

p53 Recovery and Apoptosis Induction via Inhibition of E6-PDZ Interaction

Aida Abate¹, Michael Belmares¹, Chamorro Somoza¹, Valli Ramasami¹, Minh Ho¹, Pauline Henry¹, Ken Mendoza¹, Jingjing Wang¹, Wei Yuan¹, Johannes Schweizer¹ and Peter Lu¹
¹Arbor Vita Corporation, Sunnyvale, CA, USA

Introduction

Cervical cancer is the second most common cancer diagnosis in women worldwide, with approximately 500,000 cases and over 200,000 deaths annually. Oncogenic variants of human papilloma virus (HPV) have been correlated with cervical cancer at a frequency of > 99%, and also cause penile/anal and head and neck cancers. We have recently determined that all oncogenic E6 proteins bind to the PDZ protein MAGI1-d1 while E6 proteins from non-oncogenic variants do not bind to PDZ proteins. The interaction of oncogenic E6 proteins to MAGI1-d1 is critical for both HPV-induced carcinogenesis and for maintenance of the cancerous state in HPV-positive cells.

Our central hypotheses for this study are that (1) HPV-mediated oncogenesis involves interaction between the HPV-E6 protein and the cellular PDZ protein MAGI1 and (2) inhibitors of this interaction will be effective cervical cancer therapeutics. In support of these hypotheses, we first provide evidence that all oncogenic E6 variants bind to MAGI1. We then demonstrate a pathway by which E6-MAGI1 binding via JNK activation leads to enhanced cellular production of E6, which in turn causes p53 degradation and oncogenesis. Then, to identify small molecules that bind MAGI1-d1, a total of approximately 650,000 test compounds from the Chemical Diversity Library (ChemDiv, San Diego CA) and the Blanca Pharmaceutical Library (Blanca Pharmaceutical, Mountain View CA) were screened virtually, and 184 small molecules were selected from the "possible hits lists" for experimental testing. The 184 small molecule compounds identified computationally were tested *in vitro* via a competitive colorimetric ELISA for inhibition of the MAGI1-E6 interaction. Small molecules were screened for inhibition of the interaction between the C-terminal peptide of E6 and MAGI1. A reduction in OD at 450 nm by a small molecule was interpreted as blockade of binding of the labeled E6 peptide (bound biotinylated E6 peptide yields yellow color via binding to HRP-streptavidin).

AVC-7 was one of the three compounds that inhibited the interaction of HPV-E6 with MAGI1 with an IC50 of 87 μ M. Inhibition by AVC-7 was PDZ specific: e.g., it did not inhibit ligand binding to Shank1, or individual domains of PDZ-PSD95 (data not shown). Finally, we show that AVC-7 reduces E6 protein levels and restores p53 levels in HPV-infected cancer cells, inducing their apoptosis. The activity of this initial small molecule is highly structure specific, suggesting the potential for its derivatization to yield a human cancer therapeutic.

Results

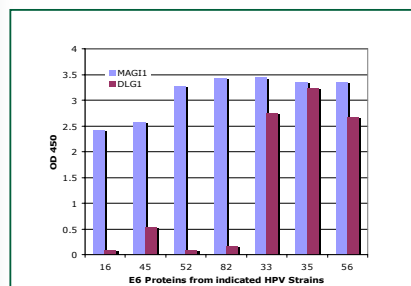


Fig. 3. MAGI-1 binds all oncogenic E6 proteins while DLG1 only binds a subset of them. Biotinylated C-terminal peptides (2 μ M) corresponding to the indicated HPV types were tested for binding to MAGI1 and DLG1 (in addition to other PDZ proteins; not shown) via a modified ELISA assay. Y-axis indicates yellow color resulting from PDZ-ligand binding.

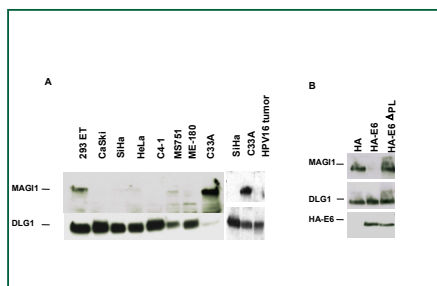


Fig. 4. (A) MAGI1 and DLG1 protein levels in HPV-positive or HPV-negative cervical cancer cells and cervical cancer biopsy. Total cell lysates analyzed by Western blot. (B) Cells were transiently transfected with pmk1t-HA-E6, pmk1t-HA-E6 Δ PL or the control pmk1t-HA expression vector. Shown are MAGI-1, DLG1 and the E6 protein expression levels.

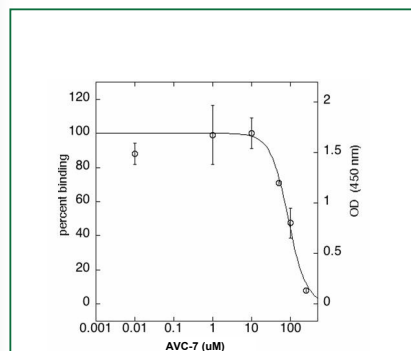


Fig. 6. AVC-7 inhibits the interaction of MAGI1's PDZ domain and E6's C-terminal peptide (biotinylated to enable detection) with increasing concentration (IC50 = 87 μ M).

