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Apoptosis, 5-fluorouracil sensitivity and expression of apoptotic proteins in a human ectocervical cell carcinogenesis model using different media

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Abstract

Apoptosis has received widespread attention for its essential roles in biology, medicine and cancer. We previously found that normal, human papillomavirus (HPV) 16-immortalized and their transformed endocervical cells were increasingly resistant to apoptosis induced by a cancer therapeutic drug. Here, analogously, another common anticancer drug, 5-fluorouracil, in an ectocervical cell carcinogenesis model induced apoptosis in primary human ectocervical cells (HEC), whereas HPV18-immortalized HEC (HEC-18) and transformed HEC-18 (HEC-18T) were more resistant. Growth in serum/low density lipoprotein (LDL)-containing medium reversed resistance to 5-fluorouracil-induced apoptosis, particularly in HEC-18T. Cell viability results confirmed these findings. Using Western blots to compare protein levels with those of HEC not treated with 5-fluorouracil, the fold changes in HEC-18 and HEC-18T in LDL-free medium were 1.6–6.1-fold lower for pro-apoptotic p53, Bak and Bax. Four anti-apoptotic proteins were altered −2.1 to +14.6-fold for Bcl-2 and BAG-1 isoform p33 and p29. For BAG-1 p50 and p46, HEC-18 were weakly expressed and HEC-18T were moderately higher. Grown in LDL-containing medium, the differences in pro-apoptotic protein levels were mostly reversed. Expression was 1.4–32-fold higher in HEC-18 and HEC-18T of p53, Bax, BAG-1 p29, BAG-1 p33 and total BAG-1. These results showed that HEC carcinogenesis results in resistance to 5-fluorouracil-induced apoptosis, associated with reduced expression during carcinogenesis of pro-apoptotic proteins and increased expression of specific anti-apoptotic proteins.

Keywords: 5-Fluorouracil; Apoptosis; Apoptosis regulating protein; Carcinogenesis, cervical; Growth medium

1. Introduction

The common anticancer drug, 5-fluorouracil, is currently being used as an oral prodrug to achieve optimal response and postoperative survival in oral adjuvant chemotherapy of cervical cancer patients (Thomas, 2000; Morris et al., 1999). 5-Fluorouracil must be enzymatically activated to its cytotoxic form by the targeted tumor cells. 5-Fluorouracil is an analogue of pyrimidine nucleosides that blocks the synthesis of deoxythymidylic acid by thymidylate synthetase and disrupts normal RNA function. Dihydropyrimidine dehydrogenase is the enzyme for the rate-limiting step for 5-fluorouracil catabolism that accounts for more than 80% of its elimination (Milano and Etienne, 1994). The effective cytotoxic level of available 5-fluorouracil is thus principally determined by its catabolism by dihydropyrimidine dehydrogenase.

The cellular and molecular mechanism of 5-fluorouracil chemotherapeutic effects remains unclear. Induction by 5-fluorouracil of apoptosis in cervical cancer and proteins that regulate apoptosis have been proposed to play essential roles. Experimental data suggested the involvement of p53, the Bcl-2 family and BAG-1 in chemotherapy-induced apoptosis (Hengstermann et al., 2001; Harris, 1996; Jaattela, 1999; Yang et al., 1998b; Ding et al., 2000a,b; Violette et al., 2002). For premalignant and malignant lesions, understanding the roles of these factors and how their expression may be regulated may lead to developing optimum 5-fluorouracil treatment and circumventing resistance.

