Extracellular Monomeric Ubiquitin, the major component of Transferon Oral®, binds to CXCL12, revealing a new regulatory role on the CXCR4/CXCL12 axis

González-Martínez AP[1,2,3], Aguilar-Alonso F[1,2], Fragozo A[1,2], Domínguez-Sánchez K[1,2], Pavón L[3], Vallejo-Castillo L[1,2], Almagro JC[1,2,4], and Pérez-Tapia SM[1,2,3].

[1]Unidad de Desarrollo e Investigación en Bioterapéuticos (UDIBI), Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico City, Mexico. [2]Laboratorio Nacional Para Servicios Especializados de Investigación, Desarrollo e Innovación (I + D + I) Para Farmoquímicos y Biotecnológicos, LANSEIDI-FarBiotec-CONACyT, Mexico City, Mexico. [3]Departamento de Inmunología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico City, Mexico. [4]GlobalBio, Inc., Cambridge, MA 02138, USA.
anethpaola13@gmail.com; lavallejos@ipn.mx; sperezt@ipn.mx.

INTRODUCTION

Transferon Oral® is a Dialyzable Leukocyte Extract (DLE) with immunomodulatory properties. Its major components are two monomeric Ubiquitin (Ub) forms: Ub1-76 and Ub1-74[1]. Although Ub is critical for intracellular signaling, it has been reported to have immunomodulatory activity as a partial extracellular CXCR4 agonist[2]. CXCL12 is a vital signaling chemokine known to bind and actívate the G-protein coupled receptors CXCR4 and CXCR7. Dysregulation of the CXCL12/CXCR4 axis can lead to various diseases, including autoimmune disorders, cancer, and chronic inflammation. Therefore, chemokine activity regulation is critical to maintaining immune homeostasis and preventing illness. One such mechanism is the release of Chemokine-Binding Proteins (CBPs) to modulate chemokine activity by altering receptor binding or bioavailability[3]. In this study, we found by ELISAs that Extracellular Monomeric Ubiquitin (EmUb) does not bind to CXCR4 but to CXCL12, suggesting that Ub may regulate the CXCL12/CXCR4 axis as a CBP to CXCL12.

OBJECTIVE

To evaluate de interaction between EmUb and CXCL12/CXCR4.

METHODS

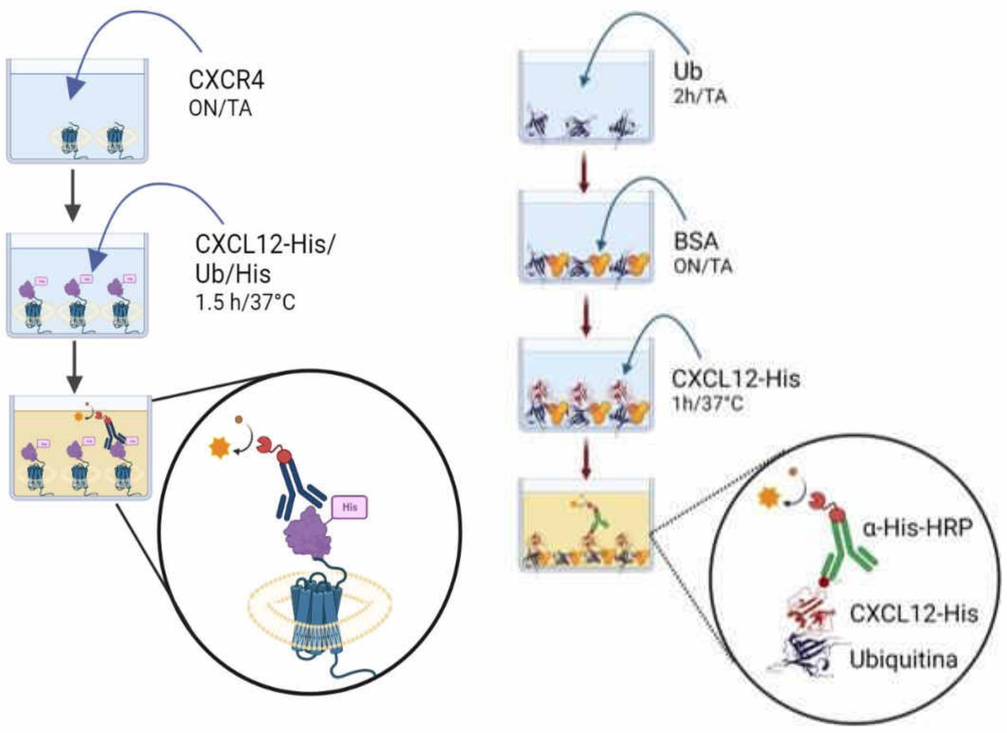

Figure. 1 Sequential steps of the indirect ELISA assay methodology used in the present study.

