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Expression and Purification of Recombinant Protein to Generate a Monoclonal Antibody to the PX domain of Tks5 α isoform in Cancer Cells

By
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An undergraduate honors thesis submitted in partial fulfillment of the

requirements for the degree of

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Thesis Adviser

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Abstract

Tyrosine kinase Src substrates, Tks4 and Tks5, are involved in the formation of podosomes and invadopodia during cell migration and cancer cell metastasis. Most recently, the α isoform of Tks5 has been discovered highly expressed in cancer cells, suggesting that the Tks5 α isoform plays essential roles in the regulation of invadopodia. In the Tks protein family, only the Tks5 α isoform contains a PX domain, which binds an important second messenger, PI (3,4) P2. Using a monoclonal antibody as an antigen-specific-probe to detect the protein of interest could be a useful tool that would provide a highly specific recognition at the molecular level. This project aims to develop a highly specific rabbit monoclonal antibody (mAb) to target the human Tks5 α isoform (specifically the PX domain) in cancer cells. In this study we describe the first step of the entire process, immunogen preparation. The results showed that the recombinant protein, hTks5 α PX domain, was successfully expressed in BL21 *E.coli* competent cells and the purified antibody has high immunoreactivity. Other promising results obtained from western blot and immunofluorescence analyses indicated the developing antibody samples have high targeting effects on human Tks5 α isoform PX domain.

Introduction

The impact of cancer worldwide is devastating. For example, cancer is the number two cause of death in the United States in 2013 (Xu et al., 2016). Many studies have investigated how cancer cells invade the body to produce tumor growth and metastasis. Specifically, important research is being done on the role of podosomes and invadopodia in cell migration and metastasis. Both podosomes and invadopodia behave invasively. Both structures are actin rich protrusions of ventral plasma membrane structures that associate with cell attachment and degrade the extracellular matrix, ECM (Gimona et al., 2008; Murphy & Courtneidge, 2011). Podosomes are found in normal cells while invadopodia arise in cancer cells (Gimona et al., 2008; Murphy & Courtneidge, 2011) (see Figure 1). There is much debate in the field regarding the distinction between these two membrane structures — the overall architecture and function display some similarities but the morphological and molecular structures are dissimilar (Murphy & Courtneidge, 2011). For instance, both structures are transient membrane structures that are regulated by several key proteins including the scaffolding proteins (Tks4 and Tks5), the metalloprotease (MT1-MMP), and actin regulators cortactin and (N-WASP) (Murphy & Courtneidge, 2011). However, the half-life of actin turn-over in these two structures are very different. Invadopodia are more stable in comparison to podosomes (Murphy & Courtneidge, 2011). Invadopodia can protrude further to the ECM while podosomes only have minimal protrusion (Murphy & Courtneidge, 2011). In order to characterize the detailed roles of invadopodia in tumor progression and metastasis, it is important to see their localization in vivo (Courtneidge, 2012). In recent years, two crucial tyrosine kinase Src substrates, Tks4 and Tks5, were identified and discovered to be very important in cancer cell invasion and tumor growth.

Both Tks4 and Tks5 are vital for the formation of podosomes and invadopodia (Blouw et al., 2015; Courtneidge, 2012). Research has shown that Tks4 and Tks5 also involved in embryonic development (Courtneidge, 2012).

In humans, Tks4 and Tks5 are encoded by SH3PXD2B and SH3PXD2A genes respectively, and their sequence identity is 47%. (Cejudo-Martin et al., 2014; Courtneidge, 2005). Both Tks4 and Tks5 contain Src phosphorylation sites, proline-rich motifs, Src homology 3 (SH3) domains and a phospho homology (PX) domain — the PX domain in Tks4 is followed by four SH3 domains while the PX domain in Tks5 is located at the N-terminus and followed by five SH3 domains (Cejudo-Martin et al., 2014; Courtneidge et al., 2005; Courtneidge 2012; Stylli et al., 2009) (see Figure 2). No catalytic domain was found in both Tks 4 and Tks5, so they were designated as adaptor or scaffolding proteins that interact with and bind to other proteins and lipids (Courtneidge et al., 2005). Tks4 and Tks5 have specific scaffolding functions in the formation of protrusion of membrane structures (Courtneidge et al, 2005; Courtneidge, 2012). Tks5 was recently found highly expressed in human cancer cells culture (in vitro) and tumor tissue (in vivo), suggesting Tks5 plays a critical role in regulating tumor progression and metastasis (Courtneidge, 2012). Although Tks5 does not have any catalytic activity, it most likely associates with other key regulators for the formation of invadopodia (Buschman et al., 2009; Courtneidge et al., 2005). Phosphatidylinositol 3,4 bisphosphate (PI (3,4) P2) is a phosphorylated inositol lipid, a second messenger activates many essential cell signaling pathways, which binds to the PX domain of Tks5 initiates the formation of invadopodia (Abram et al., 2003; Buschman et al., 2009; Courtneidge, 2012; Oikawa et al, 2008). The transmembrane metalloproteases ADAMs binds to the fifth SH3 domain of Tks5 (Abram et al., 2003; Buschman et al., 2009). The ADAMs play important roles in regulating cell migration, cell adhesion,