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In vitro models of cervical carcinogenesis may be useful to study the development of 5-fluorouracil resistance. 5-Fluorouracil has been reported to induce apoptosis in many tumor cell types, but few studies have addressed 5-fluorouracil-induced apoptosis in cervical carcinoma cells (Ueda et al., 1997), and especially in a multistep cervical cell carcinogenesis model system. In our previous studies using a human papillomavirus (HPV) 16-immortalized endocervical cell model for squamous cell carcinoma, cells were increasingly resistant to treatment with 5-fluorouracil (Ding et al., 2000a,b). Significantly, we have also found progressively increased resistance to 5-fluorouracil of HPV16-immortalized and cisplatin-transformed/multidrug resistant (MDR) cells (Ding et al., 2000a). Further study of the associations of apoptosis-regulating proteins in chemotherapeutic drug-induced apoptosis showed that our HPV16-immortalized and cigarette smoke condensate-transformed endocervical cells had up-regulated murine double minute-2 (MDM2) after transformation (Yang et al., 1998b). Also, changes in expression were observed for p53 (Ding et al., 2000a). HPV18 is associated with cervical adenocarcinoma (Teshima et al., 1997). This study used our carcinogenesis model system for multistage ectocervical adenocarcinoma to investigate the role of apoptosis and its induction by 5-fluorouracil in two different media and modulation by 5-fluorouracil of proteins regulating apoptosis. Apoptosis was affected with or without 5-fluorouracil treatment, depending on HPV18-immortalization and transformation. The effects on apoptosis were associated with differential changes in patterns of apoptosis-regulating proteins.

2. Materials and methods

2.1. Cell culture and cell viability assay

Primary human ectocervical cells (HEC) were prepared and maintained in serum-free medium for keratinocytes, as described previously (Tsutsumi et al., 1992; Boyce and Ham, 1985). HEC were subsequently cultured in serum-free low-calcium keratinocyte growth medium (KGM), whereas HEC-18 and HEC-18T were cultured in either KGM or high-calcium Dulbecco's modified Eagle's media (DMEM) containing 10% fetal calf serum for 3 days or to 80% confluence and then passaged. Except where indicated, 5-fluorouracil (Sigma) treatment was with 23.1 μM 5-fluorouracil for HEC, 19.9 μM 5-fluorouracil for HEC-18 and 20.3 μM 5-fluorouracil for HEC-18T (the IC_{50} values for the respective cell types used to obtain optimum effects). Cells were counted with a hemocytometer.

Cell viability was evaluated by trypan blue dye exclusion assay. Briefly, 10^5 cells/well were seeded in six-well plates, incubated for 24 h, treated with 5-fluorouracil, trypsinized and gently mixed with equal volumes of 4% trypan blue. Cell viability was quantified immediately under microscopy and calculated as the percentage of treated compared with untreated cells that excluded trypan blue dye.

2.2. Morphological analysis

Cells were cultured in triplicate wells of four-well tissue culture chamber slides for 24 h, incubated with or without 5-fluorouracil for 6 h and examined under phase contrast microscopy.

2.3. DNA fragmentation apoptosis assays

Cells were seeded into six-well plates at 10^5 cells/cell, incubated with or without 5-fluorouracil for 0, 24, 48 and 72 h, washed in PBS and lysed in 10 mM Tris–HCl, pH 7.4, 0.4 M NaCl, 1% sodium dodecyl sulfate (SDS) and 0.2 mg/ml proteinase K at 37 °C for 4 h. Lysates were incubated with 0.2 mg/ml RNase A and T_1 at 37 °C for 1 h and phenol/chloroform-extracted. Apoptotic fragments were resolved by 1.5% agarose gel electrophoresis.

2.4. Western blot analysis

Antibodies used were rabbit polyclonal antibodies for Bak; mouse monoclonal antibodies (mAbs) for Bcl-X_L and $p21^{WAF1}$ (Santa Cruz Biotechnology), Bax, PCNA and MDM2 (Oncogene), p53 and Bcl-2 (DAKO), BAG-1 (Yang et al., 1998a) and β-actin mAb (Sigma); and secondary antibodies (Amersham).

Proteins were analyzed, as described previously (Yang et al., 1998b), using 8–12% SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

2.5. Statistical analysis

The statistical significance of results was analyzed using Student's *t*-test.

