CXCL12 Oligomerization Dictates Divergent Signaling and Migratory Responses in Intestinal Epithelial Cells

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Abstract

Chemotactic migration classically exhibits a bell-shaped response over a narrow concentration range. Previous studies have shown the chemokine, CXCL12, exists in a monomer-dimer equilibrium with heightened concentrations resulting in preferential accumulation of homo-dimeric chemokine. Little is understood how CXCL12 oligomerization induces distinct cellular effects through the same cognate receptor, CXCR4. The objective of these studies is to determine the effects of monomeric and dimeric CXCL12 on colorectal cancer cell migration. HCT116 and HT29 human colorectal carcinoma cells were utilized to examine both chemotactic and restitution migration respectively. Previously engineered preferential monomeric (CXCL12H25R), locked dimer (CXCL122), and wild-type (CXCL12WT) were utilized in our analyses. Transwell inserts and aspiration wounded monolayers demonstrated that CXCL12H25R and CXCL122 induced a strong chemotactic and restitution migration response. In contrast, CXCL12WT was unable to elicit cellular migration. In agreement with those data, filamentous actin formation was induced by CXCL12H25R and CXCL122, but not CXCL12WT. Intracellular calcium flux, a classical hallmark of G-protein coupled receptor signaling, was elicited equally by each oligomeric variant. This response was blocked with addition of CXCR4-specific inhibitors indicating each of the variants engaged the same cognate receptor. Similarly, CXCL12WT dose-dependently inhibited CXCR4, but not CXCR1, mediated chemotaxis. The lack of migration was subsequently shown using nuclear run-on assays to reflect distinct interactions of the monomeric and dimeric ligand for CXCR4 homotypic receptor dimers. These latter data support the notion that double occupancy of dimeric receptor limits migration while single occupancy of the same receptors evokes strong migratory responses. Together these data underline the importance of CXCL12 oligomerization in dictating epithelial migration and suggest CXCL12WT could serve as a potential inhibitor of colorectal cancer metastasis.

Summary

• Monomeric but not dimeric CXCL12 promotes cellular migration of HCT116 and HT29 cells. Lack of migration was similarly seen at heightened concentrations of the wild-type chemokine. Filamentous actin formation, indicative of cellular migration, is increased with stimulation with monomeric and wild-type CXCL12 at 10nM doses.
• Both monomeric and dimeric CXCL12 are active agonists of CXCR4 and promote intracellular calcium flux
• Dimeric CXCL12 dose dependently inhibits migration towards wild-type CXCL12 but does not affect CXCR1 mediated chemotaxis
• Monomeric and dimeric CXCL12 have distinct interactions with the N-terminus of CXCR4

Our results provide evidence for agonist-biased chemokine receptor signaling and demonstrate that the biphasic nature of the chemotactic migration response reflects differential CXCR4 signaling evoked by monomeric and dimeric CXCL12.

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Figures

Dimeric CXCL12 fails to induce cellular migration

Monomeric but not dimeric CXCL12 promotes cellular migration of HCT116 and HT29 cells. Lack of migration was similarly seen at heightened concentrations of the wild-type chemokine. Filamentous actin formation, indicative of cellular migration, is increased with stimulation with monomeric and wild-type CXCL12 at 10nM doses.

Dimeric CXCL12 blocks CXCR4 mediated chemotaxis

(A) 10nM CXCL12 or (B) 100nM CXCL8 chemokines were added simultaneously with increasing concentrations of CXCL12 to the bottom well of the transwell system containing HCT116 chemotaxis was quantified. Data shown are mean ± SEM from three independent experiments. Data shown are mean ± SEM from three independent experiments. Dashes represent basal migration. Asterisk indicates statistically significant difference from vehicle with 10nM CXCL12 control (P<0.05).

CXCL12 oligomerization mediates distinct interactions with the N-terminus of CXCR4

(A) %/H heteronuclear NOE of CXCL12-CXCR4 complexes in the absence (gray) and presence (black) of 500M CXCL12, or CXCL12 WT. CXCR4 residues 4–9 (red) are weakly associated with CXCL12 WT, but interact strongly with CXCL12 H25R indicating a unique 1:1 complex stoichiometry. B) Stoichiometry of the CXCL12-CXCR4 complex shifts from 1:2 to 2:2 as the CXCL12 concentration exceeds the receptor dimerization in the presence of CXCR4 WT (500M).

Dimeric CXCL12 blocks CXCR4 mediated chemotaxis

(A) HCT116 cells were stimulated with 10nM doses of CXCL12 WT or CXCL12 WT. Cells were stained with phalloidin to visualize filamentous actin formation. Lysophosphatidic acid (LPA) and PGE2 were used as positive and negative controls respectively. Images shown at 1000x magnification. B) Data shown are mean fluorescence intensity per cell ± SEM of 90 cells per treatment. Asterisk indicates statistically significant difference from vehicle control (P<0.05).

CXCR4 mobilization of intracellular calcium

(A) HCT116 cells were stimulated with 10nM doses of the indicated CXCL12 variant and calcium flux was measured using a spectrophotometer (ab648). B-D HCT116 cells were pre-treated with either a pharmacological antagonist (AMD100) or neutralizing antibody (12G5) to CXCR4. IgG isotype matched antibody served as control. Data from panel A are shown as mean ± SEM of three independent experiments performed in duplicate. Data from panels B-D are representative of three independent experiments.

Filamentous Actin formation is induced by monomeric CXCL12

A. HCT116 cells were non-migratory following stimulation of dimeric CXCL12 or 1000nM CXCL12 WT. Asterisk indicates statistically significant difference from vehicle control (P<0.05).

Dimeric CXCL12 fails to induce cellular migration

A. Chemotactic migration of HCT116 cells was analyzed using a transwell assay. Chemotaxis was measured with 10nM doses of monomeric (CXCL12 WT), dimeric (CXCL12 H25R) or wild-type (CXCL12 WT) chemokine. Data are mean ± SEM from three independent experiments and a total of 30 quantified fields of view per treatment. B. HT29 confluent monolayers were wounded using a vacuum aspirator and stimulated daily for three days with 10nM doses of either CXCL12 WT, CXCL12 H25R, or CXCL12 WT. Data shown are mean ± SEM from three independent experiments and a total of 18 wounds per treatment. Vehicle (NaCl) and serum served as controls for baseline and positive cellular migration respectively. Cells were non-migratory following stimulation of dimeric CXCL12 or 1000nM CXCL12 WT. Asterisk indicates statistically significant difference from vehicle control (P<0.05).

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