Molecular Genetics of Gynecologic Cancer

During the past decades, the expansion of molecular biology has had a pivotal role in understanding the basis of cancer development and progression. In addition, real advances have been made in the application of DNA recombinant technology to cancer therapy and patient management. In gynecologic oncologic fields, there are also many investigations to explore the basic pathogenesis of gynecologic cancer, such as cervical cancer, ovarian cancer, and endometrial cancer. It is now known that specific types of human papilloma viruses (HPV) are the principal etiologic agents for both cervical cancer and its precursors. However, the various kinds of alterations in oncogenes and tumor suppressor genes may play additional roles in carcinogenesis of cervical cancer. Although ovarian carcinoma is the most frequent cause of death from gynecologic malignancies, the histogenesis and biological characteristics of these tumors are not well understood. During the last several years, many key observations have been made concerning the genetic alterations associated with ovarian cancer. Recent researches including some dominant oncogenes and tumor suppressor gene mutations common to these malignancies are providing bases to elucidate the mechanisms underlying this cancer. The most important basis of endometrial cancer is that K-ras and p53 mutations are also frequently observed.

Key Words : *Genetics, Biochemical; Genital neoplasms, Female; Genes, Suppressor, Tumor; Oncogenes*

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Received : October 9, 1997

Accepted : October 14, 1997

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INTRODUCTION

During the past decades, the expansion of molecular biology has had a pivotal role in understanding the basis of cancer development and progression. Nevertheless, the application of these findings to the practice of medicine has been slower than anticipated. Recently, however, real advances have been made in the application of DNA recombinant technology to cancer therapy and patient management. In gynecologic oncologic fields, there are also many investigations to explore the basic pathogenesis of gynecologic cancer, such as cervical cancer, ovarian cancer, and endometrial cancer. In the followings, a short summary of "molecular genetics of gynecologic cancer" gives us an example of the many aspects of researches in gynecologic oncologic fields.

CERVICAL CANCER

Cervical cancer develops from precursor lesions referred to cervical intraepithelial neoplasia. It is now known that specific types of human papilloma viruses (HPV) are the

principal etiologic agents for both cervical cancer and its precursors. Although the number of known genital HPV types now exceeds 20, only certain types such as 16, 18, 31, 35, 39, 45, 51, 56, 58 are high-oncogenic-risk associated with high-grade intraepithelial lesions and cancer. Among them type 16 and type 18 HPVs are clinically the most important. The high-oncogenic-risk HPV types produce two oncoproteins, designated E6 and E7, which interact with endogenous cell cycle regulatory proteins, including p53 and Rb. The interaction of virally derived and endogenous cellular proteins converges in deregulation of cell cycle progression and appears to be critical for the development of cervical cancer. One additional event that appears to play a role in tumor progression is integration of HPV DNA into the host genome. Integration of HPV DNA frequently disrupts the E2 open reading frames, resulting in overexpression of the E6 and E7 oncoproteins and possibly causing genomic instability. However, because HPV infection is not sufficient for cervical carcinogenesis, attention has focused on molecular cofactors important to this process. Especially, various kinds of alterations in oncogenes and tumor suppressor genes may play a role in carcinogenesis of cervical cancer.

Human papilloma virus in cervical cancer

That HPV is a critical factor for cervical carcinogenesis, and that the HPV E6 and E7 genes are oncogenic are clearly established. The E6 protein appears to alter cell growth through its effects on p53, which is an endogenous, cell-derived tumor-suppressor protein. Wild-type p53 is a nuclear protein that negatively regulates cell growth and division. A transient increase of wild-type p53 is observed in response to DNA damage, and accumulation of wild-type p53 induces cell growth arrest in the G₁ phase of the cell cycle (1, 2). The p53-induced G₁ growth arrest presumably allows the cell to repair the damage to its DNA and to maintain genomic integrity before progressing to the S phase and undergoing DNA synthesis (3). But, binding of E6 protein to p53 results in a loss of p53 activity within cells. The cells expressing mutant forms of p53 fail to exhibit cell arrest in the G₁ phase after damage to their DNA and continue cell divisions. The E7 protein binds to the retinoblastoma (Rb) protein. In normal cells not infected with a high-oncogenic risk HPV, the hypophosphorylated forms of Rb protein, forms complexes with transcription factors of the E2F family. These complexes negatively regulate cell growth by repressing transcription of E2F-dependent genes. If the E2F-Rb complexes dissociate, free E2F becomes available. Free E2F stimulates the transcription of the E2F-dependent genes and allows DNA replication (4).