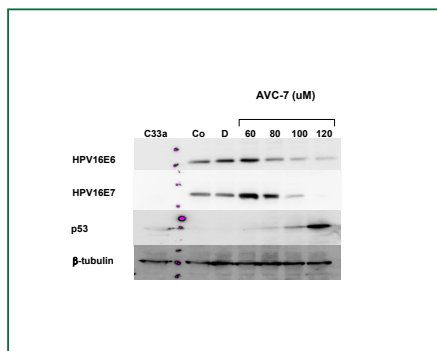


Fig. 7. The effect of AVC-7 on the expression of oncogenic proteins E6, E7 and p53 in HPV-positive CaSki cells. Cells were incubated with growth media alone (Co), vehicle control (DMSO, D) or increasing concentrations of AVC-7 for 24 h. Cell lysates were analyzed by Western blot. β -tubulin was used a loading control.

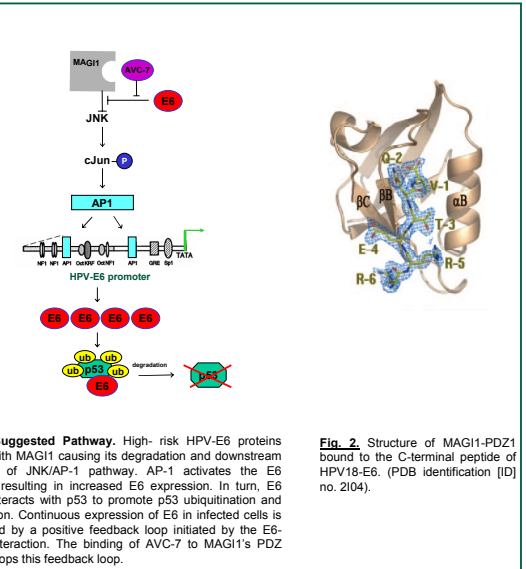


Fig. 1. Suggested Pathway. High-risk HPV-E6 proteins interact with MAGI1 causing its degradation and downstream activation of JNK/AP-1 pathway. AP-1 activates the E6 promoter resulting in increased E6 expression. In turn, E6 protein interacts with p53 to promote p53 ubiquitination and degradation. Continuous expression of E6 in infected cells is maintained by a positive feedback loop initiated by the E6-MAGI1 interaction. The binding of AVC-7 to MAGI1's PDZ domain stops this feedback loop.

Fig. 2. Structure of MAGI1-PDZ1 bound to the C-terminal peptide of HPV18-E6. (PDB identification [ID] no. 2104).

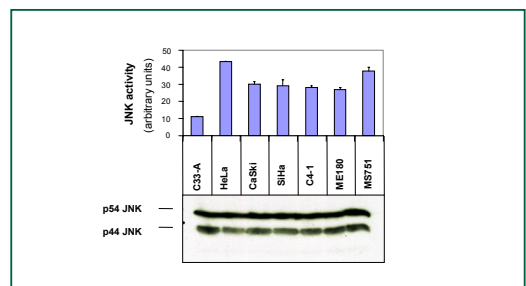


Fig. 5. Basal JNK activities and expression levels in the HPV-negative cervical cancer cell line C33A and six HPV-positive cervical cancer cell lines. JNK activity was measured by reacting cell lysates with [³²P]ATP in the presence of recombinant GST-c-Jun protein. The reaction mixture was separated by SDS-PAGE and incorporation of ³²P into GST-c-Jun visualized by autoradiography and quantified by Phosphorimaging. JNK expression was measured by Western. Differences in JNK expression were not significant and do not account for differences in JNK activity.

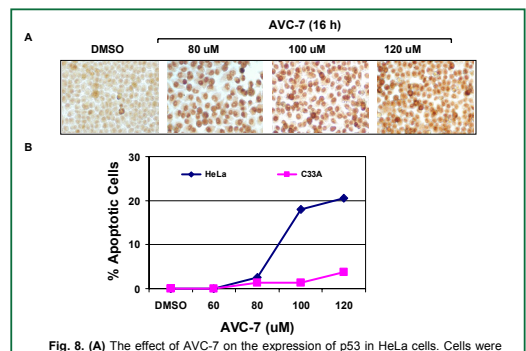


Fig. 8. (A) The effect of AVC-7 on the expression of p53 in HeLa cells. Cells were incubated with control solvent or increasing concentrations of AVC-7 for 16 h. P53 was stained with a specific antibody and visualized by using the DAB substrate. (B) AVC-7 induces selective apoptotic cell death in HPV18-positive cell line HeLa while the HPV-negative cancer cell line C33A was not affected. DNA fragmentation was measured using the DeadEnd Fluorometric TUNEL system from Promega. Data is representative of two independent experiments.

Conclusion: These results suggest that specific targeting of E6-MAGI1 interaction by AVC-7 leads to selectively induce apoptosis in HPV-positive cervical cancer cells. Further optimization of AVC-7 is a promising strategy to increase selective apoptotic cell death of cells transformed by high risk HPV. An optimized derivative of AVC-7 with improved potency can be a potential therapeutic agent to treat pre-cancerous and cancerous lesions caused by oncogenic HPV infection.

