An *In vitro* Multistep Carcinogenesis Model for Human Cervical Cancer

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Abstract

Human papillomaviruses (HPV) are believed to be the primary causal agents for development of cervical cancer, and deregulated expression of two viral oncoproteins E6 and E7 in basal cells, mostly by integration, is considered to be a critical event for disease progression. However, lines of evidence suggest that, besides expression of E6 and E7 genes, additional host genetic alterations are required for cancer development. To directly test this hypothesis, we first transduced HPV16 E6 and E7 with or without hTERT into several lines of normal human cervical keratinocytes (HCK) from independent donors and then searched for additional alterations required for carcinogenesis. Oncogenic HrasG12V (Hras) provided marked tumor forming ability in nude mice and ErbB2 or c-Myc (Myc) endowed weaker but significant tumor forming ability. Combined transduction of Myc and Hras to HCKs expressing E6 and E7 resulted in the creation of highly potent tumor-initiating cells. These results show that only one or two genetic changes occurring after deregulated expression of high-risk HPV oncoproteins might be sufficient for development of cervical cancer. [Cancer Res 2008;68(14):5699–705]

Introduction

Cervical cancers are thought to arise from cervical lesions after long persistent infection with high-risk human papillomaviruses (HPV; ref. 1). Mild and moderate dysplasia lesions show relatively low levels of E6 and E7 expression from episomally replicated viral genomes in the basal cell layer, whereas severe dysplasia and invasive cancer lesions often display high-level expression of E6 and E7 (2) with integration of viral DNA into the host cell genome whereby neoplastic development is believed to be initiated. Although life-time risk for HPV infection is ~80% for sexually active women (3), in most cases, infections resolve spontaneously due to effective immune responses, and ultimate development of cervical cancer is relatively rare. Thus, integration of the viral DNA into the host genome causing deregulated expression of E6 and E7 in basal cells is considered to be a rare but one of the critical events for cervical carcinogenesis. However, epidemiologic and experimental studies indicate that viral gene expression is not in itself sufficient to induce cervical cancer, and additional genetic and/or epigenetic events are required (4). Mutations or alteration in the expression or activity of PI3KCA (4), c-Myc (Myc), ErbB2 (5), cAP1 (6), Ras (7), and PTEN (8) have all been reported in cervical cancers. Of these, mutation of Ras and Myc amplification is frequently accompanied by development of recurrent cervical cancer (9), although whether they actually make a mutual contribution to such recurrence is still controversial (10). To evaluate the effect of different alterations on cervical cancer progression, we thought it important to create a naturally occurring cervical cancer model. Although human foreskin keratinocytes have been widely accepted as a standard cell type for assaying HPV oncogene activity, the relative oncogenic potencies of HPV16 E6 and E7 differ depending on the tissue evaluated (11). Thus, we chose normal human cervical keratinocytes (HCK; ref. 12) and established an *in vitro* model for cervical cancer by sequential transduction of defined genetic elements. The results with this model strongly indicate that only one or two genetic alterations might be sufficient to promote full transformation of HCK on a background of HPV16 E6 and E7 expression. Notably, creation of highly potent cancer initiating cells by introduction of Myc and oncogenic HrasG12V (Hras) could be achieved.

Materials and Methods

Cell culture and cell lines. Normal HCKs were obtained with written consent from patients who underwent abdominal surgery for a gynecologic disease other than cervical cancer. HCK1, HCK4, HCK7, HCK8, HCK9, and HCK12 cells derived from different donors were maintained in low-calcium disease other than cervical cancer. HCK1T and HCK12 were then grown in DMEM + 10% fetal bovine serum for adaptation to calcium and serum (13). Cervical cancer cell lines, SiHa, CaSki, and HeLa, and T24, a human bladder carcinoma cell line, were grown in DMEM (Sigma) containing 10% FBS.