In benign cervical precursor lesions, viral DNA is maintained in a free, extrachromosomal, circular form termed an "episome". However, in cervical cancer, the HPV DNA is integrated into the host genome (5). Integration is thought to have important effects on viral gene expression and the biologic behavior of HPV-infected cells. The physical act of integration commonly breaks the HPV genome in the E1/E2 region while maintaining the integrity of the E6/E7 region. Physical disruption of the E2 open reading frame (ORF) and loss of its encoded proteins would be expected to produce increased transcription from the intact E6 and E7 ORFs. But the long latency period between the initial HPV infection and cancer development indicates that HPV infection alone is insufficient for carcinogenesis. In addition, HPV-associated carcinogenesis is an inefficient process, because only a small fraction of women who are infected with high-oncogenic-risk HPV types will ever develop invasive cervical cancer. These findings suggest that additional factors and cellular events are required for cervical carcinogenesis.

Tumor suppressor genes in cervical cancer

Identification of specific chromosomal segments that are

Fig. 1. Autoradiogram from Southern blot analysis of DNA samples from tumor (T) and normal (N) tissue in patients 1-3 with cervical carcinomas. Probe used was p144 D6 (D17S34).

*: Allelic losses (loss of heterozygosity) in tumor tissues. Allelic bands (a and b).

lost during carcinogenesis can be used to identify potential tumor suppressor genes (Fig. 1). Loss of heterozygosity in invasive cervical cancers has been observed predominantly on the chromosomes 3, 5, and 11 and, less frequently, on chromosomes 1, 4, 6, 10, 17, 18, and X. Data from several studies indicate the presence of putative tumor suppressor genes on the short arm of chromosome 3 in the region of 3p13-21.3 (6, 7, 8). And some other studies suggest that a novel tumor suppressor genes may be present at 5p15.1-15.2 and 11q22-24 (8, 9).

In general, more than 90% of squamous cell cervical cancers contain HPV DNA, and p53 mutations are rarely seen. p53 mutations appear to be more common in HPV-negative cancers, although HPV-negative cancers that do not contain p53 mutations exist. These phenomenon suggests that loss of the function of p53 or Rb by binding with E6 and E7 protein is the key mechanism in cervical carcinogenesis. In most studies, HPV status was not a strong independent prognostic factor of outcome in cervical cancer patients (10); however, there appears to exist a trend for HPV-negative tumors to do worse.

Oncogenes in cervical cancer

Rearrangements, amplifications, or overexpressions of the c-myc protooncogene have been reported to be common events in cervical carcinogenesis. It is important to note that c-myc amplification is closely linked to the risk of early relapse, regardless of other prognostic factors, and is superior as a prognostic factor to nodal status (11). The overexpressions of c-erbB2 are associated with poor prognosis (12) and when increased expression

of Ha-ras there is a resistance of radiation (13).

Alterations in other cell cycle regulating genes

Expression of other cell cycle regulating genes such as bcl-1 and bcl-2 has been studied in cervical cancer. Bcl-1 (cyclin D1) is capable of binding to the Rb protein, and is overexpressed and amplified in the majority of cervical cancer cell lines (14), and its level of expression is elevated by activated CSF-1 receptor. Bcl-2 overexpression, which protects against apoptosis and differentiation, was not found to relate to HPV status, but as more likely to be seen in CIN 3 rather than low grade dysplasias; thus, its expression may be early event important to malignant transformation (15). In vitro, increased bcl-2 expression is noted in cervical cancer cell lines that contain an inactive p53 (16).