proteolysis and signaling (Edwards et al., 2008). Tks5 tyrosine residues are phosphorylated by Src in Src transformed cells (Courtneidge et al., 2005). In prostate cancer cell lines, invasive behavior increased as the activities of Src tyrosine kinase and phosphorylation of Tks5 increased (Burger et al., 2014). Tks5 is structurally related to p47^{phox} protein, one of the foremost cytosolic regulator subunits of the phagocytic NADPH oxidase, which contains one PX domain and two SH3 domains (Buschman et al., 2009; Diaz et al., 2009; Wang et al., 2015). Tks4 and Tks5 interact with p47^{phox}- related organizer to localize Nox1 in invadopodia (Gianni et al., 2011). In the absence of Tks4, Tks5 and other invadopodia proteins accumulated at the membrane, actin polymerization and ECM degradation would not occur suggesting Tks4 play a role to localize a transmembrane metalloprotease, MT1-MMP, in invadopodia (Courtneidge, 2012).

The current focus is on investigating the cellular and molecular mechanism of Tks5 in the formation of invadopodia. The most recent study on investigating the genetic disruption of mouse sh3pxd2a gene identified multiple isoforms of Tks5 (see Figure 3) and discovered the PX domain only appears in the α isoform (long isoform) of Tks5 (Cejudo-Martin et al., 2014). Different alternative transcriptions of the sh3pxd2a gene produce various isoforms of Tks5 (Cejudo-Martin et al., 2014). Another study found that the balance expression between Tks5 α isoform and Tks5 short isoform seems to be more critical for metastasis invasion than the total level of Tks5 alone (Li et al., 2013). Tks5 α isoform was found highly expressed in metastatic primary tumor- and metastasis- derived cells while Tks 5 short isoform was found mainly expressed in nonmetastatic primary tumor-derived cells, furthermore, increased expression of Tks 5 short isoform inhibited gelatin proteolysis and thus decreased the stability of invadopodia (Li et al., 2013).

In the light of the above findings, our goal was to observe the locations of Tks5 α isoform in human cancer cell samples. Tks5 polyclonal antibody (anti-Tks5 1731) was previously generated in the Courtneidge lab but we do not yet have a highly specific monoclonal antibody to target the PX domain of Tks5 α isoform. In this project, the recombinant protein (GST-tagged human Tks5 α PX domain) acts as immunogen for the development of rabbit monoclonal antibody. First, the human Tks5 α PX domain DNA fragment was obtained by polymerase chain reaction (PCR), and cloned into pGEX 4T1 using restriction sites. BL21 *E.coli* cells was used for transformation because this genetic modified lab strain is suitable for transformation and protein expression with high efficiency. The purified protein was sent to Abcam (a company that offers antibody development services), to generate a rabbit monoclonal antibody (mAb) to Tks5 α isoform PX domain. Rabbits can produce high titers of antibodies with high affinity to human epitopes despite the antigens not being able to evoke immune response in mice (Rossi et al., 2005). The process of generating a highly specific mAb takes several months. To produce antibodies, rabbits are injected with an immunogen. Though the rabbits used for generating antibodies are genetically identical, due to the complexity of biological systems they could still respond to the same immunogen differently (Antibody Purification Methods, 2016). At various stages of development, samples of antibodies are sent back to the Courtneidge lab for testing their specificity in targeting the Tks5 α isoform PX domain. These were tested with western blotting and immunofluorescence assays and have thus far been successful. The final goal of this project will be to develop a highly specific rabbit mAb, which acts as an antigen-specific-probe, to target the PX domain of the α isoform of hTks5. In this manner, the locations of hTks5 α isoform can be detected and visualized using immunohistochemistry in samples obtained from cancer patients, and thus helps draw conclusions about how cancers progress and metastasize.

