Environmental risk factors for prevention and molecular intervention of cervical cancer

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Received 25 May 2006; received in revised form 6 October 2006; accepted 31 October 2006

Abstract

Cervical cancer (CC) is potentially the most preventable and treatable cancer in human but it is a leading cause for cancer morbidity and mortality in women around the world. Therefore, more innovative prevention and treatment protocols need to be developed and implemented. With better understanding of the etiology of the disease, specific prevention protocols that involve life-style modifications to minimize the impact of environmental risk factors can be developed. It may be necessary to implement unique modification protocols for different countries. In addition, antiviral vaccine is a highly promising prevention approach. With respect to therapy, the development of more specific protocols that have fewer side effects is needed. With the availability of sophisticated molecular techniques, a new generation of targeted approach that has the potential to generate outstanding efficacy is being tested. Using the siRNA technology against the expression of human papillomavirus oncogenes, specific biological pathways that are essential to the growth and survival of the CC cells can be interrupted. Another promising approach is the molecular intervention of the estrogen pathway by blocking the expression of estrogen receptors. These molecular techniques may work by reactivating endogenous regulatory processes, e.g., the core apoptotic machinery, that can cause self-destruction of the CC cells, thus providing potentially effective molecular therapy. These topics are discussed in this review.

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Keywords: Cervical cancer; HPV infection; Cigarette smoking; SiRNA; Estrogen; Cancer prevention; Dominant negative estrogen receptor; Molecular therapy; Gene therapy

Introduction

Cervical cancer (CC) is a leading cause of cancer morbidity and mortality for women around the world. In 2005, the American Cancer Society estimated that more than 10,000 cases of invasive CC were diagnosed and 3710 women died from the disease in the US (www.cancer.org). However, approximately 85% of the incidence and mortality from CC occurs in developing countries, amounting to approximately 500,000 cases and 275,000 deaths (www.WHO.org; Parkin, 2001; Fig. 1) on a yearly basis. For some developing countries,
therefore, CC is the number one cause of cancer-related deaths in women. On the other hand, CC is potentially one of the most preventable and treatable cancer types in the population; therefore, such extensive human sufferings are inconceivable.

Many reviews have been written on CC. However, most of them have been concentrated mainly on topics such as human papilloma virus (HPV) etiology, prevention, screening, pathology, therapy, management and social–economic concerns. In this review, we will focus on the identification of environmental risk factors that are causally related to the development of CC and on how such information can be used to develop disease prevention and molecular intervention strategies.

CC – problems and opportunities

Among sexually active females, the risk for contracting HPV is exceedingly high, which translates to a lifetime risk of approximately 80% (Baseman and Koutsky, 2005). With such high infection rate, it becomes clear that the development of CC is still a rare event among HPV-infected women. Since significant increase in CC incidence occurs first in young adult females, the evidence indicates that, after infection with high risk HPV, it takes approximately 10 years for cancer to develop. The most common histological type of CC is the squamous cell carcinoma (SCC) and the second common type is the adenocarcinoma (www.cancer.org; reviewed by Snijders et al., 2006). The development of CC takes on a series of developmental changes. The development begins usually with the presence of non-invasive squamous lesions that are known as cervical intraepithelial neoplasias (CINs) or squamous intraepithelial lesions (SILs). These lesions are further staged as CIN 1 (or LSIL) for low grade lesions, CIN 2 and CIN 3 for medium to high grade lesions (or HSIL) and to carcinoma in situ. Interestingly, the low grade lesions may regress spontaneously leading to no serious consequences. When the carcinoma becomes invasive it can cause very serious consequences. Clinical management of these various lesions/carcinomas typically requires a combination of surgical and/or chemo- and radio-therapy protocols.

Although the risk factors, clinical features and stages for CC have been clearly identified, major questions remain to be resolved. Some questions are posted here. Why does a common and mostly benign infection process result in malignancy? Why are some individuals susceptible to the infection and/or development of the disease, and others are resistant? What mechanisms trigger the progression of the disease from one stage to another? How can one use the current knowledge to these questions to develop precise and effective prevention and intervention strategies? Some possible answers to these questions are discussed in this review.

Environmental factors for the development of CC

Approximately 90% of CC is associated with HPV infection, particularly with high risk HPV types such as HPV 16 and 18 (reviewed by Baseman and Koutsky, 2005). In addition, infection with high risk HPV can cause oncogenic transformation of cells in vitro (Chen
transmission of sexually transmitted diseases and induction of target site inflammation.

