The homeobox transcription factor Six1contributes to both tumor development and progression. Numerous studies have determined that the inappropriate expression of embryonic genes, in particular transcription factors, contributes to carcinogenesis.[15] SIX1 is essential for the development of numerous organs including the auditory and olfactory system as well as the kidney, by promoting proliferation, survival and migration of progenitor cells during embryogenesis. Six1 has also been shown to increase cancer cell proliferation, survival and invasion. The aberrant expression of SIX1 occurs in numerous adult and pediatric cancers. We have previously determined that our *in-vitro* model system for HPV16-mediated tumorigenesis shares many important features with cervical cancer and enables us to study the molecular mechanisms of transformation and immortalization in our cell human keratinocyte (HKc) lines. SIX1 mRNA and protein levels are overexpressed in our HPV16-transfomred HKc lines at the differentiation-resistant stage HKc/DR) compared with early passage, HPV16-immortalized HKc (HKc/HPV16) and in HKc/HPV16 compared to normal HKc. Furthermore, we have recently determined that SIX1 overexpression in HKc/HPV16 induces the differentiation-resistant phenotype characteristic of HKc/DR, and that SIX1 overexpression in HKc/DR induces tumorigenicity. In this study, we explored the role of SIX1 as a regulator of growth and transformation in normal HKc, and its role in the maintenance of growth and a transformed phenotype in HKc/HPV16 and HKc/DR. We determined that loss of SIX1 is not tolerated by HKc/DR, which appear to be "addicted" to this oncogene. Decreased SIX1 expression results in slower proliferation and decreased HPV16 E6/E7 mRNA levels. We utilized Affymetrix GeneChip Arrays to explore the gene expression changes associated with decreased SIX1 expression in HKc/DR. Ingenuity Pathway Analysis, real time PCR and functional cell-based assays determined that SIX1 is vital for cell survival; the decline in SIX1 causes a

transition from the mesenchymal phenotype characteristic of HKc/DR towards the standard epithelial phenotype (mesenchymal-epithelial transition, MET). MET is accompanied by a switch in TGF-β signaling from an EMT-inducing tumor promoter to a tumor suppressor in HKc/DR cells. Additionally, we observed that SIX1 overexpression in normal HKc extends their lifespan and induces epithelial-mesenchymal transition, EMT. In summary, our studies suggest that SIX1 is necessary for cell survival in HPV-16 –transformed cells and may potentially become a suitable therapeutic target for HPV-driven cancers.