For the development of rabbit mAb, immunogen preparation is the first step of the entire process and followed by immunization, hybridoma creation, fusion and screening, subcloning and finally, hybridoma delivery (Abcam, 2016). Immunogenicity is greatly influenced by the chemical composition of the immunogen — optimal immunogen should have B cell and T cell epitopes, and binding sites for MHC (major histocompatibility complex) class II molecules — and the level of immune response of the injected animals (Dübel, 2014). In order to produce monoclonal antisera, a purified immunogen should be homogeneous. Thus, immunogen preparation is a crucial step for the development of mAb (Antibody Purification Methods, 2016). In the present study, we describe this first step of the entire process including purification and extraction as well as quality and quantity control for the recombinant protein, hTks5 α PX domain. Because P47^{phox} is structurally related to Tks5 α we therefore also constructed and purified GST protein and p47^{phox} PX domain (recombinant protein) for the use of counter-screening agents in the ELISA test by Abcam. The idea was not to pick clones that either reacted with GST or P47^{phox} PX domain.

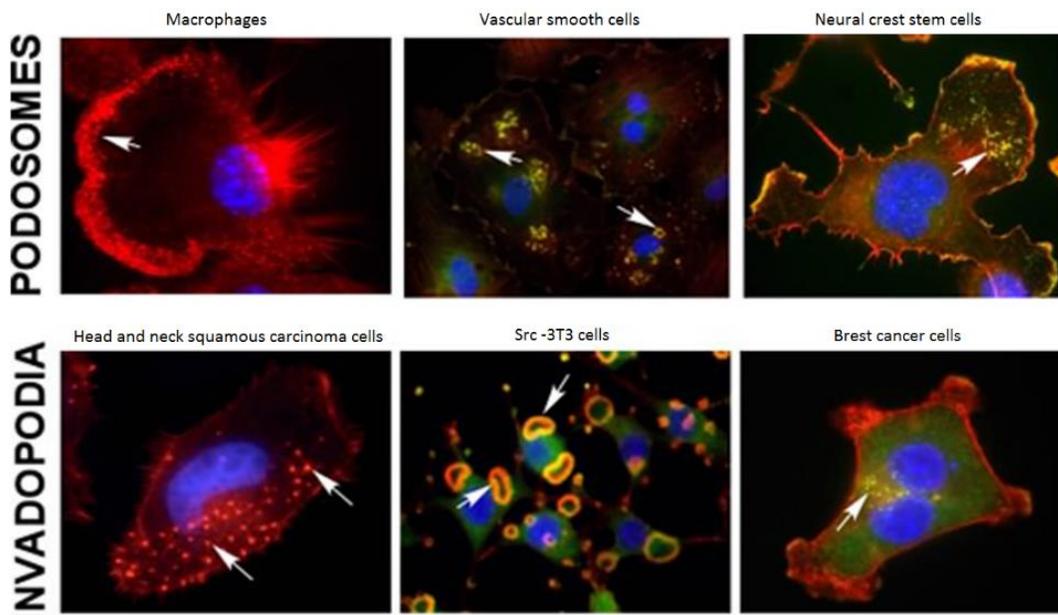


Figure 1. Images of Co-immunostaining cells. Podosomes are found in normal cell types (top). Invadopodia form in various cancer cell types (bottom). White arrows point out the locations of invadopodia and podosomes, F-actin were stained in red and cortactin were stained in green, nuclei were stained in blue (Murphy & Courtneidge, 2011).

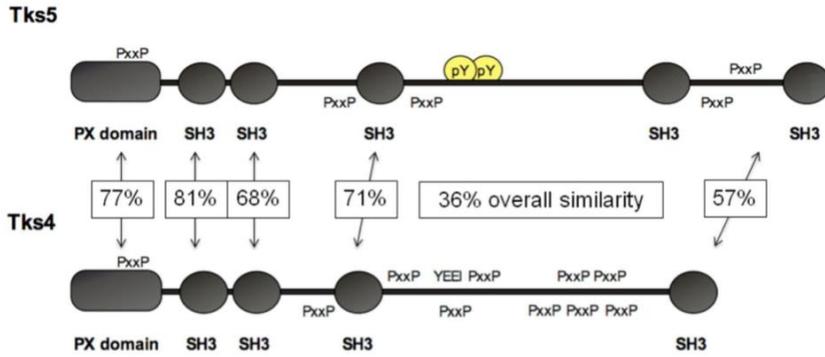


Figure 2. Schematic diagram of the architectures of Tks4 and Tks5. Both proteins contain a PX domain, the SH3 domains, Src phosphorylation sites (pY and YEEI) and proline-rich motifs (PxxP) (Courtneidge, 2012).

