ABSTRACT

Human papillomaviruses have been identified as the major aetiological factor in cervical cancer. Constitutive expression of the high risk HPV E6 and E7 oncoproteins are important for malignant transformation in infected keratinocytes. In particular, E6 and E7 bind to and inactivate the cellular tumor suppressors p53 and pRB, respectively, thus delaying differentiation and inducing proliferation in suprabasal keratinocytes to enable HPV replication. One member of the pRB family, p130 appears to be an important target for E7 in promoting S-phase entry. Recently, it has been discovered that p130 is part of a large protein complex termed DREAM. The composition of DREAM is temporally regulated during the cell cycle; being associated with E2F-4 and either p107 or p130 in G0/G1 and with the B-myb transcription factor in S/G2.

In this study, we addressed whether p130/DREAM complex is disrupted in HPV-transformed cell lines and whether this property is important for E6 and E7 function. We found that, p130-DREAM complex diminished in HPV-transformed cell lines (CaSki and SiHa) compared to control cell lines. However, p130/DREAM complex was reformed and cell cycle was arrested in HPV-transformed cell lines when E6/E7 expression was targeted by specific small hairpin RNAs. We further demonstrated that the profound G1 arrest in CaSki cells was dependent on p130/DREAM reformation by also targeting the expression of the DREAM component Lin-54 and p130. Moreover, p130/DREAM complex was completely disrupted in high risk HPV compared to low risk and cutaneous HPV when various types of HPV were expressed ectopically in T98G cells. Interestingly, one type of the cutaneous HPV, 48 has an ability to disrupt p130/DREAM quite dramatically although it binds to pocket protein with

extremely low affinity. Furthermore we found that B-myb/DREAM complex is not critical in regulating S/G2 phase in CaSki cells as determined by G2/M-phase genes in real time PCR.

Finally, we showed the ability of E7 in disrupting p130/DREAM complex through multiple mechanisms. Four different types of p130 mutants were expressed in pMSCV puro vector and transfected into CaSki and T98G cell lines. The results demonstrate that 16E7 must bind to p130 in order to induce the S/G2 phase in HPV-transformed cell line. Furthermore, the cutaneous HPV type (48E7) promotes the S phase by binding towards p21, the CDK inihibitor. The results show that continued HPV16 E6/E7 expression is necessary in cervical cancer cells to prevent cell-cycle arrest by a repressive p130/DREAM complex.