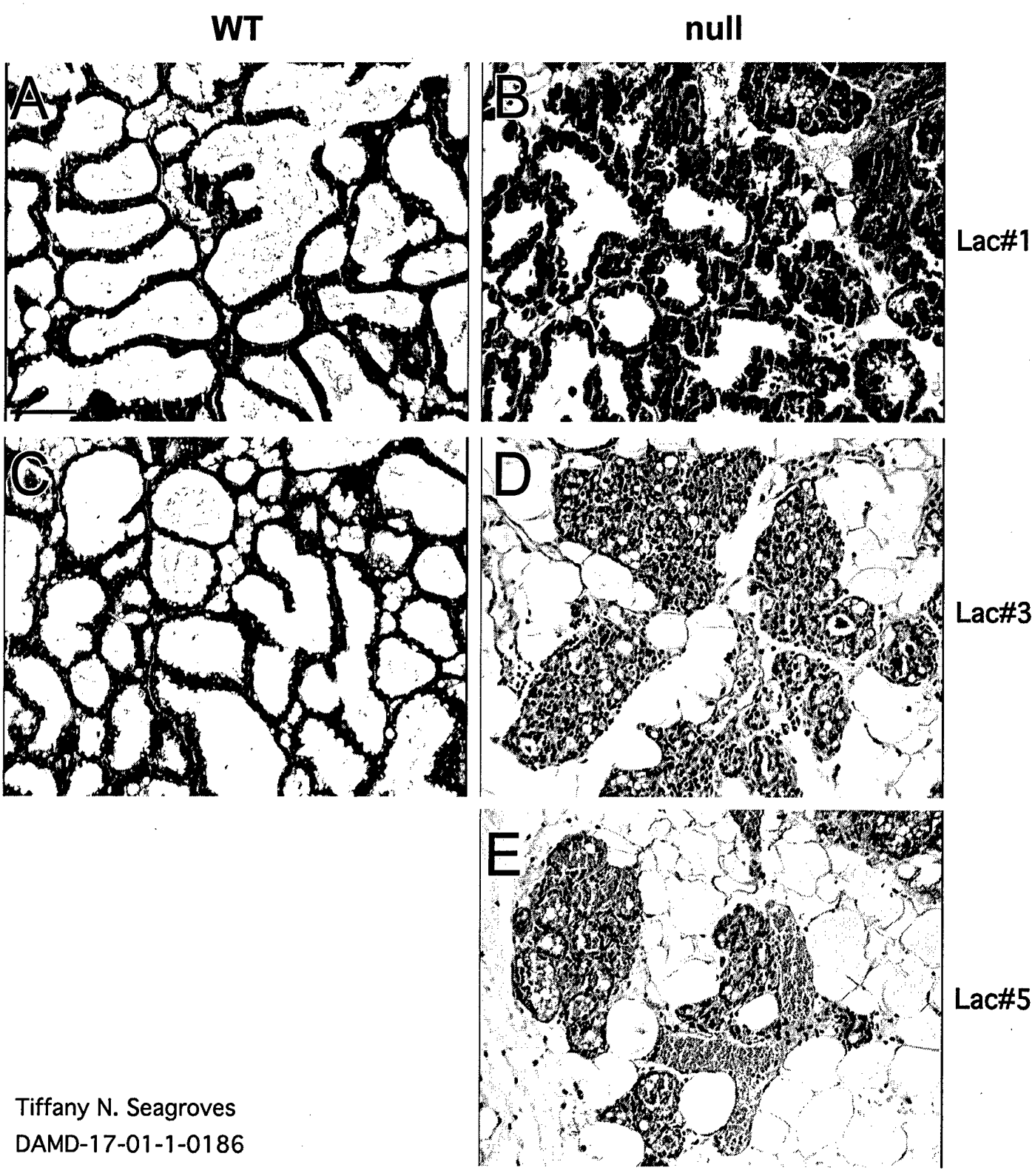
Seagroves, T.N., Hadsell, D., McManaman, J., Palmer, C., Liao, D., McNulty, W., Welm, B., Wagner, K.-U., Neville, M. and R.S. Johnson. (2003). HIF-1alpha is a critical regulator of secretory differentiation and activation, but not vascular expansion, in the mouse mammary gland. Development *130*, 1713-1724.

Semenza, G. L. (2000). HIF-1 and human disease: one highly involved factor. Genes Dev *14*, 1983-1991.

Stroka, D. M., Burkhardt, T., Desbaillets, I., Wenger, R. H., Neil, D. A., Bauer, C., Gassmann, M., and Candinas, D. (2001). HIF-1 is expressed in normoxic tissue and displays an organ-specific regulation under systemic hypoxia. Faseb J *15*, 2445-2453.

Zhong, H., De Marzo, A. M., Laughner, E., Lim, M., Hilton, D. A., Zagzag, D., Buechler, P., Isaacs, W. B., Semenza, G. L., and Simons, J. W. (1999). Overexpression of hypoxia-inducible factor 1alpha in common human cancers and their metastases. Cancer Research *59*, 5830-5835.

FIGURE 1-Effects of VHL deletion on Multiple Lactations



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