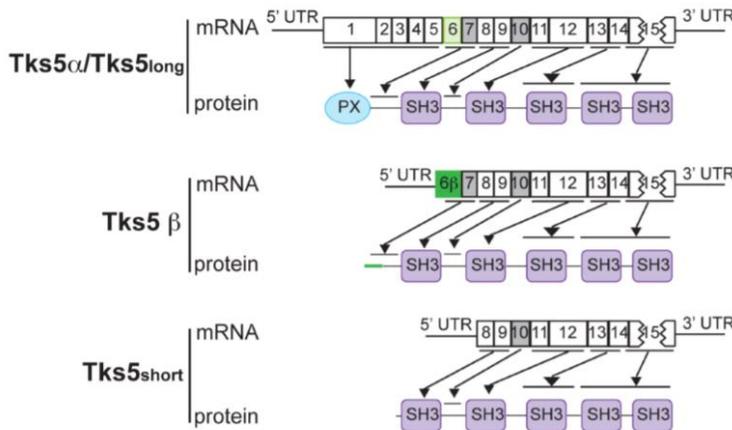


Figure 3. Schematic diagram of the architectures of Tks5 α /Tks5 long, Tks5 β and Tks5 short isoforms. Different isoforms of Tks 5 are encoded by the mouse sh3pxd2a gene. Only the Tks5 α / Tks5 long isoform contains a PX domain (blue). The SH3 domains appear on all three isoforms (Cejudo-Martin et al., 2014).

Materials and Methods

The GST vector (pGEX 4T1) was previously purchased and p47^{phox} PX domain in pGEX 4T1 was designed and constructed by a former lab member. The protein extraction, purification and analysis steps describe in here are identical for GST, p47^{phox} PX domain and hTks5 α PX domain.

Polymerase Chain Reaction and Cloning (hTks5 α PX domain)

A PCR fragment was generated using specific Tks5 α PX domain primers that contained specific RE sites for cloning (BamHI and EcoRI). DNA template used was from a previous vector containing the PX sequence (Tks5-GFP). Once generated, the fragment was ligated into a pGEX 4T1 vector containing a GST tag.

Transformation (hTks 5 α PX domain)

10 ng of the recombinant plasmid (insert: hTks5-PX; GST fusion vector: pGEX-4T1) was transformed into BL21 competent *E.coli* cells. After placing the cell mixture on ice for 30 minutes, the mixture was subjected to heat shock at 42 °C for 10 seconds and then the mixture was placed back on ice for 5 minutes. 950 μ l of SOC medium (super Optimal broth (SOB) with Catabolite repression) was added into the cell mixture to maximize the transformation efficiency followed by incubation at 37 °C for 1 hour and shaking at 250 rpm. 25 μ l and 50 μ l of transformed cells were spread separately onto two LB ampicillin plates to obtain single colonies, and incubated overnight at 37 °C.

Expression & Purification of Proteins (hTks5 α PX domain, p47^{phox} PX domain and GST)

A single colony was selected from a LB ampicillin plate and inoculated into 5 ml of LB broth containing 10 μ l of 500x ampicillin (50 mg/ml). Then the mixture was incubated in a shaking incubator overnight at 37 °C. After incubation, the 5 ml culture was then transferred into a flask containing 1 liter LB broth with ampicillin and the initial optical density (O.D.) was measured using spectrophotometer. When O.D. 595 nm reached 0.5 – 0.6, Isopropyl β -D-1-

thiogalactopyranoside (IPTG) was added to a final concentration of 0.1 mM into the culture to trigger the lac operon to induce protein expression. The culture was incubated in the shaking incubator overnight at 25 °C to prevent protein aggregation in the culture. After spinning down the cell pellets, 20 ml of lysis extraction buffer (20 mM Tris pH7.4, 1% Triton, mg/ml lysozyme, 5 m DTT and 2 tablets of protease inhibitors, Roche #11-836-170-001) was added to the cell pellets and the mixture was incubated with rotation at 4 °C for 1 hours. Ultra-sonication was used to physically lyse the cells (the cell culture was kept in ice water bath to avoid protein denaturation). The sonicator probe was placed into the ice water for 3 times each for 30 seconds at 50% amplitude and then the probe was directly submerged into the cell culture for 30 seconds with 50% amplitude to lyse the cells.

