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Regulation of CD95-mediated apoptosis in human Papillomavirus positive cervical cancer cells

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One way to escape immunological surveillance is by dys-regulation of CD95-mediated apoptosis. When cervical carcinoma cells were monitored towards apoptotic signals, human papillomavirus (HPV) type 18-positive cell lines were found to be highly sensitive to agonistic CD95 antibodies ($CD95^{S}$) or TNF-? after co-exposure with cycloheximide. In contrast, HPV16-positive cervical carcinoma cells were CD95 resistant ($CD95^{R}$) under the same experimental conditions. Somatic cell hybridization between $CD95^{S}$ and $CD95^{R}$ cervical carcinoma cell lines revealed that CD95 sensitivity was a dominant trait, which correlates with abundant c-Myc and low Bcl-$_{XL}$ expression. Although $CD95^{R}$ cervical carcinoma cells expressed even higher levels of p53 and CD95 receptor at their surface, resistance could be attributed to the inability to form a functional death-inducing signaling complex („DISC"), necessary for successful transmission of the apoptotic response. These data indicate that resistance to apoptosis may represent not only an important immunological escape mechanism during virus-induced carcinogenesis, but may also explain the different incidence rates of HPV16 and HPV18 types found in cervical cancer patients.

Furthermore, considering the possibility that cancer cells can also counterattack infiltrating lymphocytes, co-cultivation between Jurkat T-cells and HPV16/18 positive cervical carcinoma cell lines were performed. Of interest was the finding that only $CD95^{S}$ cells were capable to kill co-cultivated Jurkat cells, which support the notion that cervical carcinoma cell

lines used different ways to escape immunological control.

To understand the function of the individual oncogenes of HPV16 ($CD95^{R}$) in modulating the cellular response to CD95L or TNF-? , human keratinocytes were used as model system, which were separately immortalized either with E6, E7 or E6/E7 oncoproteins of HPV16 via retroviral gene transfer. Applying the same condition as utilized for cervical cancer cells, only E7-immortalized cells underwent apoptosis, whereas E6- and E6/E7-expressing keratinocytes were again resistant. The dominance of E6 correlated with a significant down-regulation of the CD95 gene both at the RNA and protein level. No quantitative differences in the amount of the „Fas-associated death domain“ (FADD) protein, caspase 8 (a effector caspase) or caspase 3 (an executioner caspase) could be discerned. In contrast, E7-immortalized keratinocytes revealed abundant amounts of c-Myc and p53, without significant alterations of Bcl-2, corroborating the pivotal role of c-Myc in sensitizing cells to apoptosis. Interestingly, high levels of the apoptosis inhibitory c-FLIP protein could be only detected in normal keratinocytes, whereas the protein was generally down-regulated in all immortalized cells in the order E7/E6 > E7 > E6. Although c-FLIP was also reduced in E6-positive cells, no apoptosis occurred, because the pro-apoptotic proteins c-Myc and p53 were missing. Since HPV16E6 has quite a obvious anti-apoptotic functions, therapeutic strategies which specifically inactivate E6 may help to sensitize HPV16 ($CD95^{R}$)-positive cells to apoptosis.