3. Results

3.1. Effect of 5-fluorouracil, cervical carcinogenesis and culture media on apoptosis

The effect that cervical cancer progression and different media may have on apoptosis induction by the common anticancer drug 5-fluorouracil was investigated in a carcinogenesis model system for cervical adenocarcinoma consisting of primary HEC, HPV18-immortalized HEC-18 and cigarette smoke condensate-transformed HEC-18T. Apoptosis induction by 5-fluorouracil was examined for cells cultured 72 h in different growth media. Neither the carcinogenesis stage nor the presence of serum appeared to affect the cell apoptotic morphology for HEC, HEC-18 and HEC-18T, since cells evidenced apoptotic bodies in all three stages of carcinogenesis and both media (Fig. 1). The

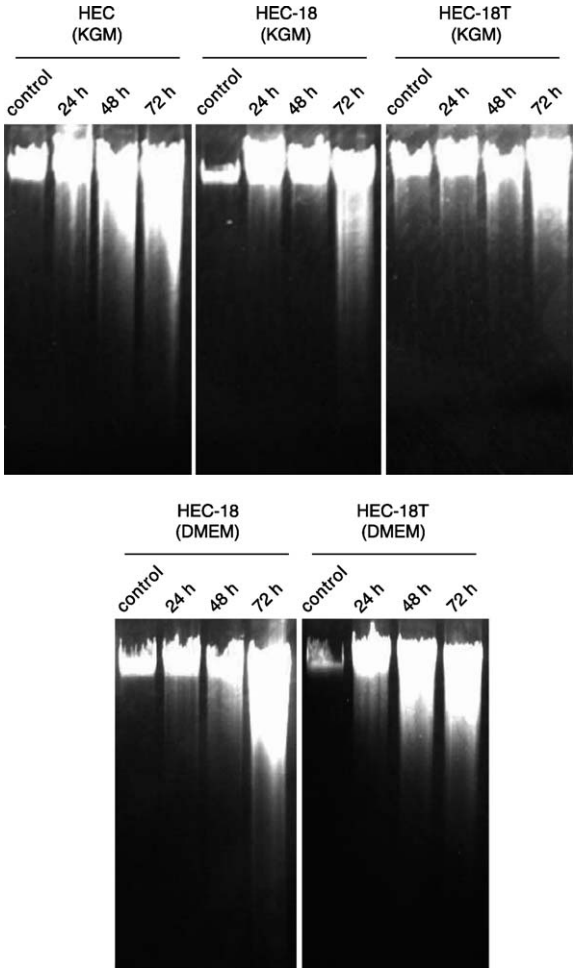

Fig. 1. Apoptotic effect, which was revealed by DNA fragmentation assays, of 5-fluorouracil treatment in different media and an ectocervical cell adenocarcinoma carcinogenesis model system. The cell system was composed of primary human ectocervical cells (HEC), HPV18-immortalized HEC (HEC-18) and cigarette smoke condensate-transformed HEC-18 (HEC-18T), which were grown in serum-free (KGM) and serum-containing (DMEM) media. 5-Fluorouracil treatment was with 23.1 μM for HEC, 19.9 μM for HEC-18 and 20.3 μM for HEC-18T.

frequency of apoptotic body formation induced by 5-fluorouracil was also comparable in all cells and both conditions. The method, however, is not best suited to quantify apoptosis, and the effect of only 72-h treatment was examined.

DNA fragmentation pattern is another hallmark of apoptosis. To better characterize the apoptosis induced by 5-fluorouracil, the cellular DNA was examined of 5-fluorouracil-treated and untreated HEC, HEC-18 and HEC-18T incubated in KGM for 0, 24, 48 and 72 h. After the comparison of DNA fragmentation apoptosis levels in different cell lines grown in different media, HEC displayed some fragmentation at 48 h and more at 72 h, HEC-18 revealed fragmentation only at 72 h and HEC-18T had even less fragmentation at only 72 h. Surprisingly, greater susceptibility of HEC-18 and HEC-18T to 5-fluorouracil-induced apoptosis was found for culture in DMEM versus KGM, with fragments visible at 48 h for HEC-18 and 24 and 48 h for HEC-18T.

3.2. Effect of cervical carcinogenesis and culture media on cell growth characteristics

Growth characteristics and the response to different media are other features of carcinogenesis that may be altered and help explain the results of 5-fluorouracil treatment. Examined for 8 days, the cell growth rate (Fig. 2) and calculated doubling times (Table 1) were progressively, although not significantly, reduced from HEC to HEC-18 to HEC-18T in KGM and from HEC-18 to HEC-18T in DMEM. Comparing growth in KGM versus DMEM, there was reduction for immortalized and transformed cells, but normal HEC were strictly dependent on KGM for growth.

