CXCL14 as Potential Binding Partner for HPV E7

by

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Abstract:

The human papillomavirus is a leading cause of cervical cancer around the world. This oncovirus uses proteins like E7 to hijack the cell cycle by binding to cellular proteins, such as pRb. Another potential binding target of E7 is CXCL14. This chemokine plays a key role in the viral immune response which would be a significant target for E7. The purification of this protein for binding studies was developed through fast protein liquid chromatography (FPLC), using a nickel column for affinity binding and a size exclusion column, and the protein sample was then visualized by NMR. The protocol was successful in purifying CXCL14 which can be used for further study in the binding of E7 to CXCL14.

Introduction:

The Human Papillomavirus (HPV) is one of the most common sexually transmitted infections (STI) around the world. There are over 200 strains of HPV which are classified into two different categories: high risk and low risk.[1] Low risk strains are those resulting in benign skin lesions while high risk strains cause cancer. High risk strains of HPV account for most cases of cervical cancer in women and are also associated with other anogenital cancers.[2] Because STIs such as HPV are so transmissable, vaccines for HPV have been formulated in attempts to prevent HPV related cancers.[3] This oncogenic virus uses proteins, E6 and E7, to disrupt the cell cycle leading to uncontrolled cell growth, thus leading to cancer.[2] However, little is understood about how the

virus can have such diverse severities as some strains result in skin lesions while others cause cancer.

The E7 protein of HPV is notable because half of the protein is intrinsically disordered. Once forming a dimer, the unstructured region of the protein is able to bind to other proteins in the cell. One protein that E7 is known to bind to is the retinoblastoma protein (pRb) which is a tumor suppressor.[1] PRb regulates the cell cycle by binding to the transcription factor E2F until the cell is ready to divide. When a cell is infected with HPV, E7 binds to pRb, releasing E2F which in turn initiates proliferation.[1] Additionally, E7 degrades pRb to prevent its rebinding with E2F, holding the cell in a state of continuous proliferation, leading to tumor growth and cancer.[1] HPV E7 can bind to other proteins in the cell, hijacking the normal function, one potential binding partner being CXCL14.

CXCL14 is chemokine primarily made in epithelial cells that functions to recruit immune response factors.[4] Chemokines are an essential component of the immune system in signaling where the immune system should target.[4] CXCL14 is a key part of the viral immune response, and when the protein is non-functional, it has been associated with the development of cancer.[5] In HPV patients, CXCL14 has been reported to be in lower concentrations.[6] This may be attributed to E7 binding and degradation similar to that with pRb. It is believed that HPV is affecting CXCL14 at a transcriptional level.[6] However, if the chemokine is still expressed, E7 may then bind and degrade it. If E7 is binding to and degrading CXCL14, it may be preventing the immune system from targeting the virus. If so, the binding of E7 to CXCL14 may contribute to the progression of HPV into cancer.

Materials and Methods:

Transformation and Growth: A transformation of CXCL14 DNA plasmid was done using BL21 competent cells. A vial of BL21 cells was thawed on ice and 50 µL of cells was mixed with 2 µL of DNA. The cells incubated on ice for 10 minutes, heat shocked for 45 seconds at 42 ℃, and incubated on ice again for two minutes. In the tube of cells, 500 µL of LB was added before incubation at 37 ℃ and 270 rpm for 45 minutes. The culture was plated on a carbenicillin plate at 100 µg/mL and incubated overnight at 37℃.

A colony with the transformed DNA was inoculated into 2.5 mL of LB with 100 µg/mL carbenicillin. The culture incubated at 37 ℃, 270 rpm for 2.5 hours and then was transferred into 100 mL of LB with carbenicillin at 100 µg/mL and continued incubation. Once the A600 reached 1.0, the culture was transferred into 3L of M9 media with an ^{15}N label. Incubation continued until the culture reached an A600 of 0.6 then was induced with 500 µL IPTG per liter. This incubated overnight at room temperature, 270 rpm. The cells were harvested by centrifugation at 3200 rpm for 30 minutes at 4 ℃ then frozen.

