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TITLE: WDR26 in Advanced Breast Cancer: A Novel Regulator of the P13K/ AKT Pathway

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14. ABSTRACT

The PI3K/AKT pathway is one of the most deregulated pathways in breast cancers (>70%), and a major contributor to tumor progression. PI3Ks and AKTs comprise of multiple isoforms that play a critical role in a wide variety of physiological progresses. Moreover, during cancer progression, different PI3K and AKT isoforms may have different and even opposite roles. Notably, PI3Kβ and AKT2 have been identified as the major isoform that contribute to breast cancer growth and metastasis. Yet, it is not yet clear how to specifically target the PI3Kβ/AKT2 without causing wide spread side effects. In this proposal, we aim to test the hypothesis that WDR26 functions as a novel regulator of the PI3Kβ/AKT2 pathway, and a previously unidentified marker/therapeutic target in advanced breast cancer, in particular, triple negative breast cancer (TNBC). Our results thus far demonstrated that WDR26 selectively interacts with Gβγ, PI3Kβ, and AKT2 and serves as a scaffold that fosters their interaction to promote PI3K/AKT activation; and in highly malignant and invasive breast tumors, upregulated WDR26 overactivates the PI3Kβ/AKT2 pathway, promoting breast tumor growth and metastasis. These findings thus pinpoint WDR26 as a potential therapeutic target for disrupting PI3K/AKT overactivation and breast cancer progression.

15. SUBJECT TERMS

Breast cancer growth and Metastasis, heterotrimeric G protein βγ subunits, G protein-coupled receptors, signal transduction, PI3K, AKT

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1. **Introduction:**

Breast cancer is the second most common cause of cancer death in women in the US. Despite recent advances in the development of new treatments (e.g., targeted therapies) limited options are available for patients with advanced breast cancer, in particular, triple negative breast cancer (TNBC). Thus, it is imperative to develop novel approaches for treating advanced breast cancer.

In over 70% of breast cancers, the PI3K/AKT signaling pathway is dysregulated. This pathway transmits signals downstream from critical cell surface receptors, including receptor tyrosine kinase and G protein-coupled receptors (GPCRs) and, when dysregulated, is believed to promote tumor progression, resistance to available therapies and cancer relapse. PI3K/AKT signaling plays a central role in driving many aggressive breast cancers, making it one of the most hotly pursued therapeutic targets for new breast cancer treatments. However, ongoing concerns remain regarding the efficacy and long-term safety of directly inhibiting enzymatic activities that control a wide spectrum of biological processes. Thus, it is imperative that new strategies be developed for regulating, with a high degree of specificity, the signals this pathway emits, so we can harness the power of the PI3Ks and AKTs to control breast cancer.

Our preliminary studies suggest that WDR26 may function as a scaffold that fosters the interaction between Gβγ, PI3Kβ, and AKT2; and in highly malignant and invasive breast tumors, upregulated WDR26 overactivates the PI3Kβ/AKT2 pathway, promoting breast tumor growth and metastasis. Moreover, WDR26 may serve as a previously unidentified, yet powerful prognostic marker /therapeutic target for advanced TNBC patients. In this proposal, we aim to define precisely the key role of WDR26 in breast tumor (in particular TNBC) development, as well as to determine, using preclinical models of advanced triple negative breast cancer, the therapeutic efficacy of targeting WDR26 with small molecule inhibitors. Our proposed studies could potentially uncover a novel and efficacious approach for developing a new PI3K/AKT-targeted therapy that would improve the outcome for patients affected by advanced breast cancer (in particular, triple negative breast cancer), including the women in the military services. This could be a major breakthrough both in our understanding of the molecular mechanisms that drive TNBC progression and in our effort to eliminate suffering and death caused by advanced breast cancer.

2. **Keywords:** Breast cancer growth and Metastasis, heterotrimeric G protein βγ subunits, G protein-coupled receptors, signal transduction, PI3K, AKT

3. **Accomplishments:**

What were the major goals of the project?

Major Goal 1: Assess how WDR26 regulates PI3K/AKT signaling in breast cancer cells.

Major Goal 2: Determine how breast tumor progression in nude mice is driven by WDR26 regulation of the PI3Kβ/AKT2 pathway.

Major Goal 3: Analyze WDR26 levels in human breast cancer specimens.

Major Goal 4: Determine the therapeutic efficacy of small molecule inhibitors of WDR26 in breast cancer.

What was accomplished under these goals?

As detailed in the last report, we have accomplished much of the major goals 1 and 2. In this report period, we focused on determining whether WDR26 selectively promotes GPCR-mediated AKT2 activation and the structural basis for the selective binding of WDR26 to AKT2 (vs AKT1), analyzing WDR26 levels in human breast cancer specimens, and screening for small molecule inhibitors of WDR26-mediated PI3K/AKT activation.

We found that downregulation of WDR26 impaired LPA- but not EGF-stimulated AKT2 phosphorylation in the triple negative cell lines, MDA-MB231 (Fig. 1) and HCC1806 (not shown). However, it had little effect on AKT1 phosphorylation (Fig. 1). We showed in the last report that WDR26 directly binds AKT2 with a higher capacity than AKT1. To test if direct

binding of AKT2 to WDR26 accounts for its selective activation by WDR26, we are determining the binding sites of WDR26 on AKT2. We have generated a series of AKT2 deletion mutants conjugated with RFP (Fig. 2) and are currently investigating which mutants retain the ability to bind WDR26 by co-immunoprecipitation assays.

