Antisense Human Papillomavirus (HPV) E6/E7 Expression, Reduced Stability of Epidermal Growth Factor, and Diminished Growth of HPV-Positive Tumor Cells

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Papillomaviruses were the first viruses found to transmit cancer. Isolates of a rabbit papillomavirus from infected cottontail rabbits were shown to produce benign papillomas in disease-free recipients (1). These viruses are small DNA viruses that display both species and tissue specificity and differ markedly in their transforming capacity. Although more than 70 different human papillomavirus (HPV) types have been identified, only certain strains of HPV have emerged as participants in the development of squamous cell carcinomas, including cancers of the cervix and the oral cavity. HPV16 and HPV18 are associated with neoplastic and preneoplastic lesions, whereas HPV6 and HPV11, although structurally similar to HPV16 and HPV18, are associated with benign growths such as warts (2–4).

The HPV genome is an approximately 8-kilobase genome that encodes early (E) and late (L) gene products (2,5). E6, E7, E1, E2, E4, and E5 are proteins that are produced early in the viral life cycle, whereas L1 and L2 are capsid proteins produced later (2,5). The E6 and E7 genes are of particular importance with respect to the ability of HPV to immortalize epithelial cells (6). The E6 and E7 genes are expressed in essentially all HPV-involved cervical tumors in vivo (7,8), in cervical cell lines prepared from human tumors, and in cells immortalized in vitro with plasmids containing the HPV genome (9–11). Moreover, human epidermal keratinocytes and cervical epithelial cells have been immortalized with plasmids containing only E6 and E7 (6,12). Although HPV immortalizes the cells, these cells do not form tumors in nude mice or grow efficiently in soft agar (9,11). Moreover, there is evidence that the progression toward tumorigenicity requires additional genetic changes that involve the activation of other oncogenes (13). HPV E7 protein in cooperation with an activated ras or fos oncogene can fully transform cells (13,14). Binding studies show that E6 interacts with p53 and that E7 interacts with pRb (15–17). Thus, the E6 and E7 proteins are thought to immortalize cells, at least in part, by interfering with the function of the p53 and pRb tumor suppressor proteins. In vitro studies indicate that the interaction of E6 protein with p53 functions to target the latter protein for degradation via a ubiquitin-dependent pathway (18,19) and that the interaction of E7 with the pRb renders the latter protein inactive (17,20). The loss of the growth-suppressive effects of p53 and pRb is thought to contribute to cell immortalization. This proposal is further supported by the observation that the E6/E7 proteins from the low-risk papillomaviruses HPV6 and HPV11 bind p53 and pRb less tightly than E6/E7 from HPV16 and HPV18 (15,19,21,22). The continued expression of HPV E6 and E7 oncogenes is necessary to maintain the immortalized phenotype. Moreover, increased affinity of E6/E7 for p53 and pRb is positively associated with increased ability to immortalize keratinocytes (15,19). Furthermore, there is a strong association between HPV-immortalized epithelial cell growth and E6/E7 expression under most conditions (23,24). However, some agents like retinoic acid can regulate HPV-immortalized cervical cell growth with no change in E6/E7 expression (12,25).

One strategy that has been used to elucidate the role of E6/E7 in the control of cell growth has been to alter selectively the expression of these oncogenes via antisense technologies. Transfection of plasmids encoding antisense HPV18 E6 and E7 RNA was shown to inhibit the growth in two HPV-positive cervical carcinoma cell lines C4-1 and HeLa and in the HPV18-positive oral carcinoma cell line 1483 as well as to inhibit the tumorigenicity of these cells in nude mice (26–30). Only HPV-positive cells were inhibited, and the effect was dependent on expression of the antisense sequences. Antisense constructs that were designed to generate RNAs with ribozyme activity were particularly effective (31–33). It has since been shown that antisense oligonucleotides specific for E6 and E7 also have inhibitory effects on HPV-positive cell growth (34). Although the antisense E7 oligonucleotide was a more effective growth inhibitor than the antisense E6 oligonucleotide, maximal inhibitory response was found when both antisense oligonucleotides were used. Moreover, prolonged growth inhibition required the continued presence of the antisense oligonucleotides (34). Reported effects on anchorage-independent cell growth and serum requirements conflicted. Cell lines either appeared to have an enhanced serum growth factor dependency (typical of normal epithelial cells) or were unaffected. The plating efficiency of the cells was unaf-
ected by antisense treatment. Most recently, the expression of HPV16 E6/E7 messenger RNA (mRNA) and the growth of the cervical carcinoma cell line SiHa were reported to be inhibited by adenovirus-mediated transfer of HPV16 E6/E7 antisense RNA (35). The growth-inhibitory effects of these antisense constructs are potentiated when the cells were also made to express either p53 (35) or RB (26).

Based on the known effects of the oncogenic strains of HPV, it has been suggested that E6 and E7 regulate cell proliferation by affecting the expression and activity of tumor suppressor proteins p53 and pRb (16,17,36). Both proteins are important participants in the cell cycle and cell death pathways. Cell cycle arrest at the G1 phase is induced by p53 and pRb (37–40). In addition, p53 can induce apoptosis during which retinoblastoma protein is selectively cleaved by an interleukin-converting enzyme-like protease. Antisense-induced changes in cell growth might therefore be expected to directly correlate with the level of E6/E7 expression. However, no changes in the levels of HPV expression were found in HeLa cells whose growth was inhibited by antisense HPV E6/E7 (27). Furthermore, von Knebel Doeberitz et al. (28) found no change in the levels of mRNA or protein of E6 and E7 in C4-1 cells transfected with HPV antisense constructs. Similarly, no changes in the levels of E7 protein were found in HPV-positive cells treated with either antisense E6 or E7 oligonucleotides (34). However, the antisera to E6 and E7 lack specificity, and it may not be surprising that there are very limited data on the levels of these proteins following treatment. Unfortunately, there are no data available on pRb and p53 levels following E6/E7-targeting antisense treatments.

Although the mechanism of action for the effects of antisense expression is not yet known, it is generally understood to be dependent on RNA:RNA hybrid formation, which in turn would reduce the amount of endogenous mRNA available for protein synthesis. However, changes in cell growth and differentiation following antisense treatment have been reported in other systems with no changes in the levels of the targeted RNAs. These results have lead to the suggestion that other RNA processes may be involved, including, among others, changes in splicing, transport, and translation.

To better define the mechanisms regulating these changes in cell proliferation, Hu et al. examined how the expression of antisense HPV18 E6/E7 or sense Rb transcription units affects epidermal growth factor (EGF) receptor (EGF-R) levels in HeLa cells. Many HPV-associated cancers, including those of the cervix and oral cavity, express high levels of EGF-R, and this elevated expression is associated with a more aggressive phenotype (41–44). Transfection of normal human cervical epithelial cells, epidermal keratinocytes, and laryngeal epithelial cells with either HPV16 or HPV18 results in a fivefold to 10-fold increase in EGF-R levels (45–47). In this issue of the Journal, Hu et al. (48) show that either the expression of antisense HPV E6/E7 or the Rb transcription unit results in the decreased expression of the EGF-R protein. Although the HeLa cell clones containing both the antisense HPV E6/E7 and Rb transcription units showed no change in the EGF-R mRNA levels, there appeared to be an increase in the EGF-R protein turnover.

The EGF family of proteins includes a number of known ligands, including EGF, transforming growth factor-α, amphi-

References


Note

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