For saturation density, HEC failed to obtain saturation in either media; the number of cells/cm^2 was 83% higher in KGM, 89% higher in DMEM for transformed HEC-18T than HEC-18 (Table 1). However, there was a reduction of saturation density in DMEM versus KGM; the reduction of the number of cells/cm^2 was 41% for HEC-18 and 39% for HEC-18T. All these differences were statistically significant

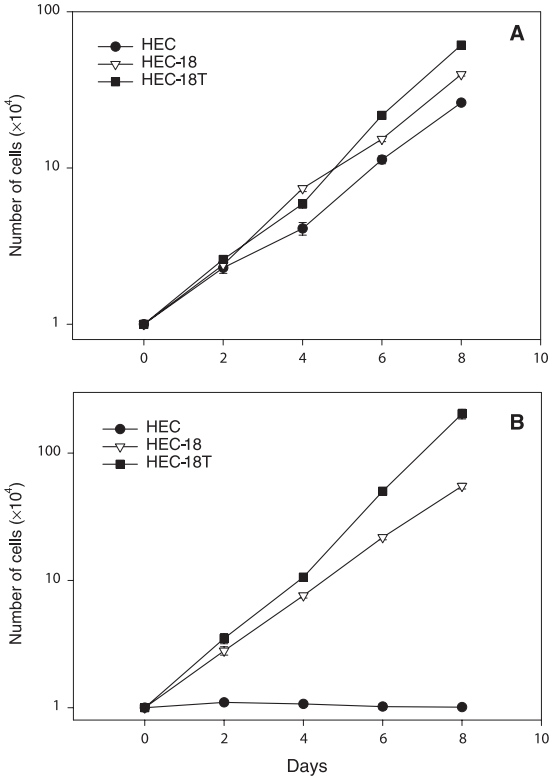

Fig. 2. Effect on cell growth of media and an ectocervical carcinogenesis model. Cell proliferation rate was examined of HEC, HEC-18 and HEC-18T in (A) KGM and (B) DMEM.

Table 1
Effect of ectocervical cell carcinogenesis and growth medium on cell growth characteristics

Growth characteristic	HEC		HEC-18		HEC-18T	
	KGM	DMEM	KGM	DMEM	KGM	DMEM
Doubling time (h)[a]	396	ND	362	32	322	253
Saturation density ($\times 10^5$)[a]	0	0	15726	929	28735	17421
Anchorage independence[b]	0	0	0	0	+	+

ND—not detected.
[a] Doubling time and saturation density values are averages of the standard deviation of three independent experiments.
[b] Anchorage independent growth represents 5/5 negative (0) or positive (+) soft agar plates.

($P<0.05$). Regarding anchorage-independent (soft agar) growth, another indicator of the malignant growth potential of cultured cells, colonies were detected only from HEC-18T, and the medium used did not affect the detection of colonies in 5/5 plates.

These results showed that during carcinogenesis, the growth characteristics changed positively in correlation with reduced sensitivity to 5-fluorouracil. However, using medium containing serum with low density lipoprotein (LDL) prevented HEC growth while enhancing growth rate and decreasing saturation density of both HEC-18 immortalized cells and HEC-18T transformed cells.

3.3. Effect of 5-fluorouracil on cell viability

To examine and quantify the effect that cervical cell oncogenesis in our model may have on determining cell viability during 5-fluorouracil treatment, trypan blue dye exclusion assay was used (Fig. 3). Cells were treated with 5-fluorouracil at different concentrations, 0, 0.1, 1, 10 and 100 μM, for constant time, 72 h (Fig. 3A), or treated with 5-fluorouracil at the same concentrations for different times, 0, 12, 24, 48 and 72 h (Fig. 3B). In both HEC-18 and HEC-18T compared with HEC, there was significantly ($P<0.05$) greater sensitivity at the IC_{50} level of 5-fluorouracil for HEC-18 and 20.3 μM 5-fluorouracil for HEC-18T. Comparing cell viability in KGM versus DMEM, there was also significantly ($P<0.05$) greater sensitivity to 5-fluorouracil. Specifically, the cell viability at 72 h was 1.4-fold greater in HEC-18 and 1.3-fold greater in HEC-18T. However, transformation of HEC-18T from HEC-18 did not affect the cell viability induced by 5-fluorouracil.