Purification: The cell pellet was resuspended in a lysis buffer of 20mM tris pH 8, 200 mM NaCl, 0.1% triton X. The solution was sonicated in a cycle of 3 seconds on, 3 seconds off ten times and then readjusted and done again. Once sonicated, the solution was centrifuged at 14000 rpm for 30 minutes at 4 ℃. The supernatant was collected and the pellet was resuspended in lysis buffer, sonicated, and centrifuged in the same process, collecting the supernatant after centrifugation.

The CXCL14 protein has a his-tag so a gravity nickel column was used. Nickel resin was used to batch bind the CXCL14 sample overnight at 4 ℃. The resin and sample were placed back into the column, washed, and then eluted by pH with a buffer 25 mM tris, 200 mM NaCl pH 6. The

elution was concentrated with a centrifuge filter at a 3K molecular weight cut off centrifuging at 2600 rpm in 30 minute intervals.

The concentrated sample was dialyzed into Buffer A of 25 mM tris pH 8, 200 mM NaCl, 10 mM imidazole then run on an Nickel-NTA column on the FPLC. The sample was loaded while running Buffer A. Half the sample was loaded and bound to the column and then eluted with a gradual increase of Buffer B of 25 mM tris, 200 mM NaCl, 500 mM imidazole pH 8. The sample was collected and concentrated. The sample was injected onto an S75 column for size exclusion chromatography on the FPLC. PBS binding buffer was used for the run on the S75.

Once the sample was collected from the FPLC, it was dialyzed into NMR buffer of 20 mM tris (pH 6.8), 50 mM NaCl, 50 mM NaCl, 0.5 mM DTT, 10% D_2O which was added after dialysis as the sample was taken for NMR.

Results and Discussion:

The transformation resulted in several colonies indicating the transformation was successful in taking up the CXCL14 plasmid. The growth of the bacterial colony reached log growth and was induced to produce protein which was then purified through centrifugation and a nickel gravity column. Purification using just size exclusion chromatography produces several peaks but was unknown as to which was CXCL14. (figure 1) All of the fractions were collected to run on the NMR to determine which was the protein of interest. Peaks 2, 3, and 4 showed no protein content when an NMR HSQC was obtained.

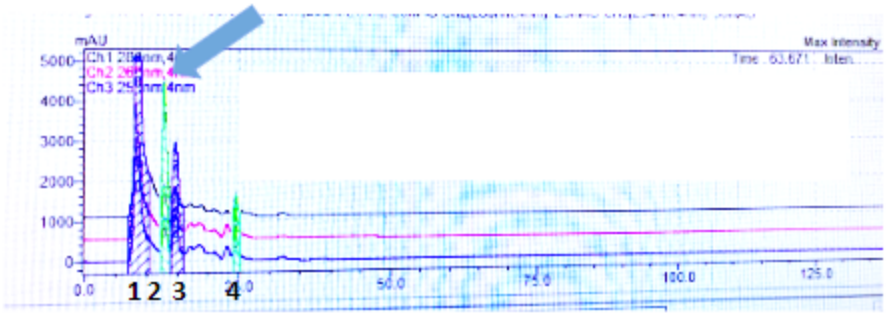

Figure 1: Size Exclusion Chromatography of CXCL14. The CXCL14 sample was run on an S75 column for size exclusion chromatography using PBS binding buffer. The intensity of absorption at 280 nm is shown over the time of the method run. Four peaks were collected, but it was determined by NMR that the second peak contained the protein of interest.

When peak 2, at around 16 minutes, was run on the NMR, the spectrum showed there was a folded protein similar to that of CXCL14. The HSQC (Figure 2) was compared to the known HSQC of CXCL14 with an ^{15}N label. The two distinct tryptophan residues at 64 and 68 are used to indicate that CXCL14 was present in the HSQC. However, there are many additional peaks in the spectra which showed the sample was not pure.