**Vector construction and retroviral infection.** Construction of the retroviral expression vectors, pCLXSN-16E6E7, pCLXSH-hTERT, and pCMVPCVpuro-myr-Akt1, has been described previously (14, 15). Human c-Myc, c-ErbB2 (a gift from Dr. T. Yamamoto, Institute of Medical Science, University of Tokyo, Tokyo, Japan) and HrasG12V (a gift from Dr. W.C. Hahn, Dana-Farber Cancer Institute, Boston, MA) were cloned and recombined into retroviral expression vectors to generate pCMVp53puro-c-Myc, pCMVp53-ErbB2, and pCMVPCV-HrasG12V. pCMVPCV was produced by replacing the puromycin resistant gene in pCMVp53puro with a segment containing blasticidin-S resistant gene. The production of recombinant retroviruses and selection of infected HCKs were described previously (16). All retrovirus-infected cells were confirmed to be free of helper virus by horizontal spread assay and by Western blots to determine the absence of viral gag protein.

**Western analysis.** Western blotting was conducted as described previously (12). Antibodies used were listed in the Supplementary Materials and Methods.
Colonies were soaked in 2.5% Triton X-100 at room temperature for 90 min, porcine gelatin (Difco) under nonreducing conditions. After electrophoresis, conditioned medium (20% FBS; termed DMEM hereafter), only those cells were exposed to calcium and serum (DMEM supplemented with 10% FBS; termed DMEM hereafter), only those cells carrying Hras in addition to E6E7 could form colonies (Fig. 1B, dark bar), indicating that the oncogenic Hras provided resistance to differentiation on the background of E6E7 expression, in line with the low level induction of a differentiation marker, involucrin, in the presence of Hras (Fig. 1C). When growth of the cells in KGM with increased cell densities (~50%) was examined, their growth slowed down when reaching confluence (~8×10^5 cells; Supplementary Fig. S1B, left). Hras plus Myc–transduced HCK1T-E cells showed fastest growth followed by those with Hras, Myc, ErbB2, or Akt. Then, when medium was replaced with DMEM on day 1, they appeared resistance to differentiation to some extent (Supplementary Fig. S1B, right) but HCK1T-vector cells could not resist calcium- and serum-induced terminal differentiation (data not shown). These data suggest that Hras plus Myc transduction to HCK1T-E resulted in the resistance of E6E7 to calcium and serum-induced terminal differentiation.

Organotypic raft culture system. The organotypic raft culture was performed as previously described (17), except that one part of type I collagen (5 mg/mL solution; AteloCell IPC; Koken) and four parts of growth medium containing human foreskin fibroblasts (HFF) immortalized by hTERT (1 × 10^6) were used for preparation of the dermal equivalent. Cells were cultivated at liquid-air interface for 2 wk and the cryosection (7 μm) was fixed with 4% paraformaldehyde and stained with H&E.

Results

Sequential transduction of oncogenes into primary HCK. Normal HCKs with an extended life span were isolated by introduction of hTERT, to provide a line that we term HCK1T (12), and they maintain normal features of cervical keratinocytes (16). We started to establish a HPV16-mediated multistep carcinogenesis model of cervical cancer with HCK1T. HCK1T transduced with HPV16 E6 and E7 by retroviral gene transfer (HCK1T-E, where E is for E6E7) showed anchorage-independent growth but failed to form tumors (12). In this study, HCK1T-E was further transduced with several oncogenes (Akt, ErbB2, Hras, Myc, and Hras plus Myc), and expression of the individual transgenes was confirmed by Western blotting (Fig. 1A). The expression levels of E6 and E7 proteins were comparable with those in cervical cancer cell lines. The expression level of Myc was lower than in HeLa cells and Hras level was comparable with that in T24 cells, a human bladder carcinoma line that has the same mutation. Increased phosphorylation of Erk confirmed the functional expression of introduced Hras. Increased Myc protein stability was found in these cells with Hras (Supplementary Fig. S1A).

