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14. ABSTRACT CXCL12 is a small molecular weight protein secreted by fibroblasts in the prostate stroma consequent to aging and activates CXCR4, a G-protein coupled receptor expressed in prostate epithelial cells. Activation of the CXCL12/CXCR4 axis has been shown to promote prostate cancer cell proliferation, motility, and invasiveness; furthermore, previous reports have shown phosphorylation of EGFR and HER2 in response to CXCL12 treatment. Here I examined the dimerization of EGFR and HER2 in response to varying concentrations of CXCL12. Although some instances of heterodimerization were observed using co-IP, there was no consistency in responses to specific concentrations and observed dimerization with CXCL12 treatment was not as robust as with EGF treatment; thus, these data are inconclusive and I am unable to report novel significant findings.					
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INTRODUCTION:

CXC-type chemokines play a role in prostate cancer initiation and progression. Chemokines are soluble, small molecular weight (8–14 kD) chemotactic cytokines that bind to their cognate G-protein coupled receptors (GPCRs) to elicit cellular responses [1]. More specifically, CXCL12 binds to the GPCR, CXCR4, which is over-expressed in primary and metastatic prostate tumors [2]. On the other hand, EGFR and HER2, members of the human epidermal receptor (HER) family, dictate downstream signaling outcomes based on either homo- or heterodimerization with members of the HER family upon stimulation, resulting in different phenotypes including increased proliferation or invasion [reviewed in 3]. GPCRs are able to activate receptor tyrosine kinases, including HER1, and this is a common mechanism of GPCR signaling in cancer [reviewed in [4]. Previous data from our lab demonstrates CXCL12 stimulation of prostate cancer epithelial cells results in phosphorylation of EGFR and subsequent activation of signaling that can occur downstream of EGFR and HER2 [5]. Moreover, our data suggest EGFR transactivation is required for CXCL12/CXCR4-mediated cellular responses, and that the mechanisms involved in CXCL12/CXCR4-mediated EGFR activation may differ between non-transformed and transformed prostate epithelial cells [5, and Begley, unpublished]. Thus, we hypothesize that CXCL12 stimulation can modulate EGFR/HER2 dimerization and, therefore, differentially activate phenotypic responses downstream of EGFR/HER2 activation depending on cell type (transformed vs. non-transformed). The proposed studies aim to elucidate the role EGFR and HER2 and their dimer configurations play in cellular responses to CXCL12. These studies could provide rationale for further investigating a dual therapeutic approach, targeting CXCR4 activation and EGFR/HER2 dimerization.

BODY:

Specific Aim 1: Determine which HER1 and HER2 homo- and heterodimer configuration transactivated by CXCR4 promotes cellular proliferation, survival, and/or motility/invasiveness in vitro. **Months 1-12*.**

Task 1: Dimerization/co-immunoprecipitation studies

2011: In order to assess heterodimers, antibodies against either EGFR or HER2 were used to pull down protein complexes and then blots probed for HER2 or EGFR, respectively, before stripping the membrane and probing for the precipitated receptor. Untreated and EGF treated cells were used as controls, showing baseline and positive heterodimerization, respectively. An IgG isotype control was used to monitor non-specific binding. Several instances, although inconsistent with reciprocal pull downs, of heterodimerization between EGFR and HER2 were observed (Figure 1). Experiments utilizing other cell lines (BPH-1, N15C6 and PC3) were to be run after completion of studies with C4-2B and LNCaP.