The homogenized lysate was subjected to centrifugation at 10,000 xG for 20 minutes at 4 °C. The cell debris was removed (20 µl cell lysate was saved for positive control) and then 5 ml of glutathione sepharose 4B (GE #17-0756-01) was added into the lysate and incubated at 4 °C with rotation for 2 hours. Prior adding to the lysate, the glutathione sepharose 4B was prepared by three washing with 45 ml of PBS and spun at 500 xG for 5 minutes. The GST-tagged recombinant protein (bound to sepharose beads) was collected by using a chromatography column with 50 ml of 25 mM glutathione elution buffer (50 mM Tris HCL, 25 mM Glutathione at a final pH of 8.0). The protein was collected into seven separate elutions in 15 ml conical tubes each containing 5 ml of eluted protein. A small aliquot (20 µl) of each elution was run on an SDS-PAGE gel (10%) to detect the present of protein in the eluate after staining with Coomassie Blue stain. Based on the appearance of the protein band intensities, the appropriate elutions were pooled together for the concentration step using Amicon Ultra Centrifugal Filters (MWCO 10 kDa) according to the manufacturer's instructions. A second SDS-PAGE gel (10%)

was used to determine the concentration of the concentrated protein by (by Coomassie staining) comparing with a known concentration protein (bovine serum albumin, 2mg/ml).

Western Blot Analysis Proteins (hTks5 α PX domain, p47^{phox} PX domain and GST)

2X Laemmli loading buffer was added into 20 μ l of purified protein and 20 μ l bacterial lysate was used as positive control. All the samples were boiled at 98 °C for 10 minutes before being separated on a 15% SDS-PAGE gel and the protein was then transferred from the gel onto a nitrocellulose membrane using semi-dry electroblotting apparatus at 0.17 A for 30 minutes with 1x transfer buffer (232 g of Tris, 116 g of glycine, 148 ml of 10% SDS to 1L ddi H₂O. 20% methanol was added before used). After blocking with 5% skim milk in TBS/(0.1% TWEEN), the membrane was incubated with an anti-GST antibody (1:1000 in TBST and 0.5% BSA) for 1 hour at room temperature followed by washing with TBS/T buffer for 3 times and each time for 5 minutes. After washing, the membrane was incubated with goat-anti-rabbit Alexa-fluor 680 (1:1000 in TBST and 0.5% BSA) for another 1 hour and washed another three times with TBS/T followed by imaging on the LI-COR imager.

Results

Expression & Purification of Recombinant Protein (hTks5 α PX domain)

The presence of GST-tagged recombinant protein (hTks5 α PX domain) in a Coomassie staining SDS-PAGE gel was observed as shown in Figure 4. On the lysate lane, an intensive protein bands appears at 40 kDa. The molecular weight of GST-tagged hTks5 α PX domain is

about 40 kDa. The molecular weight of GST protein is about 25 kDa (see Figure 7) and the PX domain is about 15 kDa. Different intensities protein bands appeared on lane #1 to #7, but the most concentrated eluted protein was on lane #2. The presence of bands indicated the eluted protein was successfully collected through the chromatography column with glutathione elution buffer.

Different volumes of the concentrated protein and bovine serum albumin (BSA) were separated on SDS PAGE gel and stained with Coomassie Blue stain (see Figure 5). The intensity and thickness of protein bands appeared on the gel corresponded to the different volume of protein being loaded in the gel. The most intensive band that appeared on the BSA lane was loaded with 20 μ l BSA. The concentration of BSA was 2 mg/ml. Therefore, by comparing the appearance of the band that we loaded with 1 μ l of recombinant protein, the concentration of the recombinant protein could be deduced to be relatively high, approximately 50 mg/ml. When necessary we also determined protein concentration by a BCA assay (Pierce Biotechnology).

Western Blot Analysis Proteins (hTks5 α PX domain, p47^{phox} PX domain and GST)

Western blot analysis was used to identify the recombinant protein and GST protein. As shown in Figure 6 and 7, the GST-tagged hTks5 α PX domain, p47^{phox} PX domain and GST were recognized by anti-GST antibody (goat-anti-rabbit Alexa-fluor 680 antibody was used as secondary antibody against anti-GST). Figure 6 shows two bands appeared on each lane. The molecular weight of the GST tag was about 25 kDa and the GST-tagged hTks5 α PX domain was about 40 kDa (see Figure 7). The intensity of protein band demonstrated that the recombinant protein had high immunoreactivity. Figure 7 also shows a protein band for GST- tagged hTks4 α

PX domain (the recombinant protein was previously prepared by a lab member) is about 40 kDa, similar to GST-tagged hTks5 α PX domain, and the protein also reacted with anti-GST.