Clonogenicity, resistance to calcium- and serum-induced terminal differentiation. HCK1T cells with or without oncogenes were examined for their cell density–dependent growth and resistance to calcium- and serum-induced terminal differentiation (20). When cells were seeded sparsely in a serum-free culture environment (KGM), HCK1T-vector cells did not form any colonies, but transduction of HPV16 E6 and E7 was sufficient to confer the capacity for colony formation (Fig. 1B, white bar). When sparsely seeded cells were exposed to calcium and serum (DMEM supplemented with 10% FBS; termed DMEM hereafter), only those carrying Hras in addition to E6E7 could form colonies (Fig. 1B, dark bar), indicating that the oncogenic Hras provided resistance to differentiation on the background of E6E7 expression, in line with the low level induction of a differentiation marker, involucrin, in the presence of Hras (Fig. 1C). When growth of the cells in KGM with increased cell densities (~50%) (3×10^5 cells per 35-mm dish) was examined, their growth slowed down when reaching confluence (~8×10^5 cells; Supplementary Fig. 1B, left). Hras plus Myc–transduced HCK1T-E cells showed fastest growth followed by those with Hras, Myc, ErbB2, or Akt. Then, when medium was replaced with DMEM on day 1, they appeared resistance to differentiation to some extent (Supplementary Fig. 1B, right) but HCK1T-vector cells could not resist calcium- and serum-induced terminal differentiation (data not shown). These data suggest that Hras plus Myc transduction to HCK1T-E resulted in the resistance...
to both contact inhibition and differentiation in DMEM, and other cells showed certain degree of growth retardation but started to regrow thereafter.

**Organotypic raft culture and gelatin zymography.** In three-dimensional organotypic raft culture (17), the addition of oncogene(s) to HCK1T-E cells increased the thickness of hyperplasia (Fig. 2A). Hras plus Myc-transduced cells featured histologic changes with invasion into the collagen raft that is reminiscent of cancer cells invading the submucosal layer (Fig. 2A). In line with the differentiation-resistant phenotypes observed in Fig. 1B and Supplementary Fig. S1B, less expression of the differentiation marker was observed in only upper layers of epidermal hyperplasia (Supplementary Fig. S2A).

Because Hras and Myc-transduced HCK1T-E cells, which had been adapted to DMEM, showed a more pronounced invasion phenotype (Fig. 2A), secretion of matrix metalloproteinases (MMP) in the conditioned medium was examined by gelatin zymography assays (Fig. 2B). Pro–MMP-2 was detected with all cell preparations cultivated in DMEM. Pro– and active MMP-9 were detected in only Hras plus Myc–transduced cells, implying a role of MMP-9 in the invasion phenotype (21).

**Anchorage-independent growth.** Because serum and calcium adaptation has been used to select differentiation-resistant keratinocytes induced by HPV genes (22, 23) and such selection has been considered to be important for the acquisition or maintenance of the transformed phenotype of keratinocytes (13, 24), DMEM-adapted cells were used for the following assays. After adaptation, Hras- and Hras plus Myc–transduced HCK1T-E cells showed similar growth and others showed slower growth compared with their growth in KGM (Supplementary Fig. S1C). Then, anchorage-independent growth was assayed in soft agar medium with DMEM (Fig. 2C). Hras introduced cells, and to a lesser extent, ErbB2, Myc, and Akt cells showed enhanced anchorage-independent growth. Notably, Hras plus Myc cells formed exceedingly large colonies, suggesting strong cooperation between Hras and Myc, although Akt also showed weaker cooperation with Hras.

**Tumorigenicity in nude mice.** Matrigel is used to enhance the tumorigenicity of the injected cells (25), and we experimentally confirmed the effects of the Matrigel (Supplementary Fig. S2B). Then, the DMEM-adapted cells mixed with Matrigel were injected s.c. into nude mice. Among them, Hras showed high activity to induce tumor formation (100%; 12 of 12, within 4 weeks; Fig. 2D), and ErbB2 (100%; 6 of 6; 2-month latency), Myc (100%; 12 of 12; 5-months latency), or Akt (50%; 6 of 12; 9- to 10-month latency; data not shown) showed weaker but significantly greater tumorigenicity than HCK1T-E vector cells, which failed to form tumors during 12 months of observation (0 of 7). Additional transduction of Myc or Akt to the HCK1T-E–carrying Hras resulted in acceleration of tumor growth. Remarkably, tumor size reached ∼500 mm³ just within 10 days in the Myc case (Fig. 2D). Presence of poorly differentiated malignant cells was confirmed in these tumors (Supplementary Fig. S2C). From these data, we conclude that expression of oncogenic Hras, ErbB2, or Myc can readily confer tumorigenic potential to HCK1T-E, and the combination of Hras and Myc strongly enhance the tumorigenicity.

