Does HPV E7's binding affinity to PTPN14 determine its degradation ability?

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Introduction

Human papillomaviruses (HPV) are a family of diverse double-stranded DNA viruses. Over 200 HPV genotypes have been identified. HPVs are classified as high-risk (cancer-causing) or low-risk (not cancer-causing). Viral proteins encoded by the high-risk HPV genome can lead to cell transformation and cancer. One of the main carcinogenic proteins is E7.

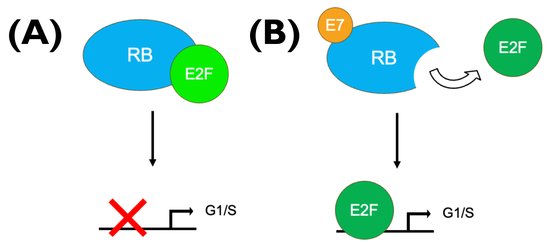

Figure 1. HPV E7 transformation mechanism with RB. (A) RB binds to transcription factor E2F and controls the cell cycle and proliferation. The G_1/S checkpoint is maintained. (B) E7 binding RB prevents the binding of E2F, allowing free E2F to bind to promoters and initiate transcription. The G_1/S checkpoint is bypassed.

One carcinogenic activity of high-risk HPV is to bind and degrade the retinoblastoma protein (RB1), allowing passage through the G_1/S checkpoint of the cell cycle[1] (Figure 1). E7 binding to RB is necessary but insufficient for transformation. Our lab is testing the hypothesis that E7 must also bind and degrade the tumor suppressor PTPN14 to promote cell growth[1].

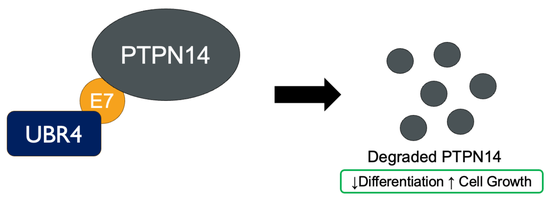

Figure 2. HPV E7 transformation mechanism with PTPN14. HPV E7 recruits a ubiquitin ligase UBR4 in addition to binding PTPN14 to degrade PTPN14. Degradation of PTPN14 by E7 leads to increased cell growth and reduced differentiation.

E7 requires UBR4 to degrade PTPN14[1] (Figure 2). PTPN14 is an important tumor suppressor because it controls cell growth and increases differentiation[1]. Different HPV E7 proteins reduce PTPN14 levels to different extents. My goal is to measure the binding affinity of PTPN14 to different HPV E7 proteins and test whether there is a correlation between binding affinity and PTPN14 levels.

Results

Varying Degradation of PTPN14 by E7

Different HPV E7 proteins reduce the steady-state level of PTPN14 to different degrees (Figure 3). There is no current data on the relationship between binding affinity and degradation. If a correlation was found between the two, then that would give an indication for why high-risk HPV E7s degrade better than low-risk ones.

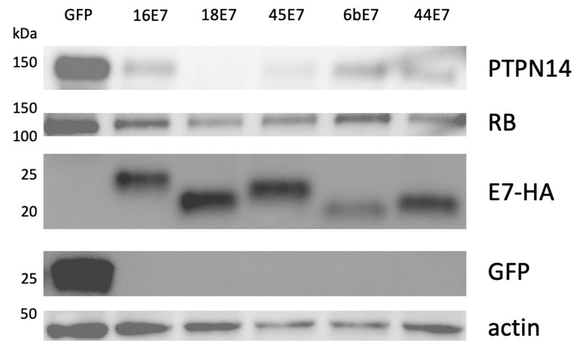

Figure 3. Western blot of Primary Human Foreskin Keratinocytes (HFK) transduced with various HPV E7 genes. E7 proteins are tagged with HA. HPV 16, 18, and 45 are high-risk genotypes found in HPV-related cancers. HPV 6b and 44 are low-risk genotypes.

Protein Expression of E7 and PTPN14

Genes coding for the C-terminus portions of E7 and PTPN14 proteins were cloned into expression vectors that add N-terminal tags necessary for protein purification (Figure 4). Vectors were then transformed into BL21(DE3) *E. coli* and induced overnight. (Figure 6). Purified protein samples were collected in several elution fractions and analyzed on a gel with Coomassie staining (Figure 7).

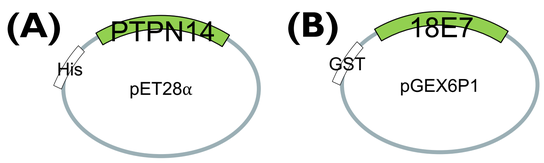

Figure 4. Vectors containing genes for PTPN14 and HPV 18E7. (A) PTPN14 contained within a pET28α backbone that N-terminally tags PTPN14 with His[2]. (B) HPV 18E7 contained within a pGEX6P1 backbone that N-terminally tags E7 with GST.

