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ELEXIS Based Screen of Human LIGs with ErbB1-4

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ABSTRACT

The Epidermal Growth Factor Receptor (ErbB1-4) family of tyrosine kinases has been associated with many cancer types. Members of the human LIG family, transmembrane proteins containing leucine-rich repeats and immunoglobulin domains, have been proposed to interact with ErbB1-4. Using the documented *Drosophila* LIG, Kekkon 1, and EGFR interaction, an extracellular interaction assay, ELEXIS, was developed to screen for novel human LIG/ErbB1-4 interactions. Any human LIGs interacting with ErbB1-4 could have the potential to be used as a cancer therapeutic.

ACKNOWLEDGEMENTS

I would like to thank Professor Duffy for his guidance and support throughout this entire year. From the lab techniques to the theory behind the project, Duff was an excellent mentor and support system throughout the entire year. He provided such great insight to laboratory procedures and taught me to think critically in terms of my research.

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Other people who have helped and supported me throughout the course of the project are Victoria Scott, Melissa Mobley, and Katie Crowley.

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INTRODUCTION

The nervous system consists of billions of neurons connected into circuits that allow the integration and propagation of neural activity. To develop neural circuits in the body, axons of nerves extend to innervate a target cell. Specifically, in the peripheral nervous system, nerve axons are extended from another neuron in the spinal cord to innervate muscle or skin cells. This process of growth and branching requires signaling peptides and their receptors for coordination. Researchers have found a family of signaling proteins, known as LIGs, which appear to interact with certain tyrosine receptor kinases to promote neural growth (Mandai et al. 2009).

Leucine-Rich Repeats and Immunoglobulin-Like Domain Containing Proteins

Leucine-rich repeats and immunoglobulin-like domain containing proteins, or LIGs, are transmembrane proteins that contain both leucine-rich repeats, or LRRs, and at least one immunoglobulin-like domain, or Ig-like domain. LRRs and Ig domains are two of the most commonly identified sequence structures in the metazoan proteome; however, relatively few proteins contain both. Typically, molecules containing both LRRs and Ig domains are involved in protein-protein interactions (MacLaren et al. 2004). Figure 1 shows the general structure of a LIG protein with its LRRs and Ig-like domains.

Figure 1. General structure of a leucine-rich repeats and immunoglobulin-like domain containing protein. The red loop contains the LRRs and the white loops contains the Ig domain.

One of the best known internal repeating motifs in proteins are leucine rich repeats. LRRs are structural motifs that span 20 to 30 amino acids in length, most of which are leucine. The N-terminus contains a conserved 11 amino acid residues that has the sequence LxxLxLxxNxL, where x is any amino acid, L is leucine, and N is asparagine (de Wit et al. 2011). This forms a β-strand and a loop, which connects to the C-terminus (Kajava 1998). These repeats come together in tandem to form a LRR domain. The domain takes the shape of a solenoid where the β-strands, as β-sheets, form the inner concave site and the overall horseshoe shape of the solenoid. The outer surface of the solenoid is formed by a variety of structures, including α-helices, polyproline II helices, and β-strands (Bella et al. 2008).

Although one structure of a typical LRR has been described here, many different LRRs structures exist in nature. The structural diversity of LRRs allow proteins to have diverse functions and to be found in all types of life forms (Bella et al. 2008). The solenoid shape has been found to be involved in protein-protein interactions; the concave side of the solenoid is found to be the ligand binding site. LRRs are found to be involved in the connectivity of neural circuits, hormone-receptor interactions, cell adhesion, and cell signaling (de Wit et al. 2011).

Ig-like domains consist of 80 amino acids that form two antiparallel β-sheets linked by β-turns or loops. These turns and loops are held together by disulfide bridges. Similar to the immunoglobulin domains in antibodies, Ig-like domains can contain variable, or V, regions or constant, or C, regions. However, unlike Ig-domains in antibodies, Ig-like domains can contain a V region, C region, or a combination of the two (Brümmendorf and Rathjen 1994; Williams and Barclay 1988).

Like LRRs, Ig-like domains also play a role in protein-protein interactions and are found in a variety of different structures. For example, the Ig-like domain-containing proteins in T-cells and antibodies are involved in antigen recognition while LIG proteins have been found to be involved in cell-cell adhesion. Proteins with Ig-like domains are also involved in ligand-receptor interactions in development, differentiation, activation, and regulation (Brümmendorf and Rathjen 1994).