We have conducted studies in Colombia, USA, and Venezuela to identify risk factors for CC and to provide some explanations regarding the wide differences in CC incidence/mortality among the three countries, as illustrated in Fig. 1 and in www.who.org. The focus of our series of investigations was on identifying life style and heritable (acquired and genetic susceptibility, respectively) risk factors for CC (Au et al., 2003; Au, 2004). The life style risk factors are summarized in this section. Patients with HSIL or invasive cancer were recruited from each of the three countries (approximately 100 per country). The pathological status of the tumors was systematically validated. The patients were matched with local healthy controls. All volunteers were interviewed based on a structured questionnaire. Bilingual interviewers were employed to conduct the interviews. The questionnaire specifically requested information on the number of sexual partners, age of first sexual activity, smoking habits, exposure to other combustion products, etc. In addition, biological specimens such as Pap smear and peripheral blood samples were collected for the analysis of the presence of different types of HPV and of different genotypes.

The collected data were used to calculate the odd ratios for risk for CC. The calculation was based on comparing the data from the patients with those from the controls for each country. A summary of the findings is shown in Table 1.

As shown in Table 1, infection with high risk HPV, especially HPV 16 and 18, is the most significant risk factor for CC, as reported in the literature. However, the levels of risk are not the same among the three countries with the highest risk in the USA although the rates of infection with different high risk HPV were similar. Having more than two active sexual partners is a significant risk factor for Venezuela but not for Colombia or USA. Having sexual activities before the age of 18 is a significant risk factor only for Venezuela (Sierra-Torres et al., 2003a). Cigarette smoking is a significant risk factor for the USA but not for

Table 1. Life style susceptibility risk factors for CC

<table>
<thead>
<tr>
<th>Susceptibility</th>
<th>Odd ratios (95% confidence interval)</th>
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<tbody>
<tr>
<td></td>
<td>Colombia</td>
</tr>
<tr>
<td>High risk HPV</td>
<td>19 (8.2–44.2) a</td>
</tr>
<tr>
<td>Number of sex partners</td>
<td>1.5 (0.7–3.4)</td>
</tr>
<tr>
<td>Early sexual activities</td>
<td>1.6 (0.7–3.4)</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>NA b</td>
</tr>
<tr>
<td>Wood smoke</td>
<td>7.3 (3.0–19.4) a</td>
</tr>
</tbody>
</table>

aSignificant at p <0.5.
b = Not applicable.

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Venezuela. Few Colombian women smoked, therefore risk from smoking could not be determined. On the other hand, many of these women engaged in extensive cooking activities and wood was used as the source of fuel. Therefore, their exposure to wood smoke is substantial and it is a significant risk factor for CC (Sierra-Torres, et al., 2006). The latter observation is consistent with that in Honduras (Velema et al., 2002). It should be emphasized that our investigations were conducted under identical laboratory conditions. It is clear from the data that various life-style (acquired) risk factors contribute differentially to the risk for CC. This indicates that we can make use of the country-specific information to develop different life-style modification protocols for specific and effective reduction of the disease.

Another important contributing risk factor is chronic estrogen exposure since the cancer risk is increased 2–4 times for women with extended use of oral contraceptives and 4 times for women having more than 7 children (Elson et al., 2000; Moreno et al., 2002; Moodley et al., 2003). Although estrogen is a human carcinogen for a variety of cancers (www.iarc.org), the mechanism towards the development of CC is least understood. However, in a unique K14E7 transgenic mouse model that expresses the HPV16, administration of estrogen is needed to cause the initiation of CC and the continued availability of estrogen is essential to the growth of the tumors (Brake and Lambert, 2005). Similar observations were reported in another transgenic mouse model, HPV-18 URR E6/E7 (Park et al., 2003). Therefore, from population and animal studies strongly implicates the need of estrogen for induction and growth of CC. The outcome is most likely based on its interaction with HPV to stimulate cell proliferation and to prevent apoptosis.

Environment and genetic interactions

The primary oncogenic mechanism of HPV is the release of the viral E6 and E7 proteins that bind predominantly to the products of two cellular tumor suppressor genes, p53 and pRb, respectively (Almadori et al., 2002; Al Moustafa et al., 2004) and abolish their normal function of regulation of cell proliferation and initiation of apoptosis (Horner et al., 2004; Munger et al., 2004). When the function of the p53 and RB genes are compromised, cells may accumulate DNA damage from repeated exposure to environmental mutagens or from other mechanisms but still survive and proliferate (Mehes et al., 2004; Huang et al., 2005). Their survival and proliferation allow the affected cells to evolve and acquire additional changes to become autonomously growing cells. Subsequent clonal expansion of some of these latter cells is the predominant pathway to the development of cancer. This simplistic description of the cancer process serves to emphasize the importance of genetic and environmental interactions for the development of CC.