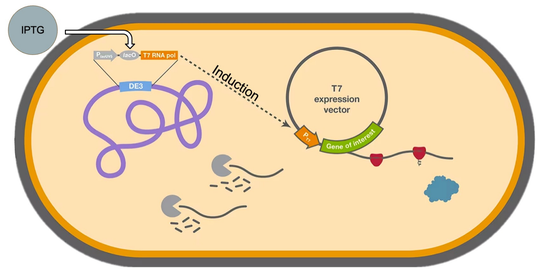

Figure 5. BL21(DE3) *Escherichia coli* protein induction system. IPTG induces the production of T7 RNA pol which will transcribe the plasmid vector's gene of interest. *Image adapted from ThermoFisher Scientific.*

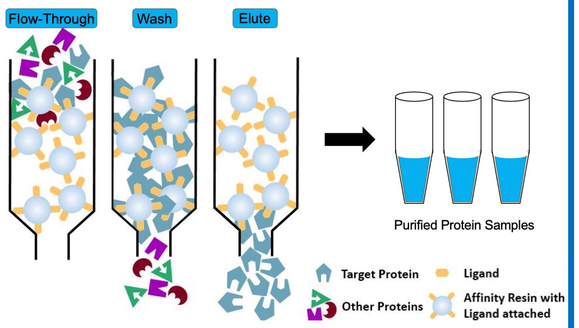

Figure 6. Affinity resin protein purification. Nickel-NTA (Ni-NTA) affinity resin binds His-tagged proteins and Glutathione affinity resin binds GST-tagged proteins. Target proteins were eluted and stored at -80°C. *Image adapted from Caframo Lab Solutions © 2016.*

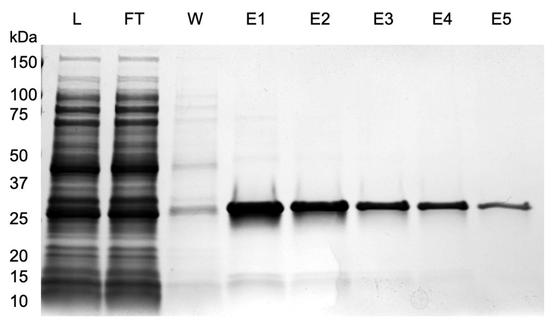

Figure 7. SDS-PAGE analysis of HPV 18E7(54-105) protein purification. Samples of bacterial lysate (L), flow-through (FT), wash (W), and elution fractions (E1-E5) were collected and Coomassie stained to determine effectiveness of column protein purification.

Future Directions

Measuring Binding Affinity using Surface Plasmon Resonance (SPR)

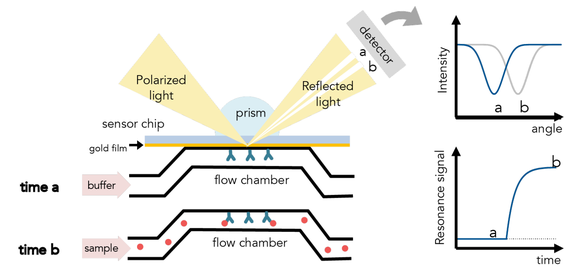

Figure 8. SPR flow chamber visualized model and binding interaction signaling results. The His tag on PTPN14 is immobilized in the flow chamber while the HPV E7 flows through. The SPR machine provides quantitative data on the binding and dissociation between the immobilized and free-flowing proteins. *Image source: Harvard Medical School, Center for Macromolecular Interactions.*

Prepared proteins will be flowed through the Biacore T200 SPR machine at different concentrations to determine the dissociation constant between HPV E7 and PTPN14 (Figure 8).

Figure 9. Sequence alignment of several HPV E7 proteins. RB binds to the LxCxE motif (highlighted in yellow). PTPN14 binds to the conserved Arginine (highlighted in red). N-terminal portions of each E7 will be removed when producing proteins (orange).

My plan is to grow more E7 proteins in addition to C-terminus HPV 18E7 and collecting data on all the binding affinities using SPR. Based on the N-terminal deletion of the 18E7 vector, other E7s will be prepared similarly according to sequence alignment (Figure 9).

References

1. White EA, Münger K, Howley PM: *High-Risk Human Papillomavirus E7 Proteins Target PTPN14 for Degradation*. mBio 7(5): e1530-16, Sep 2016.
2. Yun H-Y, Kim MW, Lee HS, Kim W, Shin JH, Kim H, et al. (2019) *Structural basis for recognition of the tumor suppressor protein PTPN14 by the oncoprotein E7 of human papillomavirus.* PLoS Biol 17(7): e3000367. https:// doi.org/10.1371/journal.pbio.3000367

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2. Thanks to Bonsu Ku from the Korea Research Institute of Bioscience and Biotechnology for providing the PTPN14 plasmid used for protein purification for my project (Figure 4A).