The LIG proteins can be divided into families that are involved in different signaling processes. One certain family of LIGs, known as the LINGOs, has been found to play a role in regulation of the differentiation and myelination of oligodendrocytes (Mi et al. 2008). Four proteins exist in the LINGO family: LINGO-1, LINGO-2, LINGO-3, and LINGO-4, but emphasis will be placed on the first three LINGOs. LINGO-1 contains 11 LRRs and 1 Ig-like domain. Chen et al 2015 found that LINGO-1-RNA interference-treated neural stem cells facilitates functional recovery after spinal cord injury and represents a promising potential strategy for the repair of spinal cord injury (Chen et al. 2015). LINGO-2, comprised of 12 LRRs and 1 Ig-like domain, is also found to play roles in the central nervous

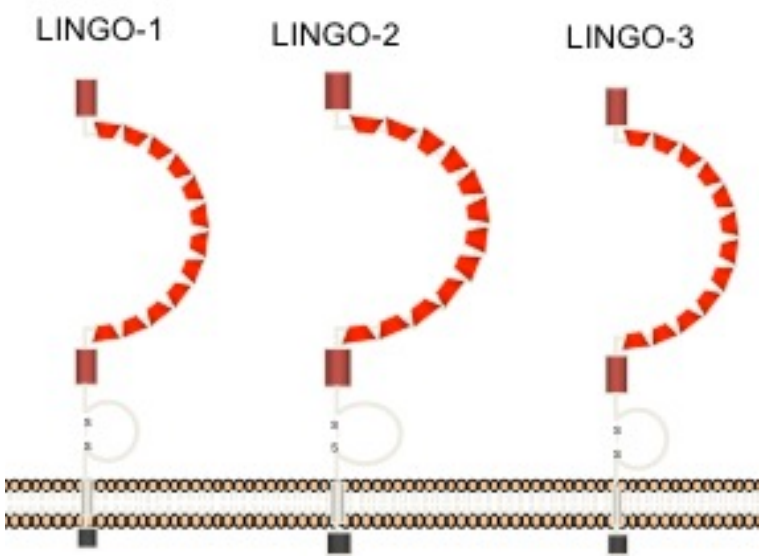

Figure 2. Structure of the LINGO family of LIG proteins.

system; LINGO-2 was found to play a role in diseases of the nervous system including Parkinson's disease. LINGO-3, comprised of 11 LRRs and 1 Ig-like domain, is still under investigation with its specific function being currently unknown (Homma et al. 2009). Figure 2 shows the structure of the LINGO family of LIG proteins.

Another LIG found to potentially play a role in neural development is LINX. LINX, also known as immunoglobulin superfamily containing leucine-rich repeat 2 or ISLR2, contains 5 LRRs and 1 Ig-like domain. Figure 3 shows the structure of LINX. It is found in the central nervous system and thought to interact with RTKs (Mandai et al. 2009).

Figure 3. Structure of the LIG protein LINX.

Although LIGs have multiple functions, the two that will be focused on are their role in the nervous system and their interactions with receptor tyrosine kinases, or RTKs. As stated previously, LIGs are known to promote neural growth by signaling for the growth of neural cells during development. They have also been found to aid in synaptic plasticity in developed nervous systems.

Epidermal Growth Factor Receptors

The Epidermal Growth Factor Receptors, or EGFR/ErbB, are a family of Tyrosine Kinase Receptors, or RTKs. There are four ErbB proteins identified in humans - EGFR/ErbB-1/HER1, ErbB-2/HER2, ErbB-3/HER3, and ErbB-4/HER4. The ErbB receptors are specifically known for the role they play in cancer, especially breast cancer, and neural and early embryonic development (Normanno et al. 2006). These

cell surface receptors are made of an intracellular domain, a short hydrophobic transmembrane domain, and an extracellular ligand-binding domain (Blume-Jensen and Hunter, 2001; Scaltriti and Baselga, 2006; Normanno et al, 2006; Figure 4). Within the extracellular region, the ErbB receptors contain four subdomains, named domains I-IV. Domains I and III are important for ligand binding, which is discussed further in this chapter, and are composed of beta-helices and leucine-rich sequences. Domains II and IV are cysteine rich. The extracellular region is connected to the intracellular region by the transmembrane domain. The intracellular domain, which is highly conserved, is composed of a tyrosine kinase domain that is surrounded by a juxtamembrane region and a carboxyl (C-) terminal tail (Normanno et al. 2006). Figure 4 shows the structure of an ErbB receptor. The structure of these receptors allows signals to be transmitted across the plasma membrane to activate gene expression and induce cellular responses (Yarden, 2001). ErbBs are involved in cell signaling that helps regulate functions such as proliferation, differentiation, migration, and apoptosis by relaying information to the nucleus (Schlessinger and Lemon, 2006).

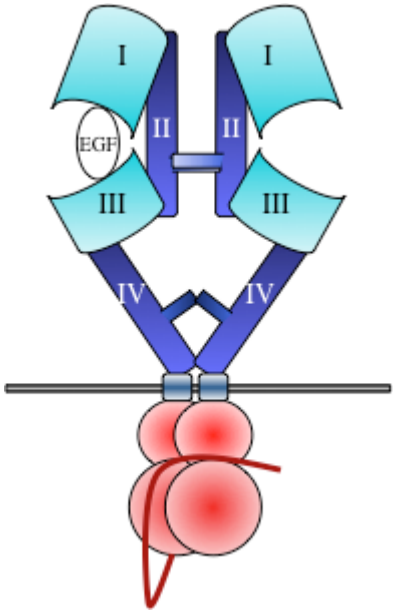

Figure 4. Structure of ErbB receptor.