3.4. Effect of growth medium LDL on apoptosis

HEC and their immortalized and transformed cells are cervical cells, which are affected by LDL in induction of apoptosis (Wang et al., 1995) or calcium depending on ATP availability (Eguchi et al., 1997). Therefore, the effect was examined of higher calcium and LDL, which are found in DMEM versus KGM and associate with greater sensitivity to apoptosis, for their role in the differential ectocervical cell susceptibility to 5-fluorouracil-induced apoptosis. Apoptosis induced by 5-fluorouracil treatment was examined in HEC-18 and HEC-18T cultured in KGM, DMEM containing LDL or KGM containing calcium. Comparing apoptotic DNA fragmentation for either HEC-18 or HEC-18T, 5-fluorouracil-induced visibly more apoptosis for cells cultured in KGM containing versus not containing LDL (Fig. 4). However, there was no involvement of the LDL receptor since Western blots and flow cytometry revealed no differences in HEC-18 or HEC-18T protein levels using KGM versus DMEM (data not shown). On the other hand, cultured in the higher calcium concentration KGM, there was little difference from using only KGM (Fig. 4). These results suggested that LDL is the factor in serum-containing DMEM determining ectocervical cell sensitivity to 5-fluorouracil-induced apoptosis.

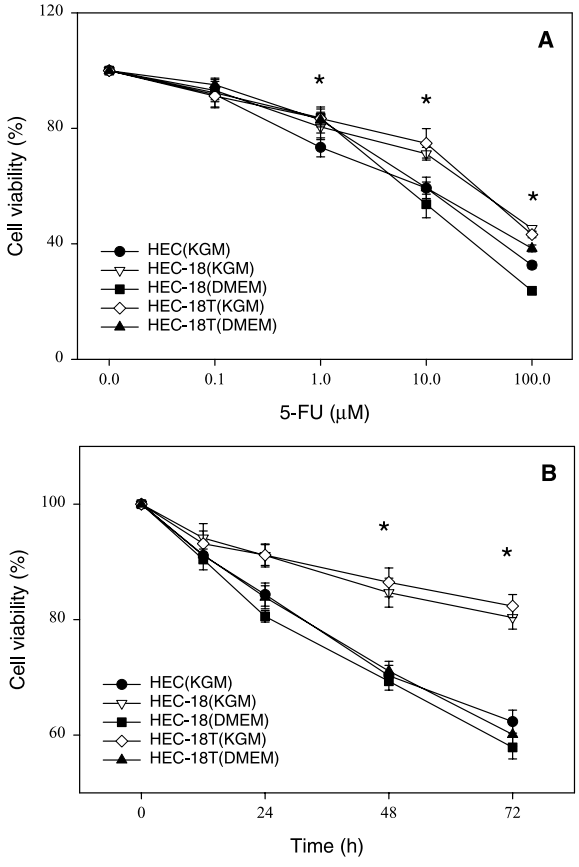

Fig. 3. Effect on cell viability of 5-fluorouracil treatment of media and ectocervical carcinogenesis. (A) Dose dependence. Results shown used the indicated concentrations of 5-fluorouracil. *$P<0.05$, significantly different for the same cells grown in KGM versus DMEM. (B) Time dependence. Conditions and symbols are as in Fig. 1 and in (A).

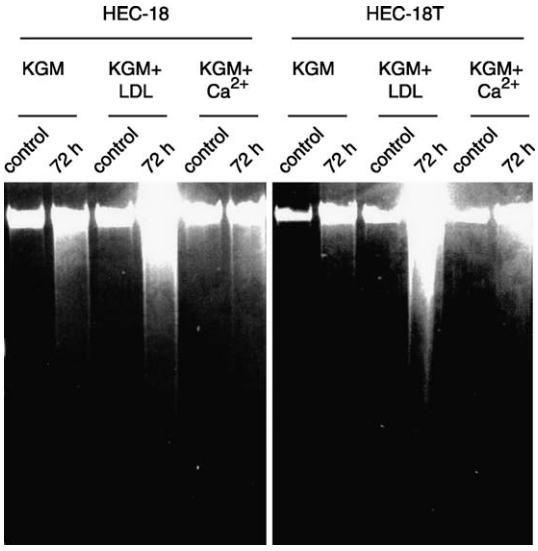

Fig. 4. Effect of LDL versus calcium present in DMEM on apoptosis in an ectocervical carcinogenesis model. KGM was supplemented (+) with LDL or calcium (Ca^{2+}) to mimic DMEM conditions. Conditions and labels are as in Fig. 1.