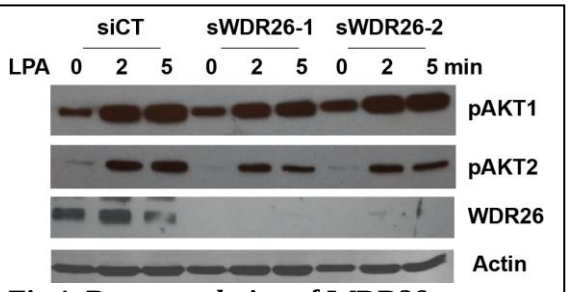

Fig 1. Downregulation of WDR26 selectively impairs LPA-stimulated AKT2 phosphorylation in MDA-MB231 cells.

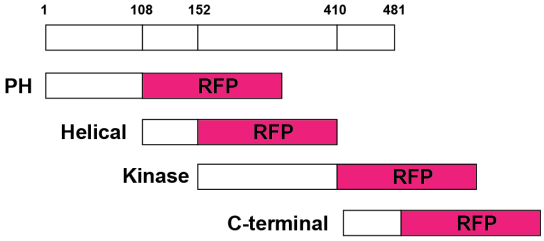

Fig 2. Generation of AKT2 deletion mutants.

ne WDR26 expression level in human breast cancer samples, we have tested the specificity of WDR26 antibodies for IHC staining, using WDR26-deficient MDA-MB231cells and mouse hepatocytes. We found that several commercially available WDR26 antibodies could not detect significant difference in the level of WDR26 in control and WDR26-deficient cells, suggesting that they are not suitable for IHC assays. To obtain WDR26b antibodies suitable for IHC staining, we are currently generating both rabbit and mouse antibodies against WDR26.

To identify small molecule inhibitors of WDR26 that perturb Gβγ-mediated PI3K/AKT activation, we screened a number of small molecules that are known to bind Gβγ, including M119F, M119K and M158C, for their ability to block LPA-stimulated PI3K/AKT activation in MDA-MB231 cells. As compared to gallein, all of these inhibitors did not affect LPA-stimulated AKT activation (Fig. 3), suggesting that they are unlikely potent inhibitors of WDR26. Therefore, our future studies will focus on characterizing gallein as an inhibitor of WDR26.

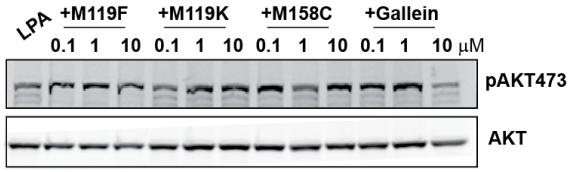

Fig 3. Effects of small molecule inhibitors on LPA-stimulated AKT phosphorylation in MDA-MB231 cells. Cells were pretreated for the indicated inhibitors at the indicated concentrations for 30min and then stimulated with LPA (10 μM) for 5min.

What opportunities for training and professional development has the project provided?

This project has provided financial support for two research interns and two postdoctoral fellows as detailed in the "individuals worked on the project table. It gave these individuals the opportunities to learn how to perform basic and translational research in breast cancer.

How were the results disseminated to communities of interest?

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?

Identify the WDR26 antibodies suitable for immunohistochemical staining of WDR26 in human breast cancer samples; complete analysis of WDR26 and phospho-AKT expression in human breast cancer samples in major goal 3; complete the studies of determining the therapeutic efficacy of small molecule inhibitors of WDR26 in breast cancer in major goal 4.

4. Impact:

What was the impact on the development of the principal discipline(s) of the project?

Our results thus far identify WDR26 as a novel regulator of GPCR-dependent activation of the PI3K/AKT signaling axis in breast tumor cells, and pinpoint WDR26 as a potential therapeutic target for breast cancer.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. **Change or problems:**

Changes in approach and reasons for change: nothing to report

Actual or anticipated problems or delays or plans to resolve them: as detailed above, we have not been able to identify a WDR26 antibody suitable for immunohistochemical analysis of WDR26 levels in human breast cancer samples. Currently, we are trying to generate both polyclonal and monoclonal antibodies against WDR26 in the lab.

Changes that had a significant impact on expenditures.

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals biohazards and/or select agents

Nothing to report.

6. **Products:** nothing to report.
7. **Participants & other collaborating organizations:**

What individuals have worked on the project?

Name:	*Songhai Chen*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	0000-0001-7316-7125
Nearest person month worked:	*1*
Contribution to Project:	Dr. Chen has provided overall administration and direction of the project.

Name:	*Jianling Bi*
Project Role:	*Postdoc*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*7*
Contribution to Project:	*Dr. Bi has been involved in determining the mechanisms by which WDR26 promotes AKT2 activation..*

Name:	*Wei Wang*
Project Role:	*Postdoc*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Wangl has been involved in characterizing WDR26 expression in human breast cancer samples*

Name: *Maddison Lensing*
Project Role: *Research Intern*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *5*

Contribution to Project: *Ms. Lensing has been involved in screening small molecule inhibitors of WDR26.*

Name: *Felix Yang*
Project Role: *Research Intern*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *3*

Contribution to Project: *Mr. Yang has contributed to the construction and characterization of AKT deletion mutants.*

Has there been a change in the active other support of the PD/PI?
Dr. Chen was awarded an R01 from NCI.

R01 CA207889 Chen (PI), Weigel R (Co-PI) 05/01/17-04/30/22
NIH/NCI
"Role of Gi/o-GPCR signaling in breast cancer progression"
This project aims to investigate how Gi/o-GPCRs cross-talk with HER2 signaling to promote tumorigenicity of tumor-initiating cells and drive HER2+ tumor progression and drug resistance
Role: Contact PI

What other organizations were involved as partners? None.

8. **Special Reporting Requirements: n/a**
9. **Appendices:** none.