The activation of ErbBs is ligand dependent. Ligands are ErbB-specific growth factors that are produced by cells either by the same cell with the receptor, known as autocrine secretion, or by surrounding cells, known as paracrine secretion. Each ligand

can be divided into one of three groups: ligands that specifically bind to EGFR including epidermal growth factor (EGF), transforming growth factor alpha (TGF-alpha), and amphiregulin (AR); ligands that bind to both ErbB-3 and EGFR including betacellulin (BTC), heparin-binding EGF-like growth factor (HB-EGF), and epiregulin (EPR); and ligands that bind to both ErbB-3 and ErbB-4 including neuregulins (NRGs). None of the ligands bind to the extracellular domain of ErbB-2; domains I and III are structurally similar and prevent ligand binding. Because of this, ErbB-2 is the preferred dimerization pair for the other ErbB receptors. Although it has ligand binding, ErbB-3 lacks tyrosine kinase activity in its intracellular domain (Normanno et al. 2006). Figure 5 shows the structure for each ErbB receptor.

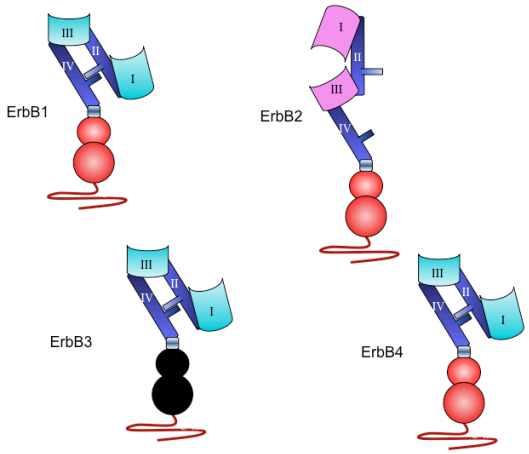

Figure 5. Family of Epidermal Growth Factor Receptors ErbB-2, pictured on the top right, has no known ligand associated, which is indicated by the blue extracellular domain. ErbB-3, pictured on the bottom left, although it has ligand activity, does not have tyrosine kinase activity, indicated by a darkened intracellular domain.

When these ligands bind to the extracellular domain, the ErbB receptors induce the formation of dimers that activate the intrinsic tyrosine kinase domain. This receptor activation causes phosphorylation of tyrosine residues that serve as docking sites for proteins. The docking of proteins leads to an activation of intracellular signaling pathways and eventually a cell response (Normanno et al. 2006). Figure 6 shows ErbB receptor activation.

Studies on the crystal structure of the ErbB family receptors show that they distinguish themselves from other RTKs with respect to their dimerization and activation

process. ErbB receptor dimerization is promoted by a beta hairpin known as a dimerization loop that protrudes from subdomain II and mediates interaction with another ErbB receptor, forming 1:1 receptor/ligand complexes (Normanno et al. 2006). Only subdomains II and IV are involved with receptor dimerization; subdomains I and III are involved with ligand binding. Without a bound ligand the receptors stay as monomers with the dimerization loop hidden (Lemmon et al, 2014). Ligand binding to domains I and III brings the two subdomains together, causing a conformational change in subdomains II and IV that exposes the dimerization loop. This change also causes a self-association between ErbBs (Normanno et al. 2006; Figure 6).

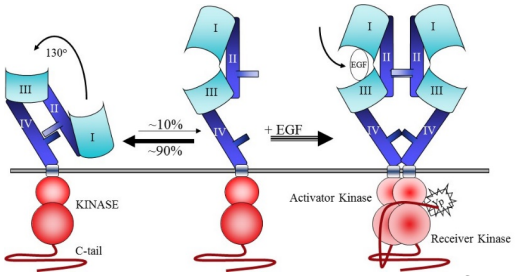

Figure 6. ErbB receptors mechanism of action in tumor cells. ErbB receptors are activated through the binding of specific ligands that are produced by either the mutated cells or by surrounding cells. Binding of ligands to the extracellular domain of ErbB receptors results in receptor dimerization, tyrosine kinase activation and phosphorylation. The activated ErbB receptors interact with different signaling molecules to transmit the signal in the cell.

MATERIALS AND METHODS

Generating LIG Constructs for Bait Proteins

AP-tagged LIG constructs were designed *in silico* using GCK software for LINGO1, LINGO2, LINGO3, and LINX and then constructed using standard molecular techniques and the Gateway cloning system. Using 5' and 3' primers, the pUAST-AP destination vector was generated by amplifying the protein tag from Dscam 7.27.25 DNA samples obtained from the Zipursky lab (Wojtowicz, et al. 2007) by previous members of the Duffy lab. sLIG constructs were then subcloned using a BP Gateway™ (Invitrogen) reaction to form an entry clone, or pENTR. These pENTR constructs were sequenced confirmed by Eton BioScience and analyzed by the Sequencher software. Each sLIG pENTR construct was then subcloned into the pUAST-AP destination vector through a LR Gateway™ (Invitrogen) reaction to produce an expression clone with the gene of interest and the AP tag.