OVARIAN CANCER

Although ovarian carcinoma is the most frequent cause of death from gynecologic malignancies, the histogenesis and biological characteristics of these tumors are not well understood. The majority of patients with ovarian cancer present with advanced disease. Unfortunately, advanced cases of these malignancies are usually lethal despite modern therapeutic modalities. Most of the recent breakthroughs in understanding the molecular basis for this disease has been in the area of hereditary epithelial ovarian cancer syndromes, which affect up to 5% of ovarian cancer cases. Much work is still needed to understand the biology underlying sporadic ovarian cancers, which invariably present as advanced stage disease and have a poor long-term outcome. During the last several years, many key observations have been made concerning the genetic alterations associated with ovarian cancer. Recent research describing loss of heterozygosity of different chromosomal loci, some dominant oncogenes, and tumor suppressor gene mutations common to these malignancies is providing a basis for the molecular genesis of these cancer. These informations should lead to new developments of early detection and treatment, including molecular therapy such as gene therapy.

Tumor suppressor genes in ovarian cancer

We have also studied the loss of heterozygosity (LOH) in ovarian cancer to find a novel tumor suppressor gene from 1987 (17, 18, 19). And during the last 2 years, studies of LOH in ovarian cancer have revealed a number of regions with high frequencies of allelic loss. Integrated with many studies about LOH, a high frequency of allelic

deletion (>33%) based upon more than 50 tumors in at least three separate studies has been documented for seven chromosome arm; 6p, 6q, 13q, 17p, 17q, 18q and Xp (20). A number of other chromosome arms have frequencies of allelic loss in the range 25-33% which may be above background rates; 4p, 8p, 9p, 11p, 14q, 16q, 19p and 21q. Among them the most important allelic loss area in ovarian cancer is p53 locus of 17p. We reported p53 mutation in epithelial ovarian carcinoma as well as borderline ovarian tumor. Allelic deletions on 17p or p53 mutations are very important genetic changes in ovarian cancer (21). Functional wild-type p53 has been shown to be required for sensitivity to a variety of chemotherapeutic drugs and radiation, playing a crucial role in the execution of the common end pathway of apoptosis. p53 mutation of one allele predisposes the cell to the loss of wild-type p53 function, which has been shown in ovarian cancer cells to lead to the development of cisplatin resistance (22). Of ovarian cancer specimens that express nuclear p53, 90% of them have p53 mutations, leading to prolongation of p53 protein half-life. In ovarian cancers, the overexpressed or mutant p53, which is seen in 50% of advanced cases (23), is associated with high grade and poor survival, but not with clinical chemoresponsiveness (24). The apparently pivotal role of p53 in the regulation of cellular proliferation has led to speculation that gene therapy, designed to reintroduce wild-type p53 genes into cancer cells that have mutant p53 genes, might be successful in suppressing tumor growth. In this regard, viral vectors expressing wild-type p53 have been used to introduce into tumor cells (25).

The BRCA1 gene may fit a tumor suppressor gene model, in that mutations in this gene frequently lead to loss of function and the gene is located in a region frequently associated with loss of heterozygosity in breast and ovarian cancers. Mutations of BRCA1 and BRCA2 can account for many of the familial cases of breast-ovarian cancer syndrome and site-specific ovarian cancers (26), but not for the sporadic ovarian cancers. In sporadic ovarian cancer cases, no mutations of BRCA1 were detected, while frequent allelic losses that included the BRCA1 region of chromosome 17q were demonstrated (27). These data suggest the involvement of additional tumor suppressor genes in the etiology of the large majority of sporadic ovarian cancers, including one possibly localized centromeric to the BRCA1 gene (28), and the other on 17p (29).

Vandamme et al. (30) have identified two deletion units in the region 11p13 and 11p15.5. These regions correspond to the location of WT-1 and WT-2 genes, raising the possibility that the same genes could be involved in ovarian carcinogenesis.

In chromosome 6, allelic losses were found on both

p and q arms. Especially the 6q 24-27 has been reported to have frequent loss of heterozygosity at the estrogen receptor gene locus which has been strongly suggested as one of the putative tumor suppressor gene (31).

Oncogenes in ovarian cancer

The protein products of nuclear protooncogenes function mostly as transcriptional regulators, interacting with the promoter regions of target genes, other proteins or participating in transcriptional region. Little is known about the role of most of these genes in ovarian cancer.