There are many factors that can influence the mentioned cancer process. Inherited (genetic) susceptibility may play an important role for CC. We have investigated the contribution of polymorphic chemical metabolizing genes and of polymorphic genes for the immune system to CC. The US population in our study has a high frequency of cigarette smokers and our data indicate that having the null GSTM1 genotype is a significant risk factor for CC (OR = 3.4; CI = 1.0–11.8; Sierra-Torres et al., 2003b). For Colombian women who did not smoke but who were exposed to wood smoke, a small but non-significant risk is associated with the null GSTT1 genotype (OR = 1.4; CI = 0.57–3.44; Sierra-Torres et al., 2006). It is well-known that enzymes from the GSTM1 and GSTT1 genes are responsible for the detoxification of epoxides which are reactive metabolites from polyaromatic hydrocarbons. The latter products are derived from combustion of organic matters such as cigarettes and wood. Therefore, the association is consistent with the exposure of the two populations. In addition, in the Colombian population, a significant risk is associated with the c2/e2 variant for the CYP2E1 genotype, after adjusting for wood smoke exposure (OR = 6.3; CI = 1.10–36.38; Sierra-Torres et al., 2006). Enzyme from this variant gene is responsible for increased activation of small organic compounds to generate more reactive products. Since smoking is not a significant risk factor for the Venezuelan women, we investigated variations in the immune system as a risk factor for this population. We found that having the HLA-DQBI*0402 allele is a significant risk for CC (p = 0.004, RR = 5.067; Dao et al., 2005). It is possible that women with the variant genotype may have reduced clearance of HPV after infection (Maciag et al., 2000). Data from these limited investigations indicate that genetic susceptibility factors can contribute significantly to CC but they can vary from one country to another. These variations may be dependent upon the ethnic make-up of the study population and their impact may be influenced by exposure to other risk factors.

Another major contributing factor is estrogen exposure, either through the endogenous or exogenous sources. Estrogen activates soluble intracellular estrogen receptors (ERa and ERb) which stimulate transcription of a variety of genes (Revankar et al., 2005). The cellular consequences include alteration of cell adhesion and migration, increased cell proliferation and resistance to drug-induced apoptosis (Chen et al., 2004; Revankar et al., 2005). The estrogen effect may also be mediated via “crosstalk” with p53: “the overall effects of p53-ER crosstalk are negative, leading to the inactivation of p53 as well as estrogen receptors”, thus the lack of cell cycle
control (Sengupta and Waslyyk, 2004; Schiff et al., 2005). In addition, estrogen can interact with HPV in infected cells. For example, exposure of HPV-positive CC cells to estrogen stimulates the expression of HPV E6 and E7 mRNA and cell proliferation (Kim et al., 2000; Brewer et al., 2005; Nair et al., 2005), and prevents the induction of apoptosis (Chen et al., 2004). HPV-negative C-33 CC cells have limited response to estrogen under their experimental conditions (Kim et al., 2000). Therefore, HPV and estrogen can certainly cooperate to stimulate cell proliferation and to prevent the expression of apoptosis, leading to the development of CC.

**Prevention, intervention and gene therapy**

Since the major risk factors and the mechanism for development of CC are better known nowadays (Fig. 2), and the cancer tissues are readily accessible, CC is probably the most preventable and treatable cancer in females (Schiffman and Castle, 2005). In prevention, the use of the recently developed HPV vaccine is successful in some studies (Campbell, 2005) but not in others (Vandepapelier et al., 2005). The efficacy of this promising CC prevention approach will be better known after its use in the population. In addition, as shown in the data from our investigation, country-specific lifestyle modification programs may need to be implemented in order to achieve effective prevention of CC, e.g. more emphasis for smoke-exposure prevention and management of sexual activities for specific countries. Pap smear test needs to be more vigorously promoted to achieve early diagnosis for intervention of CC. The fact that the simple Pap smear test has not been widely adopted by women around the world indicates the need for effective therapeutic procedures for CC. Innovative approaches are needed to significantly enhance the effectiveness of therapeutic protocols (Lee et al., 2005a; Saxena et al., 2005). With advanced genetic and molecular technology, effective and precise molecular therapeutic protocols can be developed. Since HPV infection is the necessary cause for the development of CC, this pathway has been the target for the development of molecular intervention protocols. A focus has been concentrated on the use of the interference RNA (siRNA) technology to block the expression of HPV mRNA (Butz et al., 2003; Jiang et al., 2004; Yamato et al., 2006). This recently developed technique utilizes the endogenous mechanisms for post-transcriptional gene silencing which has been clearly demonstrated in plants and animal cells. Specifically, small double stranded RNAs (synthetic 21’–22 nucleotide length) that share complementary sequences with the targeted cellular mRNA are transfected into cells. For CC cells, the siRNAs target the HPV E6 and E7 mRNA. The published studies indicate that the transfected siRNAs were able to specifically and significantly reduce the expression of viral E6 and/or E7 mRNA. The reduction led to a concomitant reactivation of the p53 pathway and subsequent expression of apoptosis in CC cells. Although the mechanism for the expression of apoptosis, thus potential efficacy in therapy, has not been identified, the effect may result from the reactivation of normal built-in cellular activities. Since CC cells have chromosome aberrations and genomic instability, the reactivation of p53 and/or Rb would spark the recognition of the damage as a serious stress signal. In response to the stress signal, the p53 and Rb proteins would initiate a cascade of cellular activities that culminate into the blockage of cell proliferation and initiation of apoptosis (Vousden, 2005; Spierings et al., 2005; You et al., 2005). The cascade of activities may include the inhibition of proteins that stimulate cell proliferation (e.g. cyclins) and the expression of proteins that contribute to apoptosis (e.g., Bcl2, Bax, caspases). Several key apoptotic proteins and models have been proposed (Spierings et al., 2005; Lakhani et al., 2006) but the one which is utilized by CC cells needs to be elucidated.