Transfection of LIG Expression Clones

S3 *Drosophila* cells were thawed and maintained as described by *Cherbas, et al* (Cherbas, 1998). To transfect cells with the DNA constructs, cells were first counted to 3.125×10^6 cells/ml and 1.6 ml of cells were seeded in 6-well cell culture plates. The seeded cells were incubated for 24 hours under normal growth conditions of 25°C without CO_2 to obtain 100% confluence. The cells were then co-transfected with both the constitutative *Arm*-GAL4 driver and the respective LIG pUAST responder constructs (LINGO1-AP, LINGO2-AP, LINGO3-AP, and LINX-AP) following the protocol described

by *Wojtowicz et al* (Wojtowicz, et al. 2007). Figure 7 shows the procedure used to transfect cells with AP-tagged LIG constructs.

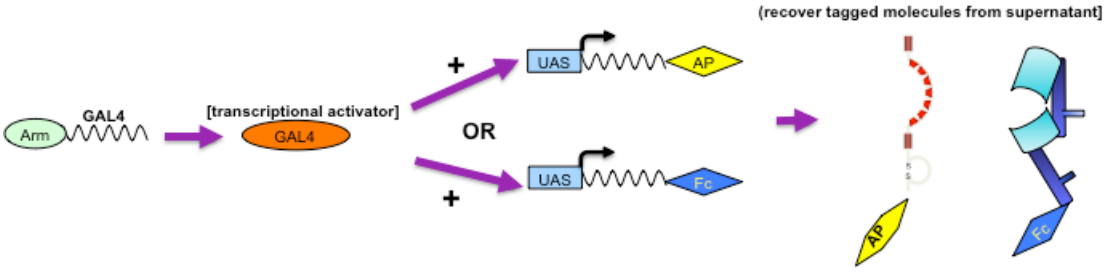

Figure 7. GAL4/UAS system used in co-transfection with AP-tagged LIG constructs for protein

After incubating for 1 week, the growth media was collected and gently centrifuged to pellet any suspended cells. The supernatant was collected, filtered with a 0.22 µm PES filter, vortexed, and aliquoted into eppendorf tubes. Each protein sample was then stored at 4° C.

Bait Quantification

Each AP bait sample was quantified by hPLAP enzymatic activity in a kinetic assay against an hPLAP enzymatic standard curve. To obtain the hPLAP standard curve, a dilution series of 100 U/L, 75 U/L, 50 U/L, 25 U/L, 10 U/L in of hPLAP in cell culture supernatant was generated. The activity of each AP bait sample was assessed against the standard curve by creating a dilution series of 100 µl, 75 µl, 50 µl, 20 µl, and 10 µl of each AP bait sample in cell culture supernatant. The activity of each sample at each concentration against the activity of cell culture supernatant was assayed by adding an equal volume of PNPP (Pierce) substrate to both the hPLAP standards and AP-tagged protein samples and tracked every minute over a 20 minute time frame at

405 nm using a Victor3 plate reader and Wallac software. The reaction was then kept in the dark and incubated at room temperature until 75 minutes when the plate was measured at 405 nm using a SpectraMax plate reader and Softmax Pro software. The reaction was stopped at 86 minutes with 50 µl of 2 N Sodium Hydroxide (NaOH) and measured at 405 nm using a SpectraMax plate reader and Softmax Pro software. The velocities of each hPLAP and AP bait sample concentration over 10 minutes were converted into a standard curve assessing hPLAP velocity vs. hPLAP concentration (U/L) that was used to quantify the hPLAP concentration in each AP bait sample. These velocities were used to quantify and normalize the AP-tagged protein samples against the hPLAP standard curve. The amount of each AP bait sample to use in each protein interaction was normalized to 502 uU for each interaction.

Protein Interactions

To determine if AP-tagged LIGs interact with Fc-tagged ErbB family of receptors, an ELISA based screening assay was developed (Wojtowicz, et al. 2007). This enzyme linked extracellular interaction screen, or ELEXIS, is diagrammed in (Figure 8).

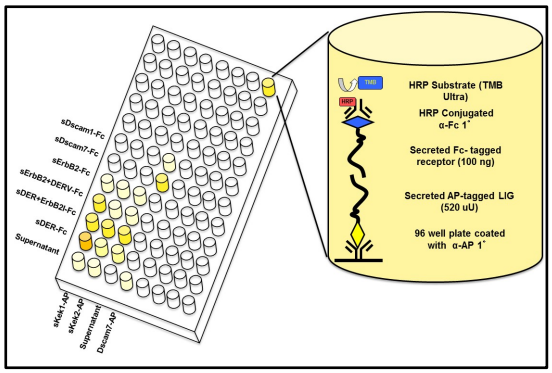

Figure 8. Structure of an ELEXIS assay.