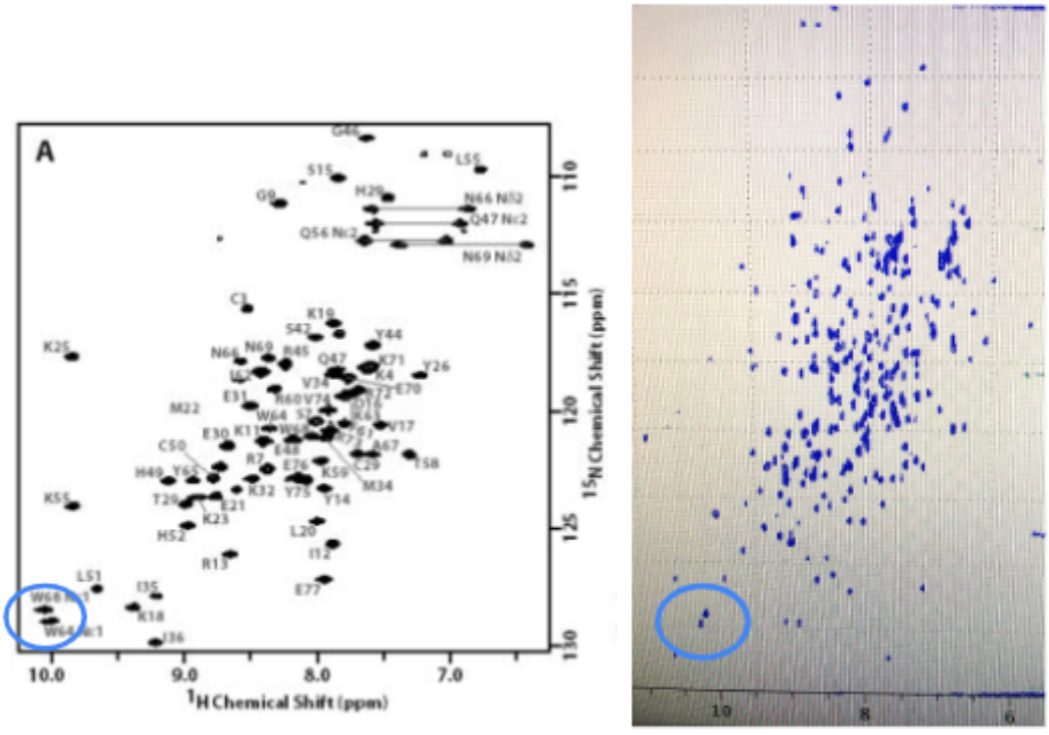

Figure 2: NMR HSQC of CXCL14 Image A shows an HSQC of CXCL14 previously published, which is used as a reference for the structure of the purified protein.[7] Figure B shows

the HSQC obtained during this purification project which indicated CXCL14 was present through the W64 and W68 residues, but not pure.

To further purify the sample, the Ni-NTA column was included in the purification process before the size exclusion chromatography. The chromatogram in figure 3 indicated CXCL14 eluted at about 45 minutes as the large gradual peak was due to the increasing imidazole content. The collected fractions were then purified using size exclusion chromatography. The chromatogram in figure 3 indicated some protein content but at too small of concentrations to collect. However, the sample was pure with only one significant peak where CXCL14 is expected to elute. CXCL14 was purified which can be used in further studies for potential binding to HPV E7.