3.5. Effect of cervical carcinogenesis and growth media on expression of apoptosis-regulating proteins

The expression level of important apoptosis inducing or inhibiting proteins in the cervical carcinogenesis model was potentially the molecular basis for the differential sensitivity to apoptosis induced by 5-fluorouracil. Therefore, apoptosis induction associated with multistage cervical carcinogenesis and medium was examined by Western blot analysis. Grown in KGM, compared with proteins from HEC by densitometry, HEC-18 and HEC-18T were decreased for pro-apoptotic p53 (6.1- and 30-fold, respectively), Bak (both cell lines 1.6-fold) and Bax (2.3- and 4.8-fold, respectively). Anti-apoptotic proteins were highly enhanced in HEC-18 and HEC-18T for Bcl-2 (7.0- and 14.6-fold, respectively) and BAG-1 isoform p33 (4.6- and 7.3-fold, respectively), but not changed for Bcl-X_L and decreased for BAG-1 p29 (2.1- and 1.6-fold, respectively) (Fig. 5; Table 2).

Comparing the proteins in cells grown in the DMEM containing LDL versus KGM, levels in HEC-18 and HEC-18T were increased for pro-apoptotic p53 (6.5- and 33-fold, respectively) and Bax (2.0- and 7.6-fold, respectively) and little changed for Bak (Fig. 5; Table 2). Anti-apoptotic proteins were decreased for Bcl-2 (5.4- and 1.4-fold, respectively) and increased for BAG-1 isoform p29 (2.7- and 3.2-fold, respectively) and BAG-1 p33 (1.4- and 2.3-fold, respectively), but not changed for Bcl-X_L.

Therefore, consistent with the decreased sensitivity to 5-fluorouracil-induced apoptosis of HEC-18 and HEC-18T compared with HEC, for apoptosis-enhancing proteins, there were lower p53, Bak and Bax. The correlation for apoptosis-inhibiting proteins was with enhanced levels of Bcl-2, total BAG-1 and its p33 isoform (Table 2). The increased apoptosis sensitivity of HEC-18 and HEC-18T in DMEM compared with KGM correlated with reduced level of Bcl-2 and increased pro-apoptotic p53 and Bax.

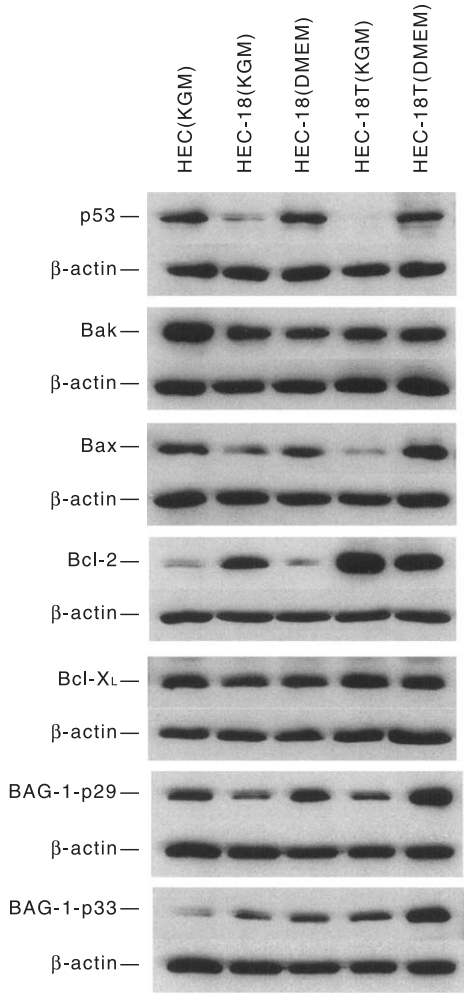

Fig. 5. Effect on expression of apoptosis-regulating proteins in different media and an ectocervical carcinogenesis model. Western blots are shown for 10-μg proteins. Conditions and labels are as in Fig. 1. The blots were also analyzed for expression of β-actin protein as controls for comparative densitometry.