Each interaction well was incubated with a mixture of 3 µg/mL Ms-anti-AP (8B6.18 Thermo) in 1x PBS (10 mM Na_2HPO_4, 2 mM KH_2PO_4, 2.7 mM KCl, and 137 mM NaCl) overnight at 4° C on a rocking platform. Wells were washed 4x1min

with 300 µl of wash buffer PBST (1x PBS and 0.05% Tween20) at room temperature on a rocking platform. 400 µl of Casein block solution (1% Casein in 1x PBS) was added to each well and incubated for 1.5 hours at room temperature on a rocking platform. Each interaction solution was generated during the blocking incubation and contained 100 ng of each Fc-tagged interacting prey sample, 520 uU of the AP bait sample, a final concentration of 2 µg/mL of the HRP conjugated Ms-anti-Fc detection antibody, and supernatant to a total volume of 50 µl (to interact the Dscam proteins (Dscam7 and Dscam1) we used 67.67 -15.6 ng of Fc and 11 ng of AP). The block solution was removed and the interaction mix was added into each well and incubated for 4 hours at room temperature on a rocking platform protected from light. Wells were washed 4x1 min with 300 µl of PBST at room temperature on a rocking platform protected from light. For detecting the presence of an interaction 100 µl of 1-Step TMB Ultra HRP Substrate (Pierce) was added to each well and tracked at 590 nm for 1 hour at room temperature using a Victor3 plate reader and Wallac software. The reactions in each well were stopped with 100 µl of 1 M Phosphoric Acid (H_3PO_4) stop solution and the endpoint absorbance was detected at 450 nm. Each protein interaction was compared to the highly confident and previously published interaction between sdEGFR-Fc and sKek1-AP or the homodimerization interaction of sDscam7 as a benchmark for a true positive interaction. Negative interactions were determined by comparing each protein interaction with the non-functional interaction between sdEGFR-Fc and sKek2-AP or the heterodimerization interaction between sDscam7-AP and sDscam1-Fc. False positive interactions were determined by comparing the interaction to both AP-tagged bait with supernatant and Fc-tagged prey with supernatant.

RESULTS

Generating AP-tagged LIG Constructs

To determine the possible interaction between LIG proteins and the ErbB family of receptors, prey and bait constructs needed to be generated for both sets of molecules. Prior work in the lab had generated constructs for the ErbB family. However, similar constructs for the LIG proteins were incomplete. Because the focus was on creating the bait proteins, DNA constructs for AP-tagged LIG proteins were generated. The pENTR vector for LINGO1, LINGO2, LINGO3, and LINX were generated by previous members of the Duffy lab and sequenced to ensure that the constructs were correct. The pENTR clones for LINGO1, LINGO2, LINGO3, and LINX were used to perform LR reactions with AP-containing pUAST vectors to obtain the expression constructs for the AP-tagged versions of LINGO1, LINGO2, LINGO3, and LINX. All constructs were verified by restriction digestion and sequencing. In addition, the Fc constructs for LINGO3 and LINX were generated and confirmed, but were not used here.

Verifying and Quantifying Protein Expression

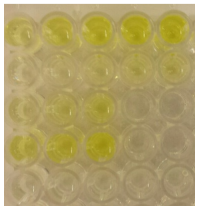

Figure 9. Picture of a typical AP activity assay. The first row contains the hPLAP standard curve, the middle rows contain the AP-tagged protein dilution series for 3 different protein samples, and the bottom row contains supernatant harvested from cells not transfected with AP-tagged LIG constructs.

After co-transfection with Arm-GAL4 into S3 *Drosophila* cells, the LIG proteins were verified and quantified using an ELISA-based AP activity assay. This assay used PNPP substrate with the AP-tagged proteins and an AP-conjugated antibody as a standard to assess the alkaline phosphatase activity of the

protein. The absorbance signals of the antibody dilutions at 405nm were plotted as a standard curve of PNP product conversion, using absorbance, as a function of AP activity levels. Figure 9 shows the color of a typical plate after the activity assay; Figure 10 shows the graph of the hPLAP standard curve.

The absorbance signals of each AP-tagged protein dilutions were plotted as a linear regression of absorbance as a function of time. Figures 11 and 12 show the activity plots for sLINGO3 and sLINX activity respectively. To determine the amount of protein present in the harvested supernatant, the readings for the undiluted sample were used for calculations. The protein concentration for LINGO3-AP was 18.3 pg/uL while the protein concentration for LINX-AP was 880 pg/uL. Based on these activity tests, both LINGO3-AP and LINX-AP appear to be produced and stable.