In addition, Migration assays on FaDu Cells were also performed. A standardized wound was created by applying consistent pressure using a sterile pipette tip to generate a linear scratch across a cell monolayer. Following the wounding, cells were treated with CXCL12, AMD3100, and mUb. Cells were then EMEM supplemented with 5% FBS at 37 °C and 5% CO2. Time-lapse imaging was performed to capture images 24 and 48 hours after wounding.

RESULTS AND DISCUSSION

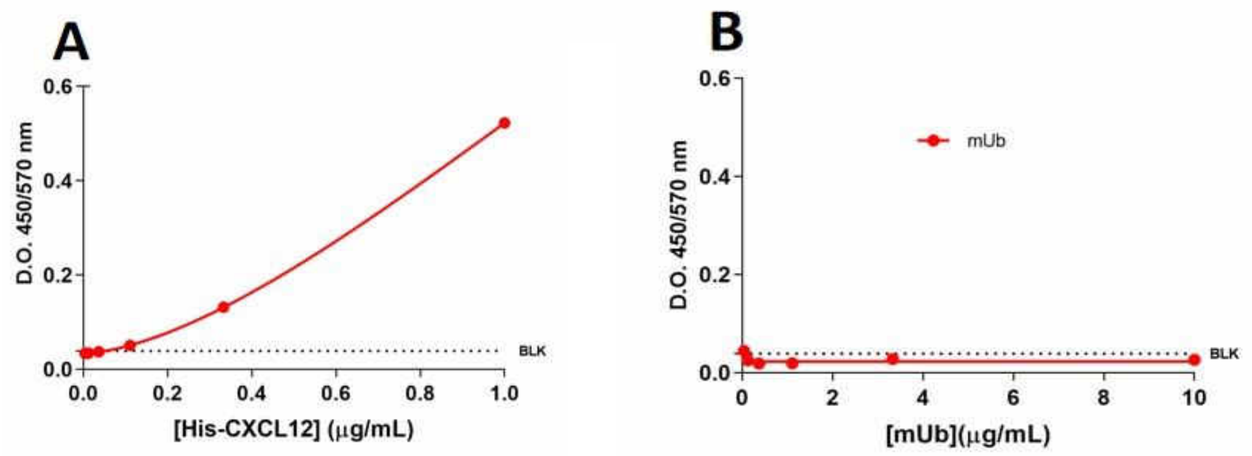

Figure. 2 Binding of CXCR4 to His-CXCL12 or His-mUb. A Binding curve depicting the interaction between His-CXCL12 and CXCR4, showed a clear and significant dose-dependent effect. **B** Despite conducting the assay under the same established conditions, no evidence of interaction between CXCR4 and mUb was detected. These findings suggest that, within the tested parameters, CXCR4 and Ub do not interact or exhibit a discernible binding affinity.

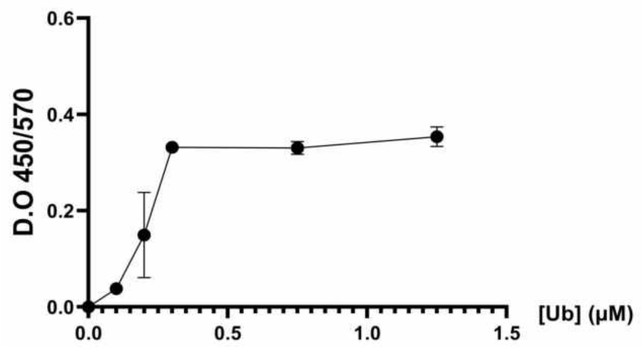

Figure 3. Binding of CXCL12 to mUb . When using a coated plate with different concentrations of ubiquitin and a constant concentration of His-CXCL12 as ligand, it was noted a direct correlation between the [Ub] and the optical density related to CXCL12 detection. The observed low recovery of CXCL12 (judged by the low absorbance levels) implies that binding between ubiquitin and CXCL12 is weak, which could potentially allow for the modulation of the inflammatory response mediated by the CXCL12/His axis in an in vivo system.