Table 2
Effect of carcinogenesis and media on relative expression of apoptosis-regulating proteins in a human ectocervical cell model of adenocarcinoma[a]

Protein	HEC-18		HEC-18T	
	KGM	DMEM	KGM	DMEM
p53	16.3 ± 1.2	106 ± 3.0	3.3 ± 1.2	107 ± 0
Bak	61.0 ± 1.7	53.5 ± 2.7	61.6 ± 0.9	70.4 ± 2.5
Bax	43.7 ± 0.3	86.4 ± 2.8	20.7 ± 2.8	157 ± 15
Bcl-2	697 ± 86	130 ± 11	1460 ± 21	1050 ± 63
Bcl-X_L	93.7 ± 4.8	98.4 ± 3.4	107 ± 4.0	106 ± 5
BAG-1 p29	47.6 ± 3.3	129 ± 7	62.8 ± 3.5	199 ± 2
BAG-1 p33	455 ± 81	625 ± 142	732 ± 108	1680 ± 100

[a] The percent level values of cellular proteins were quantified relative to those of HEC after normalization using the β-actin control. Densitometry was used for the data in Fig. 5. The data are shown as the mean ± standard deviation of three independent experiments.

4. Discussion

In this study, a model system consisting of HEC, HEC-18 and HEC-18T was chosen to mimic the progression of cervical carcinogenesis. The apoptotic effects were examined of anticancer agent 5-fluorouracil and the molecular mechanism. To develop a full model system mimicking multistep progression of cervical cancer, HEC-18T were established by our laboratory using cigarette smoke condensate treatment on HEC-18 (Nakao et al., 1996). HEC-18T reconstructed severe dysplasia in the organotypic (raft) culture system and produced invasive squamous cell carcinomas on nude mice (Nakao et al., 1996). Therefore, this in vitro model system of HPV18-related squamous cell carcinomas derived directly from uterine ectocervix, consisting of normal, immortalized and transformed cells, has been chosen for further elaboration in this study.

Regulation of apoptosis is an important defense against the emergence of cancer. In early carcinogenesis, target cell populations are predisposed to genetic lesions leading toward malignancy. Apoptotic destruction of predisposed cells may limit the number of cells available for malignant progression (Wyllie, 1992). Drugs that specifically affect the genetic and biochemical signals of programmed cell death should promote apoptotic destruction of cancer cells. The challenge oncologists currently face with is to exploit the information about mechanisms of aberrant apoptosis regulation in disease states for the development of new therapeutic approaches for cancer. Continued elucidation of the fundamental mechanisms responsible for multistep carcinogenesis and regulation of apoptosis will undoubtedly play a crucial role in this effort.

Analogous to treating ectocervical cells in culture, 5-fluorouracil was shown to be highly active in randomized control trial against the cervical high-grade lesions found in HIV-1-infected patients (Maiman et al., 1999). Although apoptotic effects of 5-fluorouracil on a variety of human cancer cells have been widely studied, it was reported only once, to our knowledge, that 5-fluorouracil can induce apoptosis in human cervical cancer cells (Ueda et al., 1997). More data were required to elucidate the mechanism of 5-fluorouracil-induced apoptosis and its role in multistage cervical carcinogenesis.

In this study, the apoptotic effects of 5-fluorouracil have been first investigated on our model system for multistage cervical carcinogenesis. Our data showed altered DNA fragmentation, growth potential and growth characteristics (Figs. 1 and 2; Table 1). Cell survival assays confirmed the DNA fragmentation results showing increased apoptosis with carcinogenesis. Previously, transformed oral keratinocytes demonstrated enhanced growth potential when the medium was changed from KGM to DMEM (Li et al., 1992). This report also showed that culture in DMEM instead of KGM reversed the sensitivity to 5-fluorouracil-induced apoptosis.