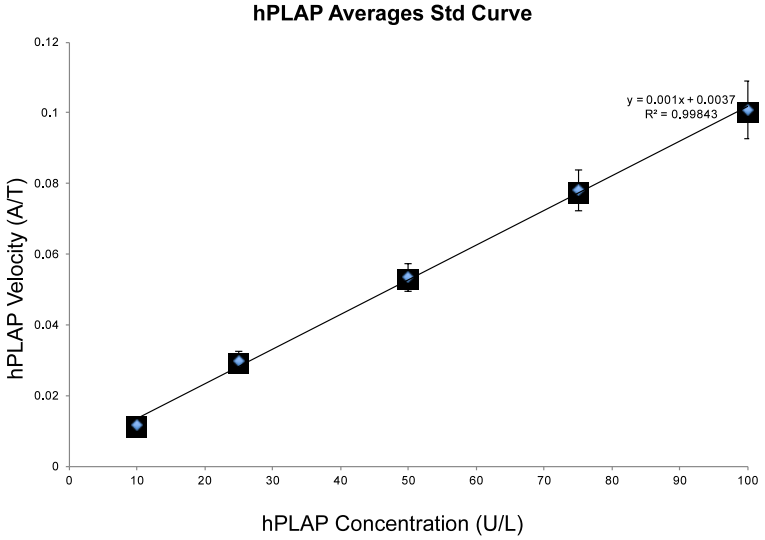

Figure 10. Graph of hPLAP standard curve used to determine concentration of AP-tagged LIGs in harvested supernatant.

In contrast, the signals for LINGO1-AP and LINGO2-AP were too low to determine if protein had been expressed and therefore quantified. Based on this, it is not clear whether or not those proteins were expressed and therefore whether or not we would be capable of expressing them.

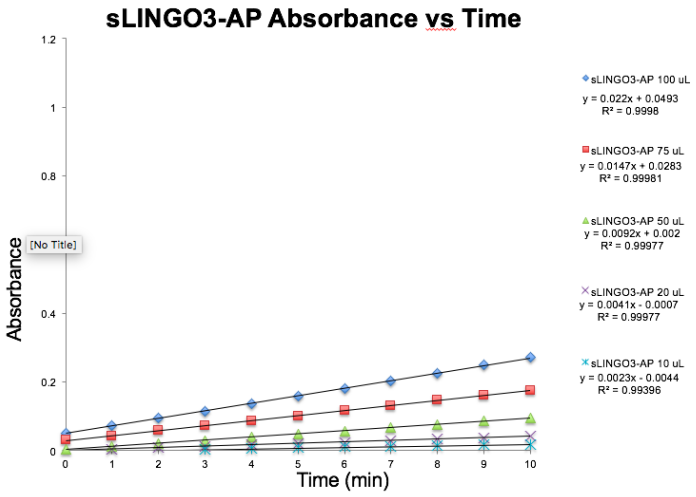

Figure 11. Activity Plot for AP activity test using harvested sLINGO3.

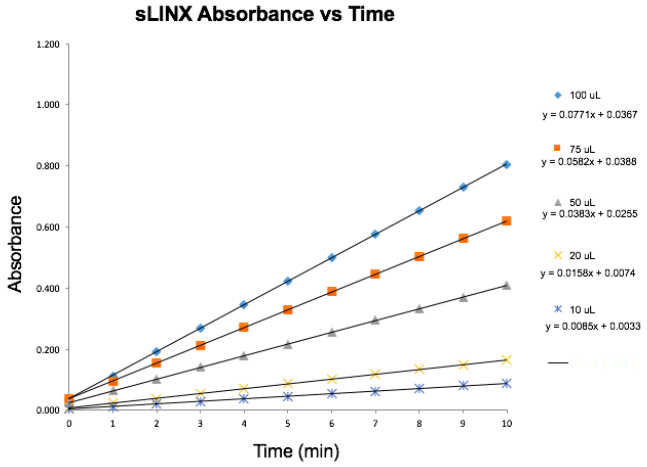

Figure 12. Activity Plot for AP activity test using harvested sLINX.

Testing Interactions

The obtained and quantified secreted versions of LINGO3-AP and LINX-AP were tested for interaction with the ErbB family of receptors. Kek1/DER and Dscam7/Dscam7 interactions were used for positive controls and Kek2/DER and Dscam7/Dscam1 interactions were used for negative controls. All LIG proteins and ErbB receptor proteins were run against supernatant as a baseline reading. The secreted versions of Fc-tagged ErbB1, ErbB2, ErbB4, DER, Dscam7, and Dscam1 and the secreted versions of AP-tagged Dscam7, Kek1, and Kek2 were provided by Alex Putnam of the Duffy lab.

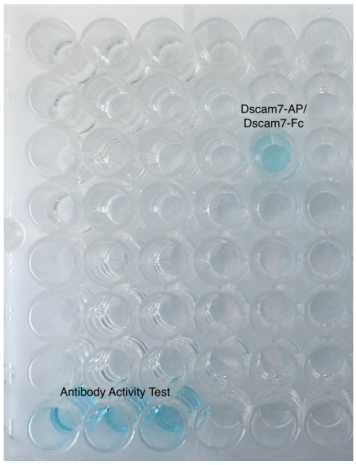

Figure 13. Picture of a typical ELEXIS assay with labeled positive interaction control and antibody activity test.

In the assay, LIG/ErbB interactions are displayed using absorbance readings at 590 nm from the conversion of TMB substrate and at 490 nm once the reaction is stopped. If the proteins are interacting in the well, the well will turn blue and then yellow once the reaction is stopped. If little to no proteins are interacting in the well, little to no color is observed. Figure 13 shows the color differences for the wells in a typical ELEXIS plate.