**Confirmation of carcinogenesis steps with independent primary HCK with or without introduction of hTERT.** HCK1T was established by hTERT transduction, although E6 together with E7 can activate telomerase. Thus, we tested whether hTERT transduction is required for transformation of HCK with an independent batch of primary HCK, termed HCK12. HCK12-E cells with an extended life span were established by transduction of E6 and E7 genes as described previously (12). HCK12-E cells were further transduced with oncogenes, Hras, Myc, and ErbB2, and expression of transgenes was determined by Western blotting, and a similar result with that of HCK1T was obtained, although E6 and E7 expression levels were higher in HCK12-E cells (Fig. 3A). Then, HCK12-E cells were adapted to DMEM. Hras and ErbB2 enhanced anchorage-independent growth (Fig. 3B) and induced tumorigenic potential (Fig. 3C) of HCK12E cells. Myc also induced tumorigenic potential with a similar latency as observed for HCK1T-E cells (100%; 6 of 6, ∼4 months). Combination of Hras and Myc further enhanced tumorigenic potential with less influence observed in HCK1T-E cells (Figs. 2D and 3C). Then, hTERT was introduced to the cells to examine its contribution to tumorigenicity. Although it is not essential, hTERT introduction markedly enhanced tumorigenicity of HCK12-E cells with Hras (Fig. 4A; 1 × 10⁶ cells per site), although not reaching that of HCK12-E cells with Hras plus Myc (Fig. 3C; 1 × 10⁶ cells per site; Fig. 4B; 2 × 10⁶ cells per site), which showed no significant difference on additional transduction of hTERT (Fig. 4B). Although HCK12-E and HCK12-E cells with Hras displayed certain degrees of telomerase activity (Fig. 4C), the degree was much lower than those with hTERT and HCK12-E cells with Hras plus Myc, in which endogenous hTERT should be directly activated by Myc in cooperation with E6 (26). These results suggest that exogenous hTERT introduction is not an essential step for in vitro cervical carcinogenesis and that Myc enhances the tumorigenic potential of Hras-transduced HCK cells in both hTERT-dependent and hTERT-independent manner.

**Cancer-initiating features of several batches of HCK cells with E6E7, Hras, and Myc.** Because transduction of Hras plus Myc to HCK1T-E and HCK12-E cells conferred very high tumorigenic potential surpassing that of cervical cancer cell lines such as SiHa or HeLa (data not shown; ref. 12), we speculate that a relatively high number of cancer-initiating cells might be contained. To determine the frequency of cervical cancer–initiating cells within these cells, a limiting dilution assay was performed as previously described (Supplementary Fig. S3; refs. 27, 28). Even when only 10 Hras plus Myc–transduced HCK1T-E cells (DMEM adapted) were injected, they formed huge tumors within 40 days (100%; 6 of 6; Supplementary Fig. S3C).

To examine whether the additional changes (genetic/epigenetic) that were essential for tumorigenesis occurred during prolonged culture and/or DMEM adaptation, we determined the tumor induction ability of Hras plus Myc without DMEM adaptation using several batches of HCK-E cells derived from different patients (HCK4 and HCK4T; HCK4 infected with hTERT, HCK7, HCK8, HCK9, and HCK1T; a-c, triplicate, determining the repeatability of HCK1T). The transgene expression levels did not differ profoundly in these cells (Supplementary Fig. S4). All the cells were kept in KGM throughout the experiment. In the course of a minimum period of time, E6E7, Hras, and Myc were serially transduced to the cells. Those batches showing relatively slower growth took longer to complete the transgene (HCK4T, HCK4, HCK7, and HCK9 for 3 weeks) than the others showing relatively faster growth (HCK8 and HCK1T for 2 weeks). Soon after the termination of the drug selection of the last transgene, cells were transplanted to the nude mice. As summarized in Table 1, oncogenic Hras and Myc expression in all batches of HCKs carrying E6E7 resulted in the production of highly tumorigenic cells as determined by limiting dilution assays. Each HCK was derived from transforming zone of
different patients and showed different phenotype in terms of basal involucrin expression levels (data not shown). This might be one of the reasons that the frequency of cancer-initiating cells is not identical in all HCKs. Nevertheless, the results summarized in Table I suggest that oncogenic Hras and Myc on the background of E6E7-expressing HCK results in the high frequency of cancer-initiating cells without further acquisition of spontaneous genetic or epigenetic alterations.