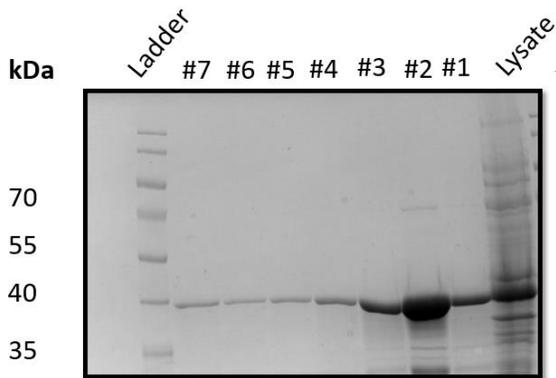


Figure 4. Expression analysis of GST-tagged hTks5 α PX domain by Coomassie staining SDS-PAGE (10%). The gel image shows the presence of recombinant protein in the eluate. Recombinant protein was collected into seven separated conical tubes, #1 to #7. An intensive band appears at 40 kDa on the lysate lane (positive control) indicated the expression of recombinant protein in BL 21 competent cells.

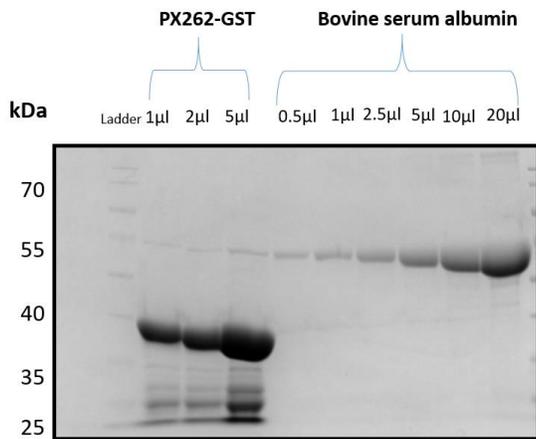


Figure 5. Concentration analysis of GST-tagged hTks5 α PX domain by Coomassie staining SDS-PAGE (10%). The concentration of bovine serum albumin (BSA) was 2 mg/ml. Compared to the intensity and thickness of the BSA protein bands, the concentration of the recombinant protein was relatively high.

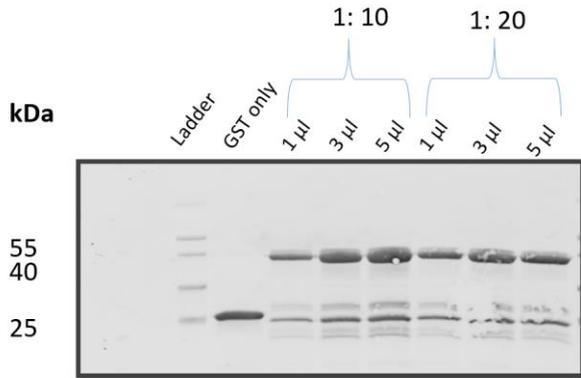


Figure 6. Western blot analysis of GST-tagged hTks5 α PX domain by 15% SDS-PAGE, anti-GST and goat-anti-rabbit Alexa-fluor 680. Two dilutions were performed (1: 10 and 1:20). The intensity and the thickness of the protein bands depended on the volume of diluted recombinant proteins.

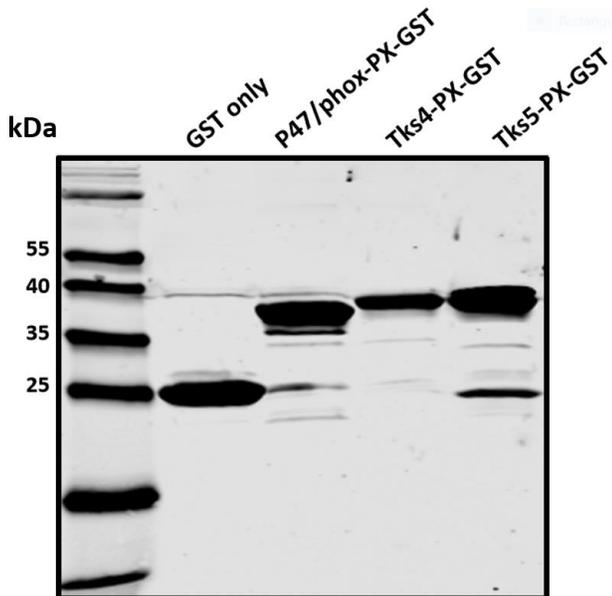


Figure 7. Western blot analysis of GST-tagged hTks5 α PX domain, GST-tagged hTks4 α PX domain, GST-tagged p47^{phox} PX domain and GST by 15% SDS-PAGE, anti-GST and goat-anti-rabbit Alexa-fluor 680. The molecular weight of GST-tagged hTks5 α PX domain and GST-tagged hTks4 α PX domain are about 40 kDa, GST-tagged p47^{phox} PX domain is about 38 kDa and the GST tag is about 25 kDa.