Figure 14 shows the fold difference for absorbance readings compared to their readings above background for the most recent ELEXIS. Compared to the fold differences for the Dscam7 dimerization interaction and the Dscam7/Dscam1 heterodimerization, the fold difference for the LINGO/ErbB and LINX/ErbB interactions over background was 1. Based on these preliminary results, in the ELEXIS assay

LINGO3 does not appear to interact with the ErbB receptors, while LINX appeared to interact with ErbB2 in some replicates, but not others.

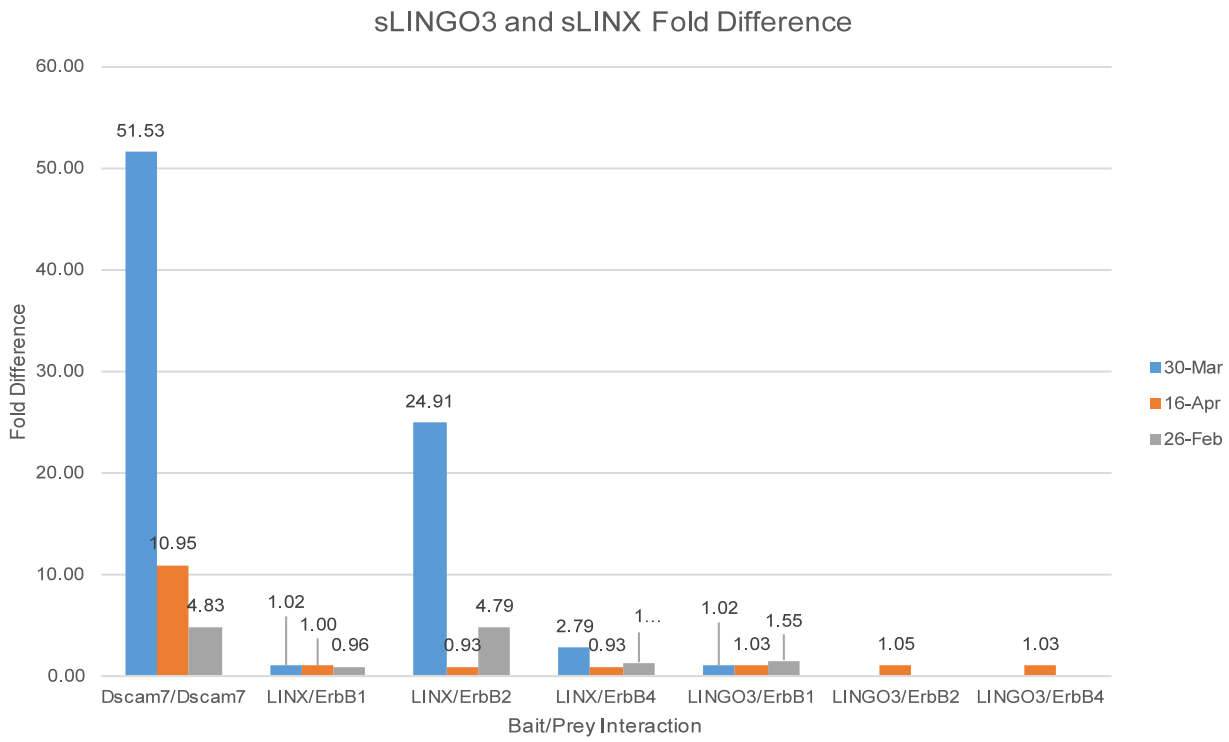

Figure 14. sLINGO3 and sLINX fold differences over background compared to the positive and negative controls.

DISCUSSION

Prior work had suggested that members of the LIG family were capable of interacting with members of the ErbB family. To further investigate this, the ELEXIS assay was used to test specific LIG/ErbB interactions. To do this, pUAST clones of secreted AP-tagged LIG proteins were produced. Out of the 10 constructs that were attempted, only 4 of the 36 AP-tagged LIG protein constructs were successfully produced: LINGO1-AP, LINGO2-AP, LINGO3-AP, and LINX-AP. These constructs were then used in transfection of *Drosophila* cells to produce secreted versions of these proteins. It was observed that the pUAST-AP expression constructs that had lower concentration readings, LINGO1-AP and LINGO2-AP, were unable to produce quantifiable amounts of protein, indicating these constructs will likely need to be re-prepped and transfected. In contrast, quantifiable secreted versions of LINGO3-AP and LINX-AP were obtained.

Once the proteins were obtained and quantified, LINGO3-AP and LINX-AP were run against ErbB1-4-Fc in an ELEXIS assay. To ensure that the assay was functional, positive and negative controls were successfully run. The positive controls gave high signal and the negative controls gave little to no signal, which indicates that the assay was functional. Preliminary data indicates minimal signal was detected for the wells containing sLINGO3 and sLINX with sErbB1, 2, and 4 and DER. Based on these results, we were unable to detect an interaction between sLINGO3 and sLINX with either the Drosophila or Human EGF Receptors.