Figure 2. Organotypic raft culture, zymography, anchorage-independent growth, and tumorigenesis in nude mice. A, assay with organotypic raft culture. The growth ability of the cells in three-dimensional organotypic raft culture was determined. The expression of E6E7 to HCK1T cells induced hyperplasia. The addition of Akt, ErbB2, Myc, or Hras to HCK1T-E cells increased the thickness of hyperplasia. HCK1T-E cells with Hras plus Myc featured histologic changes with invasion into the collagen raft, which is reminiscent of cancer cells invading the submucosal layer. Hras plus Myc–transduced HCK1T-E cells adapted to DMEM showed more pronounced invasion phenotype. B, gelatin zymography. The conditioned medium of HCK1T-E cells with vector, Akt, ErbB2, Myc, Hras, and Hras plus Myc adapted to DMEM were harvested and processed for gelatin zymography. The pro– and active forms of MMP-9 were detected in HCK1T-E cells with Hras plus Myc. C, for assessment of anchorage-independent growth of DMEM-adapted cells, aliquots (5 × 10⁴ cells) were seeded in 35-mm dishes. After 3 wk, the numbers of colonies (≥ 50 μm in diameter) were counted. D, in vivo tumor-forming abilities of HCK1T-E cells with cellular oncogene(s). The DMEM-adapted cells (P = 16) were s.c. injected into nude mice (1 × 10⁶ cells), and tumor size was measured every other day. The tumor volume (mm³) was calculated as L × W² × 0.52, where L is the longest diameter and W is the shortest diameter. Points, mean of data for 10 to 12 samples; bars, SD.
Discussion

The aims of this study were to establish models to allow in vitro reconstruction of events leading to HPV16-mediated multistep carcinogenesis and to evaluate the effect of individual alterations in this process. To our knowledge, this is the first report demonstrating that introduction of a sole oncogene could generate tumors on the background of expression of HPV16 E6 and E7 proteins in normal HCKs. The resistance to calcium- and serum-induced terminal differentiation of HCKs, as well as clonogenicity at low cell density and anchorage-independent growth in soft agar, were correlated with tumor-forming ability. The transplantation of \textit{Hras}-transduced HCK-E cells resulted in rapidly growing tumors. Furthermore, combined expression of \textit{Myc} and \textit{Hras} in HCK-E cells created highly potent tumor-initiating cells. It has been suggested that \textit{Ras} activation combined with HPV infection may be an important step in the genesis of a substantial number of cervical carcinomas (29). It has been reported that \textit{Hras} mutations are detectable in CIN 2 to 3 lesions (detected in 61 and 44% of progressing and persistence cases, respectively), and in mutated cases, progression took place in under 2 years (7). Although contradictory findings have also been reported (30, 31), our data suggest that \textit{Hras} is a critical oncogene for cervical carcinogenesis.

It has been shown that \textit{Myc} can cooperate with \textit{Ras} to transform rodent cells (32). This is the first report showing that \textit{Ras} plus \textit{Myc} cooperate to confer remarkable tumorigenicity on HCKs expressing HPV16 E6 and E7. The \textit{ErbB2} transgene presence in HCK-E cells also resulted in significant tumor formation, which is consistent with reported results for human oral epithelial cells (33). When \textit{ras} and \textit{Myc} were introduced into HCK1T cells without HPV16 E6E7, most of them died, and established cells did not show tumorigenic potential (data not shown). Furthermore, \textit{E6} and \textit{E7} shRNA introduction into \textit{Hras} plus \textit{Myc}–transduced HCK1T-E cells greatly reduced their transforming abilities (Supplementary Fig. S5), indicating that expression of \textit{E6} and \textit{E7} is a primary requirement.

A previous report indicated that active \textit{Hras} is not sufficient to induce a fully transformed phenotype in human cells expressing HPV16 E6E7 (34). In one study, \textit{Ras} signaling was found to affect the stability of \textit{Myc} protein (35) but not in another (36). We found that exogenous as well as endogenous \textit{Myc} protein levels were increased in \textit{Hras} carrying HCK1T-E cells (Fig. 1A; \textit{Myc} signal intensity, MycHras:Hras-Myc = 1.094:1.5; Fig. 3A; MycHras:Hras-Myc = 1.097:1.5). Activated \textit{Hras} signals in HCKs could increase the half-life of \textit{Myc} protein (Supplementary Fig. S1A), as previously reported for rat cell lines (35), which might explain...

