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Structural studies of HPV oncoprotein E7 and interactions with transcription factor, E2F1

Human papillomavirus (HPV) E7 is a key DNA tumor viral transforming oncoprotein related to cervical cancer around the world. In a normal cell, retinoblastoma protein pRb, a tumor suppressor protein, binds to E2F, a transcription factor, inactivating it. However in a cell with HPV, E7 interacts with pRb to impair the function of pRb, which releases E2F. With nothing there to restrain it the freed E2F transactivates genes for DNA synthesis, advancing the cell cycle into S phase, resulting in cervical epithelial host cells to progress and invasive cervical cancer. Studies of structure, dynamics, and interaction among E7, pRb, and E2Fs will provide great insights at molecular level on the progression of cervical cancer. Such studies could also suggest new strategies for interfering in E7 action, such as possible development of a clinical drug and it may help explain how antibodies in the HPV vaccine could work. Human E2F1 (aa 243-437) was expressed as a GST fusion from pGEX-6p-1-E2F1 and purified using glutathione resin. GST was removed using the PreScission 3C protease and glutathione resin. The expression of thirteen site-directed HPV 1a E7 mutants, including E7-C26S, E7-M5C/C26S, E7-E16C/C26S, E7-Y25C/C26S, E7-C26S/L39C, E7-C26S/Q44C, E7-C26S/S51C, E7-C26S/T62C, E7-C26S/S68C, E7-C26S/Q72C, E7-C26S/R79C, E7-C26S/S80C, E7-C26S/Q93C, were checked as well. We will ¹⁵N label the thirteen site-directed E7 mutant proteins and they will be purified individually and spin labeled using MTSL (1-oxy-2,2,5,5-tetramethyl-D-pyrroline-3-methyl-methanethiosulfonate). We are going to use an advance technique----- NMR paramagnetic relaxation enhancement to measure "long range" distances up to 25 Å to help us get the NMR solution structure of full-length HPV 1a E7. The label will also help us map HPV 1a E7 interaction with E2F1 by NMR.