One possibility for the lack of interaction between sLINGO3-AP and sLINX-AP and sErbB1-4-Fc is that the proteins are nonfunctional (i.e. misfolded) and therefore unable to interact with any proteins. Further work must be done to confirm the functionality of the LIGs and the ErbBs. One possible approach to this would be to perform LIG-LIG and ErbB-ErbB dimerization interactions. To do this, the Fc-tagged LIG proteins also constructed above would be useful. If an LIG-LIG or ErbB-ErbB interaction is detected, it would indicate that the proteins dimerize confirming a degree of functional activity. Confirming functionality for a given LIG or ErbB in this way data would then provide a stronger argument for the relevance of any negative interaction. Further investigation into the possible interactions of other subfamilies of with ErbB1-4 should be conducted as well.

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APPENDIX A: RAW ELEXIS DATA

Table 1. Raw data of ELEXIS assay with sLINGO3 and sLINX as bait and DER and ErbB1 as prey.

30-Mar								
	start	**10 mins**	**20 mins**	**30 mins**	**40 mins**	**50 mins**	**60 mins**	**stop**
Kek1/DER	0.108	0.154	0.203	0.233	0.248	0.256	0.260	1.071
Kek1/ErbB1	0.031	0.032	0.032	0.032	0.032	0.032	0.032	0.042
Kek1/Supt	0.033	0.033	0.033	0.034	0.034	0.034	0.034	0.043
Kek2/DER	0.036	0.044	0.047	0.049	0.049	0.049	0.050	0.117
Kek2/ErbB1	0.032	0.033	0.033	0.034	0.033	0.033	0.034	0.044
Kek2/Supt	0.031	0.032	0.032	0.032	0.032	0.033	0.033	0.042
LINX/ErbB1	0.032	0.033	0.033	0.033	0.033	0.033	0.034	0.044
LINX/Supt	0.032	0.032	0.033	0.033	0.033	0.033	0.033	0.043
LINGO3/ErbB1	0.032	0.033	0.033	0.034	0.033	0.034	0.034	0.044
LINGO3/Supt	0.032	0.032	0.033	0.033	0.033	0.033	0.034	0.043
Dscam7/Supt	0.032	0.032	0.033	0.033	0.034	0.034	0.034	0.047
Dscam7/Dscam7	0.191	0.300	0.408	0.476	0.513	0.534	0.545	2.422
Dscam7/Dscam1	0.032	0.033	0.033	0.034	0.034	0.034	0.035	0.049
Activity Test	0.041	0.042	0.043	0.044	0.044	0.044	0.044	0.071
Activity Test	0.037	0.040	0.040	0.041	0.047	0.044	0.042	0.072
Activity Test	0.035	0.038	0.039	0.040	0.040	0.041	0.041	0.065

Table 2. Raw data of ELEXIS assay with sLINGO3 and sLINX as bait and ErbB1-4 as prey.

16-Apr									
interaction	**well #**	**start**	**10 mins**	**20 mins**	**30 mins**	**40 mins**	**50 mins**	**60 mins**	**stop**
LINX/E1	A01	0.034		0.034	0.034		0.034	0.03457	0.042
LINX/E2	A02	0.030		0.031	0.031		0.031	0.03126	0.039
LINX/E4	A03	0.030		0.031	0.031		0.031	0.03113	0.039
LINX/supt	A04	0.033		0.033	0.033		0.033	0.03344	0.042
L3/E1	B01	0.031		0.032	0.032		0.033	0.03226	0.039
L3/E2	B02	0.031		0.031	0.031		0.032	0.03173	0.04
L3/E4	B03	0.031		0.031	0.032		0.032	0.0318	0.039
L3/supt	B04	0.030		0.03	0.031		0.031	0.03083	0.038
D7/supt	C04	0.031		0.032	0.032		0.032	0.03248	0.04
D7/D7	C05	0.073		0.104	0.111		0.115	0.11557	0.438
D7/D1	C06	0.031		0.031	0.031		0.031	0.03155	0.04
supt/E1	D01	0.032		0.033	0.033		0.033	0.0331	0.041
supt/E2	D02	0.033		0.033	0.034		0.034	0.03363	0.042
supt/E4	D03	0.031		0.032	0.032		0.032	0.03188	0.04
activity test	H01	0.064		0.112	0.117		0.115	0.11644	0.568
	H02	0.046		0.063	0.063		0.058	0.05387	0.482
	H03	0.076		0.107	0.108		0.106	0.10422	0.588

Table 3. Raw data of ELEXIS assay with sLINX as bait and DER and ErbB1 as prey.

26-Feb		
Interaction	**Well**	**Final Abs**
Kek1/DER	A1	0.286
Kek2/DER	B1	0.061
LINX/DER	C1	0.073
Kek1/ErbB1	A2	0.053
Kek2/ErbB1	B2	0.045
LINX/ErbB1	C2	0.045
Kek1/Supt	A3	0.045
Kek2/Supt	B3	0.046
LINX/Supt	C3	0.047
Dscam7/Supt	D3	0.047
Dscam7/Dscam7	D4	0.227