Figure 3. Confirmation of carcinogenesis steps with independent primary HCKs. A, HCK12 cells were infected with retroviruses encoding HPV16 \textit{E6E7} then \textit{Myc}, \textit{HrasG12V}, \textit{ErbB2}, or an empty vector. HCK12-E with Hras cells were further infected with and selected for retroviruses encoding \textit{Myc} or an empty vector. Western blots confirmed the expression of the transgene products. B, anchorage-independent growth assay of these HCK12-E cells were performed as in Fig. 2C. C, tumorigenic abilities of HCK12-E cells were assessed as in Fig. 2D (n = 6).
frequent overexpression of Myc and Ras mutations in cervical cancer (9, 37).

Very recently, it was reported that expression of HPV16 E6E7 and Hras are not sufficient for tumorigenic transformation of primary HCKs (38). One of the likely reasons for the discrepancy with our findings is that we injected cells as a mixture with Matrigel, which has been widely used to increase tumorigenicity (Supplementary Fig. S2B; ref. 25).

Lastly, using several batches of HCKs, we tested our hypothesis that introduction of Hras and Myc to HCKs on the background of

Table 1. Summary of frequency of cancer-initiating cells after transplantation of the indicated number of E6E7, Hras, and Myc expressing HCKs

<table>
<thead>
<tr>
<th>#Cells</th>
<th>Passages</th>
<th>1 × 10⁶</th>
<th>2 × 10⁵</th>
<th>2 × 10⁶</th>
<th>2 × 10⁷</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCK1T in DMEM</td>
<td>(p18)</td>
<td>6/6 (1)</td>
<td>6/6 (1)</td>
<td>6/6 (2)</td>
<td>6/6 (4)</td>
<td></td>
</tr>
<tr>
<td>HCK1T</td>
<td>(p17)</td>
<td>6/6 (1)</td>
<td>6/6 (2)</td>
<td>6/6 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCK1Ta</td>
<td>(p16)</td>
<td>4/4 (1)</td>
<td>4/4 (2)</td>
<td>4/4 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCK1Tb</td>
<td>(p16)</td>
<td>4/4 (1)</td>
<td>4/4 (2)</td>
<td>3/4 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCK1Tc</td>
<td>(p16)</td>
<td>4/4 (1)</td>
<td>4/4 (2)</td>
<td>2/4 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCK4T</td>
<td>(p11)</td>
<td>6/6 (1)</td>
<td>6/6 (2.5)</td>
<td>3/6 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCK4</td>
<td>(p11)</td>
<td>6/6 (3)</td>
<td>3/6 (3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCK7</td>
<td>(p8)</td>
<td>6/6 (1.5)</td>
<td>3/6 (2)</td>
<td>2/6 (2.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCK8</td>
<td>(p7)</td>
<td>6/6 (2)</td>
<td>6/6 (2)</td>
<td>6/6 (3)</td>
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<td></td>
</tr>
<tr>
<td>HCK9</td>
<td>(p7)</td>
<td>6/6 (2)</td>
<td>4/6 (2.5)</td>
<td>1/6 (2.5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE: (p), the passage of the cells used for s.c. injection. (), latency was determined as the time (wk) taken before a palpable mass could be detected. HCKs were kept in KGM except for HCK1T at the top row.
HPV16 E6/E7 expression could confer maintenance and/or creation of tumors with high-risk HPVs might be sufficient to drive for development of cervical cancer. Further efforts to introduce additional genes and/or gene combinations may provide us better matches with features of human cervical cancers. Comparison of the created cancer cells with specimens of human cervical cancer in terms of gene expression profile will be important to evaluate the faithfulness of our model. Using the present approach and other models, it might be possible to understand the role of specific oncogenes and to create effective therapeutic designs for individual patients with cervical cancer.

Disclosure of Potential Conflicts of Interest

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Acknowledgments

References

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