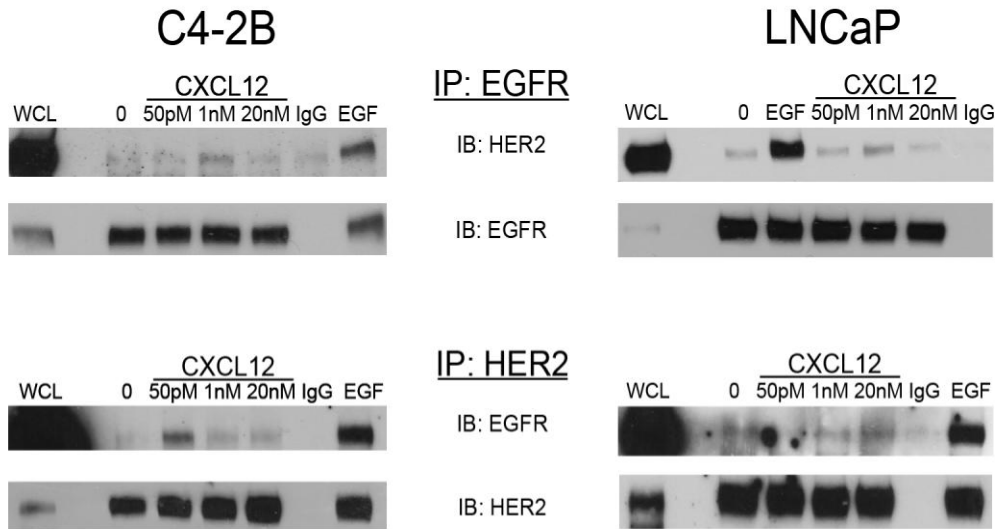


Figure 1. EGFR and HER2 Dimerization upon CXCL12 stimulation. LNCaP and C4-2B cells were left untreated or treated with 50pM, 1 or 20nM CXCL12 or 5ng/mL EGF (control) for 60 minutes in serum free media before lysates were collected and immunoprecipitations performed using 500ug total protein. IP indicates the antibody used to pull down the receptor of choice and IB (immunoblot) indicates the antibody used to probe for the receptor of choice. 10ng total protein was used for WCL (whole cell lysate) control.

In order to assess homodimers, receptor constructs containing FLAG or myc tags were being created for EGFR or purchased commercially for HER2. Transfection of C4-2B cells with tagged HER2 constructs and subsequent western blot analysis confirmed expression of HER2 and the presence of either FLAG or myc tags. Several attempts were made to tag EGFR, however, none were successful at producing the correct construct.

Task 3: Invasion/Motility Studies

2011: Data from previous invasion studies using PC3 cells in the presence of CXCL12 was able to be repeated in my hands. However, no novel data was produced. Additionally, preliminary assessment of siRNA knockdown of EGFR and HER2 in LNCaP and C4-2B cells showed siRNA to be effective. However, no experiments were run.

***Note:** Data and experiment presented here reflect work that was done over the 8-month period before my leave of absence.

KEY RESEARCH ACCOMPLISHMENTS:

- No significant accomplishments can be reported at this time.

REPORTABLE OUTCOMES:

- No reportable outcomes can be reported at this time.

CONCLUSIONS:

At this time, I am unable to report any significant and concrete conclusions based on these data. Although heterodimerization of EGFR and HER2 was observed using co-IP, inconsistency of dose responses and results appearing in reciprocal experiments do not provide strong evidence for dimerization in response to CXCL12. This may be due to the nature of co-IP (mechanical or salt disruption of EGFR/HER2 association, etc). Other methods, such as FRET or the addition of a crosslinking agent, could be employed to further study the association of these proteins in the presence of CXCL12. More time would be needed to run experiments and assess results laid out in other tasks.

REFERENCES:

1. Rot A and von Andrian UH. *Chemokines in innate and adaptive host defense: basic chemokinese grammar for immune cells*. Annual Review of Immunology 2004; 22:891-928.
2. Sun Y, Wang J, Shelburne CE, Lopatin DE, Chinnaiyan AM, Rubin MA, Pienta KJ and Taichman RS. *Expression of CXCR4 and CXCL12 (SDF-1) in human prostate cancers (PCa) in vivo*. Journal of Cellular Biochemistry 2003; 89:462-473.
3. Olayioye MA, Neve RM, Lane HA, and Hynes NE. *The ErbB signaling network: receptor heterodimerization in development and cancer*. The EMBO Journal 2000; 19(13):3159-67.
4. Bholra NE and Grandis JR. *Crosstalk between G-protein-coupled receptor and epidermal growth factor receptor in cancer*. Frontiers in Bioscience 2008; 13:1857-1865.
5. Kasina S, Scherle PA, Hall CL, and Macoska JA. *ADAM-mediated amphiregulin shedding and EGFR transactivation*. Cell Proliferation 2009; 42:799-812.