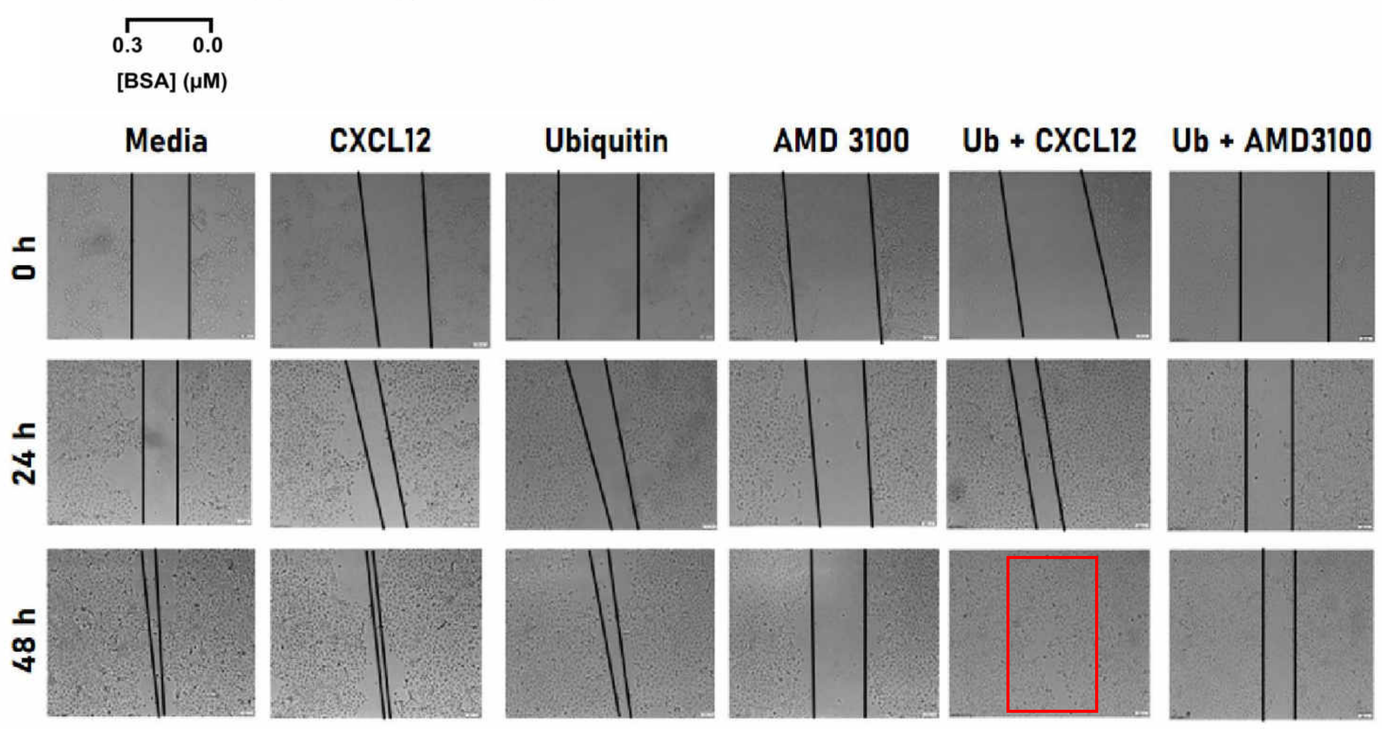

Figure.4 Effects of Ubiquitin treatment on migration. The treatment with Ub (12 µM) or CXCL12 (20 nM) did not promote migration of FaDu cells. However, treatment with AMD3100 (13 µM) , a CXCR4 inhibitor, leads to a reduction in cell migration compared to the untreated group. Interestingly, co-treatment with Ubiquitin + CXCL12 significantly enhanced migration compared to the vehicle control and even more so than the migration induced by CXCL12 or Ubiquitin alone, where no significant effects are observed. These results suggest that the Ub/CXCL12 complex may promotes migration in FaDu cells through the activation of the CXCR4 receptor.

CONCLUSIONS

Extracellular Monomeric Ubiquitin (EmUb) does not bind to CXCR4 but to CXCL12, suggesting that Ub may regulate CXCR4 as a CBP. Given the critical roles of the CXCR4/CXCL12 axis in numerous physiological and pathological processes, understanding its regulation is essential for developing novel therapeutic strategies. Further research is needed to elucidate the role of EmUb on CXCL12 activity and determine its potential as a therapeutic agent.

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