Discussion

The use of antibody probes to detect a particular protein of interest has been adapted widely in medical and biology research labs because this technology provides a highly specific molecular recognition of the target protein. For this reason, the development of a highly specific

rabbit mAb, which acts as an antigen-specific- probe, to detect the locations of the Tks 5 α isoform in cancer cell samples may provide a clue on how cancer cells invade and metastasize. In the present study, the purified hTks5 α PX domain, was used as an immunogen for the generation of mAb in rabbits. The results obtained from gel electrophoreses indicated that hTks5 α PX domain was successfully expressed and purified. The result from western blot analysis also revealed the recombinant protein has a high immunoreactivity to the anti-GST antibody. More importantly, the results from western blots on whole cell extracts and immunofluorescence (on mouse and human cancer cells) are promising (experiments performed by other lab members). Studies have shown Tks5 is highly expressed and localized to invadopodia in invasive cancer cells and tumor tissue such as breast cancers and melanomas (Courtneidge et al., 2005). Clinical study discovered that metastatic progression in lung adenocarcinomas patients associated with a high level expression of Tks5 α isoform (long isoform) and low level expression of Tks5 short isoform (Li et al., 2013). Tks5 is a large scaffolding protein and only the α isoform contains an amino terminal PX domain and five SH3 domains which functions are bind to other key regulators, such as PI (3,4) P2 and ADAMs, to control the formation of invadopodia (Abram et al., 2003; Buschman et al., 2009; Courtneidge et al., 2005; Courtneidge, 2012; Oikawa et al, 2008). Taken together, to visualize and detect the locations of Tks5 α isoform in cancer cell samples using antibody probe with high specificity will provide insights on how Tks5 α regulates the cell signaling pathways in invasive cancer cells. This may also shed light on the potential development of a new anti-metastatic strategy that could inhibit the interactions between Tks5 α isoform and other key regulatory molecules.

For the past two decades, researchers have endeavored to develop rabbit mAb in biological research because hypothetically rabbit mAb may have the combination of the best

characteristics of mouse mAbs and rabbit antisera (Rossi et al., 2005). Nevertheless, mice have been widely used as model in medical and research labs centered on a well-known discovery of genetic similarity between humans and mice is high. To develop mouse mAb as a probe to target the PX domain or SH3 domains on Tks5 α isoform merits further investigation, because it will be interesting to see the differences in targeting effects between rabbit mAb and mouse mAb in mice and human cancer cell samples using immunofluorescent and immunohistochemistry analysis. Such research will also provide insight into how cancer progresses and metastasizes in mice as compared to humans.

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References

- Abcam. Customer Service Orientation Package. Retrieved May 16, 2016, from file:///C:/Users/Owner/Downloads/E-1012%20Custom%20Services%20orientation%20packet%20A4%20(2).pdf
- Abram CL, Seals DF, Pass I, Salinsky D, Maurer L, Roth TM, Courtneidge SA. The adaptor protein fish associates with members of the ADAMs family and localizes to podosomes of Src-transformed cells. *J Biol Chem*. 2003; 278:16844–16851. [PubMed: 12615925]
- Antibody Purification Methods. (n.d.). Retrieved May 16, 2016, from <https://www.thermofisher.com/us/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/antibody-purification-methods.html>
- Blouw, B., Patel, M., Iizuka, S., Abdullah, C., You, W. K., Huang, X., Li J. L., Diaz B., Stallcup W.B., and Courtneidge, S. A. (2015). The Invadopodia Scaffold Protein Tks5 Is Required for the Growth of Human Breast Cancer Cells In Vitro and In Vivo. *PLoS ONE*, 10(3). <http://doi.org/10.1371/journal.pone.0121003>
- Burger, K. L., Learman, B. S., Boucherle, A. K., Sirintrapun, S. J., Isom, S., Díaz, B., ... Seals, D. F. (2014). Src-dependent Tks5 phosphorylation regulates invadopodia-associated invasion in prostate cancer cells. *The Prostate*, 74(2), 134–148. <http://doi.org/10.1002/pros.22735>
- Buschman, M. D., Bromann, P. A., Cejudo-Martin, P., Wen, F., Pass, I., & Courtneidge, S. A. (2009). The Novel Adaptor Protein Tks4 (SH3PXD2B) Is Required for Functional Podosome Formation. *Molecular Biology of the Cell*, 20(5), 1302–1311. <http://doi.org/10.1091/mbc.E08-09-0949>
- Cejudo-Martin, P., Yuen, A., Vlahovich, N., Lock, P., Courtneidge, S. A., & Díaz, B. (2014). Genetic disruption of the sh3pxd2a gene reveals an essential role in mouse development and the existence of a novel isoform of tks5. *PloS One*, 9(9), e107674. <http://doi.org/10.1371/journal>
- Courtneidge, S. A., Azucena, E. F., Pass, I., Seals, D. F., & Tesfay, L. (2005). The Src Substrate Tks5, Podosomes (Invadopodia), and Cancer Cell Invasion. *Cold Spring Harbor Symposia on Quantitative Biology*, 70, 167–171. <http://doi.org/10.1101/sqb.2005.70.014>
- Courtneidge, S. A. (2012). Cell migration and invasion in human disease: the Tks adaptor proteins. *Biochemical Society Transactions*, 40(1), 129–132. <http://doi.org/10.1042/BST20110685>
- Diaz, B., Shani, G., Pass, I., Anderson, D., Quintavalle, M., & Courtneidge, S. A. (2009). Tks5-dependent, Nox-mediated Generation of Reactive Oxygen Species is Necessary for Invadopodia Formation. *Science Signaling*, 2(88), ra53. <http://doi.org/10.1126/scisignal.2000368>
- Dübel, S., & Reichert, J. M. (2014). *Handbook of Therapeutic Antibodies*. John Wiley & Sons.