For the reversed susceptibility to 5-fluorouracil-induced apoptosis in DMEM versus KGM, the role was investigated for LDL in the culture medium. KGM plus an appropriate amount of LDLs was employed to study their role. The apoptotic levels of HEC-18 and HEC-18T in our model of cervical adenocarcinoma oncogenesis greatly increased after 5-fluorouracil treatment when cells were subject to KGM plus LDLs, consistent with the idea that LDL is the important mediator. We previously found that cervical cancer cell LDL receptors mediated LDL-drug uptake (Xiao et al., 1999; Kader and Pater, 2002). Receptor-independent uptake of lipoproteins is also well established in vivo, and reversible cell surface LDL interactions have been reported and may involve cellular interactions with LDL-cytotoxic drug complexes not mediated via the LDL receptor pathway (Shaw et al., 1987).

The next important issue was the apoptotic mechanism underlying these observations. Cervical oncogenesis may result from the deregulated expression of cellular apoptosis regulating genes (Yang et al., 1998b). Apoptosis is a genetically controlled process that is regulated by a finely controlled equilibrium between anti-apoptotic genes, genes involved in inhibiting apoptosis, and pro-apoptotic genes, genes involved in triggering apoptosis. A wide variety of studies demonstrate that the sensitivity of cells to apoptosis can be usually reflected by expression levels of anti-apoptotic genes and pro-apoptotic genes or their ratio. Since many such proteins participate in regulating apoptosis, their relative levels have far-reaching implications in the control and possible treatment of human cancers.

The degradation of pro-apoptotic p53 by the high-risk HPV E6 in cervical immortalized and tumor cells mimics the situation in other tumors in which p53 is mutated (Tommasino and Crawford, 1995; Brown and Wouters, 1999). Overexpression of Bax gene sensitizes K562 erythroleukemia cells to apoptosis induced by selective chemotherapeutic agents (Kobayashi et al., 1998). We previously found that endocervical cells showed unaltered Bax expression (Yang et al., 1998b). Down-regulation of p53 and Bax was associated with decreased sensitivity to 5-fluorouracil-induced apoptosis in HPV18-immortalization, while up-regulation is associated with transformation and increased sensitivity to apoptosis due to culture in LDL-containing medium (Fig. 5 and Table 2).

Bcl-2 is regarded as an anti-apoptotic gene (White, 1996). Renal epithelial cells overexpressing Bcl-2 were significantly less sensitive to cisplatin-induced apoptosis (Zhan et al., 1999). Similarly, greatly enhanced expression of Bcl-2 in HEC-18 and HEC-18T correlated with their resistance to 5-fluorouracil-induced apoptosis. On the other hand, reduced expression of Bcl-2 in HEC-18 and HEC-18T in DMEM versus KGM resulted in increased sensitivity to 5-fluorouracil-induced apoptosis.

BAG-1 is an anti-apoptotic protein identified as a Bcl-2-binding protein having four isoforms of 29, 33, 46 and 50

kDa (Takayama et al., 1995; Yang et al., 1998a). BAG-1 has been found to bind the receptors for steroid hormones and prevent their apoptosis-inducing function (Kullmann et al., 1998). The present study first demonstrated how the treatment effect of an anticancer agent, 5-fluorouracil, may be dictated by BAG-1 expression levels during apoptosis in cervical cancer cells. In agreement with BAG-1 anti-apoptotic function, the level of BAG-1 in both HEC-18 and HEC-18T correlated with anti-apoptosis. Various levels were observed of each BAG-1 isoform during carcinogenesis and in response to different growth medium, indicating that they may have different functions in regulating apoptosis induced by 5-fluorouracil. For both KGM and DMEM growth conditions, BAG-1 p29 was reduced by immortalization and enhanced above the levels seen in HEC after tumorigenesis. Only the p33 BAG-1 isoform was enhanced progressively. For both isoforms, levels were higher in DMEM than in KGM (Table 2). Therefore, the role of BAG-1 function other than anti-apoptosis requires further investigation.

In summary, expression of apoptosis-regulating proteins revealed potential explanation for the various carcinogenesis- and medium-dependent results. HEC carcinogenesis results in resistance to 5-fluorouracil-induced apoptosis, associated with reduced expression during carcinogenesis of pro-apoptotic proteins and increased expression of specific anti-apoptotic proteins. However, growth in LDL-containing medium reversed 5-fluorouracil resistance, which may have involved increased pro-apoptotic p53 and Bax levels.

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