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Role of annexin-I in cervical cancer cell proliferation

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Objective. **To investigate the role of annexin-I in human cervical cancer, we evaluated the expression of annexin-I and the relation with the proliferation of cancer cells.**

Methods. **By the immunohistochemical analysis and western blotting of annexin- I in cervical cancer tissues, we investigated the extent and distribution of the expression of annexin-I in cervical cancer tissues. To make the cells proliferate and antiproliferate, we treated the human cancer cell lines, SiHa and HeLa cell lines with tamoxifen, estradiol, and retinoic acid for 5 days. We used 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide(MTT) colorimetric assay to measure the proliferation and flow cytometry to detect the expression level of annexin-I, simultaneously.**

Results. **In immunohistochemical stain, a granular staining pattern involving the entire cytoplasm was more heavily observed in malignant lesions than normals. In western blotting, the antibodies against 35-kDa annexin-I appeared to react more strongly with the lysates of cancer tissues than normal and benign tissues. In SiHa and HeLa cell lines with the treatments of tamoxifen and β- estradiol, increased expressions of annexin-I were noted with the correlations of increased proliferation of cells. And with the treatments of all trans retinoic, decreased expressions of annexin-I were noted with the correlation of decreased proliferation of cells.**

Conclusions. **The results suggest that the expression of annexin-I might correlate with cervical cancer than normal and the proliferation of cancer cells.**

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Inactivation of p73 protein by HPV E6 protein: Novel p53-independent function of HPV E6

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HPV E6 and E7 oncoproteins play major roles by inactivation of p53 and pRb tumor suppressor proteins. Purpose of the study is to identify novel cellular target, p73, of E6 and to determine how E6 inactivates the function of p73.

Interaction between E6 and p73 were identified by yeast two-hybrid assay *in vivo* and GST pull-down assay *in vitro*. Function of the interaction was determined by transient transfections using $p21^{WAF1}$ promoter-CAT reporter plasmid. Molecular mechanisms were assessed by *in vivo* and *in vitro* protein degradation assays and gel mobility shift assays. To compare the inactivation mechanism of p73 to that of p53, several E6 mutants were used for transient transfecion assays.

Yeast two-hybrid and GST pull-down assays indicated a physical interaction between p73 and either HPV-16 or HPV-11 E6 protein *in vivo* and *in vitro*. Transactivation domain was found to be required for the interaction. Transient co-expression of E6 inhibited p73-mediated activation of $p21^{WAF1}$ promoter in a p53-defective C33A cell line. Using Gal4-p73 fusion protein, E6 inhibition of p73 transactivation function was independent of sequence-specific DNA binding, which was confirmed by an electrophoretic mobility shift assay. Moreover, E6 inhibited p73 function by interfering with the activity of amino-terminal activation domain. Protein degradation assays *in vivo* and *in vitro* indicated that p73, unlike p53, was not susceptible to E6-dependent proteolysis. Co-transfection of E6 mutants revealed that two independent functions of E6, *i. e.*, the repression of p53-mediated transactivation and degradation of p53, could be separable. These results suggest that the inactivation of p73 could be mediated by transcriptional repression through the direct interaction between E6 and p73, but not by the degradation of p73.

In conclusion, we identified p73 as a novel cellular target of HPV-E6 protein and found that E6 binds p73 through the amino-terminal transactivation domain, and inhibits its transactivation function independent of the protein degradation and DNA binding. In addition, studies with E6 mutants indicated that the interaction is sufficient for the inactivation of p73. Consequently, in addition to the inactivation of p53, the functional interference of p73 by HPV-E6 may contribute to E6-mediated cellular transformation.