- Edwards, D. R., Handsley, M. M., & Pennington, C. J. (2008). The ADAM metalloproteinases. *Molecular aspects of medicine*, 29(5), 258-289.
<http://www.sciencedirect.com.proxy.lib.pdx.edu/science/article/pii/S0098299708000551>
- Gianni, D., DerMardirossian, C., & Bokoch, G. M. (2011). Direct interaction between Tks proteins and the N-terminal proline-rich region (PRR) of NoxA1 mediates Nox1-dependent ROS generation. *European Journal of Cell Biology*, 90(2-3), 164–171. <http://doi.org/10.1016/j.ejcb.2010.05.007>
- Gimona, M., Buccione, R., Courtneidge, S. A., & Linder, S. (2008). Assembly and biological role of podosomes and invadopodia. *Current Opinion in Cell Biology*, 20(2), 235–241.
<http://doi.org/10.1016/j.ceb.2008.01.005>
- Li, C.M., Chen, G., Dayton, T.L., Kim-Kiselak, C., Hoersch, S., Whittaker, C.A., Bronson, R. T., Beer, D.G., Winslow, M.M., & Jacks, T. (2013) Differential Tks5 isoform expression contributes to metastatic invasion of lung adenocarcinoma. *Genes & development* 27: 1557–1567
- Murphy, D. A., & Courtneidge, S. A. (2011). The “ins” and “outs” of podosomes and invadopodia: characteristics, formation and function. *Nature Reviews. Molecular Cell Biology*, 12(7), 413–426.
<http://doi.org/10.1038/nrm3141>
- Oikawa, T., Itoh, T., & Takenawa, T. (2008). Sequential signals toward podosome formation in NIH-src cells. *The Journal of Cell Biology*, 182(1), 157–169. <http://doi.org/10.1083/jcb.200801042>
- Rossi, S., Laurino, L., Furlanetto, A., Chinellato, S., Orvieto, E., Canal, F., Facchetti, F., and Tos, A. P. D. (2005). Rabbit Monoclonal Antibodies. *American Journal of Clinical Pathology*, 124(2), 295–302.
<http://doi.org/10.1309/NR8HN08GDPVEMU08>
- Stylli, S.S., Stacey, T.T., Verhagen, A. M., Xu, S.S., Pass, I., et al. (2009) Nck adaptor proteins link Tks5 to invadopodia actin regulation and ECM degradation. *Journal of cell science* 122: 2727–2740.
- Wang, H., Chen, X., Su, Y., Paueksakon, P., Hu, W., Zhang, M.-Z., Harris, R., Blackwell, T., and Pozzi, A. (2015). p47phox contributes to albuminuria and kidney fibrosis in mice. *Kidney International*, 87(5), 948–962. <http://doi.org/10.1038/ki.2014.386>
- Xu, J., Murphy, S.L., Kochanek, K.D., and Bastian B.A. (2016). Deaths: Final Data for 2013. *National Vital Statistics Reports*, 64 (2) http://www.cdc.gov/nchs/data/nvsr/nvsr64/nvsr64_02.pdf