The use of siRNA to develop molecular therapy is a promising approach that should be exploited further. Besides using siRNA alone, it is possible to combine siRNA with effective chemotherapeutic drugs to achieve higher efficacy (Putral et al., 2005). Multiple siRNA may be used to block multiple targets, e.g., different HPV and helper viruses in CC (Yamato et al., 2006; You et al., 2006). Eventually, the effectiveness of siRNA as a therapeutic protocol needs to be systematically evaluated in experimental models and in clinical trials.

Another therapeutic target is the estrogen pathway. Since estrogen receptors influence the expression of HPV, blockage of the estrogen pathway may have similar effect to that of blockage of HPV that utilizes the siRNA technology (Au et al., 2005). Although molecular blockage of the estrogen pathway has not been

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**Fig. 2.** Diagrammatic presentation of the major steps in the development of cervical cancer.
reported for CC, an approach using a dominant negative vector may be useful.

The dominant negative vector approach uses a molecular technique to deliver gene-based therapy into target cells (Curiel, 2000). It involves the use of adenovirus to deliver dominant negative estrogen receptor (ER) mutant genes into cancer cells (Ince et al., 1993, 1995; Chien et al., 1999, Lazennec et al., 1999, Hallenbeck and Stevenson, 2000; Lee et al., 2001). These ER mutants exert their growth-inhibitory effect by making inactive heterodimers with wild-type ERα and ERβ (Ince et al., 1993; Lee et al., 2001). The aberrant hetero-dimers become unable to bind to the estrogen-responsive elements (ERE) in different growth-related genes or unable to activate transcription when bound to ERE. Indeed, the therapeutic activity of a dominant negative vector against uterine leiomyoma, an estrogen-dependent tumor of the uterus, has been reported by Al-Hendy et al. (2004) using both in vitro and in vivo models. Recently, the dominant negative vector was reported for the first time to be effective in blocking the expression of HPV and causing apoptosis in CC cells in vitro (Au et al., 2006).

**Conclusion and future directions**

CC is potentially the most preventable and treatable cancer in human. Therefore, success in prevention and treatment will significantly reduce the morbidity and mortality burden in females around the world. The success is, however, dependent upon overcoming several challenges: education and adoption of life-style prevention strategies, access to health services, access to health care, understanding the etiology and mechanisms for the disease, and development of innovative and effective prevention and therapeutic protocols.

In this review, we have discussed the significant role of acquired and genetic susceptibility factors (i.e. life-style and heritable factors) that contribute to the development of CC. More importantly, these factors may contribute to the documented variations in the incidence of CC in different countries. Understanding the role of these factors will allow health agencies to develop country-specific prevention protocols against the development of CC.

Scientific investigations are underway to identify the crucial biological pathways for the initiation and growth of CC. These investigations may involve the use of genomic and proteomic techniques. With such knowledge, scientists can develop precise molecular protocols to interrupt the crucial biological process and to cause destruction (apoptosis) of the cancer cells. The siRNA and, possibly, the estrogen-receptor blocker protocols can be used as models to illustrate innovative approaches for gene therapy against CC. Success of these molecular therapy approaches may be based on the reactivation of endogenous mechanism to block proliferation and of the core apoptotic machinery to initiate apoptosis in cancer cells.

Molecular intervention and gene therapy approaches are promising clinical technologies that may have certain advantages over conventional approaches. The molecular and gene approaches can reach the precise target at the cell and gene levels, thus minimizing undesirable side effects. Another possible benefit is that application of these innovative protocols can be minimally invasive thus allowing women to retain reproductive function and fertility. Finally, the development of precise and effective protocols is essential to the success in the tripartite approach to the eradication of CC: prevention, intervention and therapy.

**Acknowledgement**

The study is partially supported by grants from the John Sealy Memorial Foundation to W. W. Au, NIEHS Pilot Project Grant #ES06676 to S.A.S. and NICHD Grant R01-HD 46228 to A.A.

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