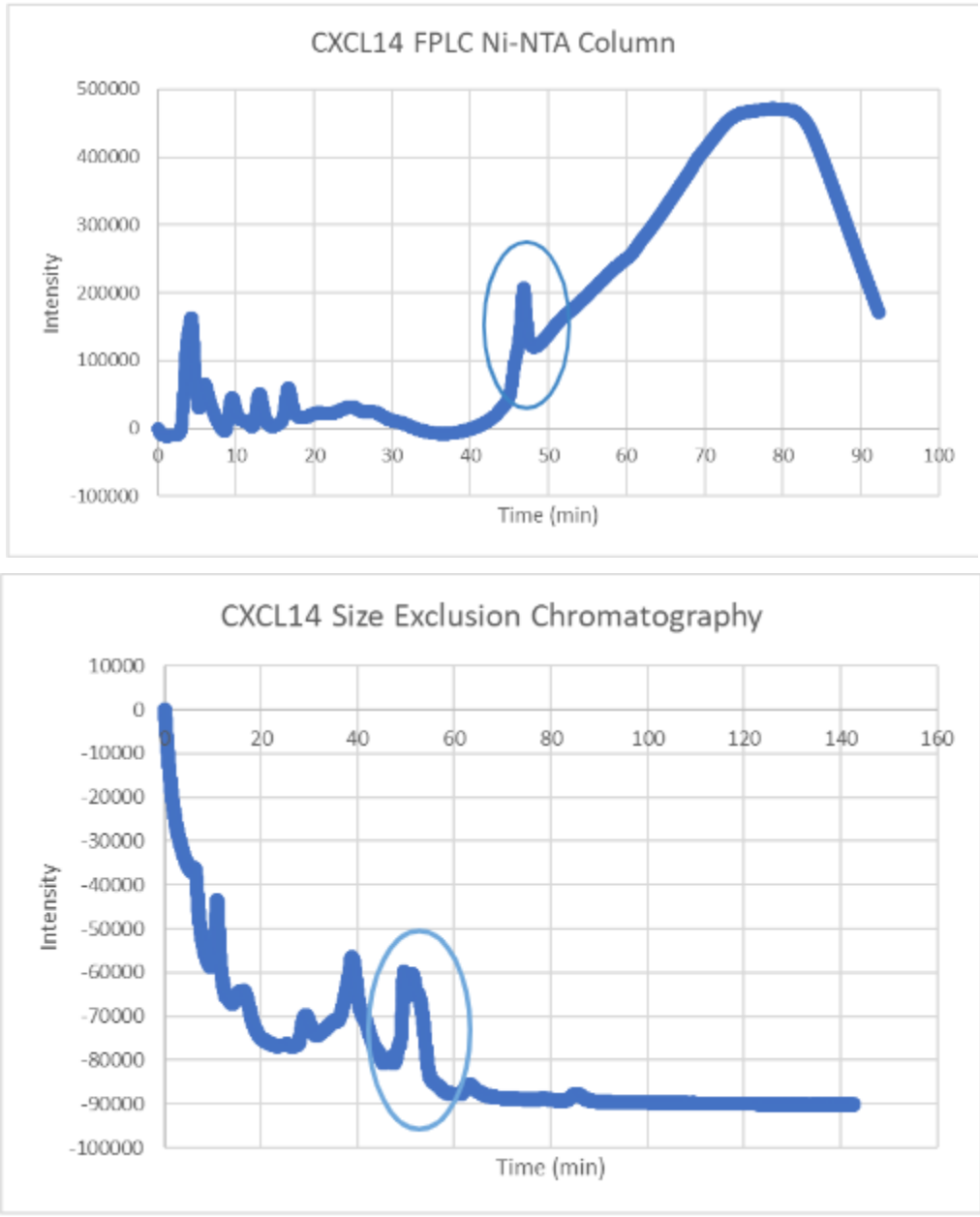

Figure 3: Purification of CXCL14 using two FPLC columns. The top graph shows the intensity of absorption at 280 nm over the time of the run. This was completed on a Ni-NTA column, beginning with a low concentration of imidazole for the protein to bind, then gradually increasing imidazole concentration to elute CXCL14. The peak at 45 minutes is the protein sample. The second graph shows the CXCL14 fractions collected from the Ni-NTA column run on the S75 for size exclusion chromatography. The intensities never reached the threshold, so no fractions were collected.

Discussion and Future Directions:

This study intended to optimize the purification of CXCL14 in high concentrations for NMR analysis using FPLC to determine if CXCL14 and E7 bind. To study CXCL14 and its potential binding to E7 through NMR, a large amount of pure protein is needed. However, the CXCL14 protein was lost during the purification process so no further NMR analysis could be done. In the future, this study could continue in optimizing the FPLC purification or find an alternative method for analysis.

This purification protocol can be completed again, but when running on the Ni-NTA column on the FPLC, a lower concentration of imidazole should be used in the starting buffer. This likely prevented some protein from sticking to the column which resulted in the low protein concentration. Once the pure sample of CXCL14 is obtained, the protein can be mixed with HPV E7 to observe the potential binding interaction. An alternative to purification could be to run a pull down assay with impure lysate to see if E7 binds CXCL14 and can pull the protein out of the lysate. If successful, the assay would show if E7 binds CXCL14 without the need to purify the protein before further study.

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