The c-myc protooncogene encodes a DNA-binding protein that plays a role in the regulation of cellular proliferation. Dividing cells exhibit increased amounts of nuclear c-myc protein, whereas quiescent cells express negligible levels. In accordance with observations in other solid tumors, amplification of this gene has been reported in approximately one third of advanced-stage epithelial ovarian neoplasms (32).

The c-fms protooncogene encodes the receptor for colony-stimulating factor (CSF). And this oncogene contribute to the autocrine and paracrine secretion of Interleukin 1 (IL-1), IL6 and TNF during the ovarian carcinogenesis. Elevated levels of CSF are also found in the serum of 70% of ovarian cancer patients (33).

The c-erbB protooncogene encodes the transmembrane receptor for Epidermal Growth Factor (EGF). The EGFR is expressed by most advanced stage ovarian cancers and EGFR positive tumors have a worse prognosis than tumors not expressing the receptor (34). And the HER-2/neu or erbB2 oncogene is related with erbB oncogene. It encodes a membrane receptor that binds a glycoprotein similar to transforming growth factor- α . Overexpression of erbB2 was found in 30% of ovarian cancer and associated with poor prognosis (35). But there are also some controversies (36), so more investigations are needed.

The Ha-ras genes have acquired the transforming activity either by mutations that result in changes of amino acids of Ha-ras oncogene products, or by the enhanced expression of normal Ha-ras gene. The p21 proteins of mutated Ha-ras genes, which lack the GTPase activity, may lead to the alterations in the control of cell growth (37). Ki-ras mutations are reported in 30% of ovarian cancer patients and these mutations are significantly frequent in mucinous adenoma of ovary. So K-ras mutations may be an important and relatively early event in mucinous ovarian tumors (38).

Molecules involved in adhesion and invasion

Metastasis is the most life-threatening aspect of cancer,

CD44R1 Amplification in Gynecological Malignancies

Fig. 2. Representative cases of RT-PCR amplification products of CD44 cDNA from the surgical specimens of ovarian carcinoma. CD44H transcript was expressed in all of the cases, and CD44R1 was expressed in lane 2 and 4.

and requires multiple genetic alterations and complex molecular cascade. Loss of normal adhesive functions by cells and gain of new adhesive functions are crucial in this metastatic cascade (39). The cell adhesion molecule CD44 is expressed when ovarian cancer cells are attached to peritoneal mesothelium, but such expression is lost when the cells are found in ascites fluid (40). We also reported that CD44 splicing variants which were associated with invasiveness of tumor cells during the carcinogenesis are very important in metastasis of ovarian cancer (41, Fig. 2). Especially, overexpression of CD44R1 variant makes the tumor cell to potentiate the capacity of metastasis and is associated with poor prognosis (42).

ENDOMETRIAL CANCER

Although endometrial cancer is the most common gynecologic cancer in the western countries, the details of its carcinogenesis are not well known. But recently a number of steps in sporadic endometrial carcinogenesis have been identified.

The most important basis of understanding of molecular events in endometrial cancer is that this is hormone-dependent tumor. Aromatase cytochrome p450 is part of the complex mechanism responsible for conversion of C19 steroids to estrogen. Its increased expression in endometrial cancers suggests a role in promotion of neoplastic proliferation (43).

Additional molecular abnormalities include overexpression of p53 and c-myc gene amplification; p53 overexpression correlated with a poor prognosis (44).

Tumor suppressor genes in endometrial cancer

Unlike most other cancers studied so far, endometrial

cancer is not well characterized by a small number of loci that are involved by loss of heterozygosity. In endometrial cancers, most chromosomes contain regions of sustained allelic loss (45, 46). Chromosome 17p is also frequently involved, which contains the p53 gene known to confer a poor prognosis in endometrial cancers when mutated or deleted, and which plays an important role in the transition to carcinoma from atypical hyperplasia. Chromosome 18q DCC gene, a putative tumor suppressor gene, frequently mutated in colon cancers. While no mutations were found in the exons of the DCC gene in endometrial cancers, chromosome 18 was found to be capable of suppressing tumorigenicity of endometrial cancer cells in nude mice by the procedure of microcell fusion, with DCC expression elevated in most of the suppressed hybrids (47). These data strongly suggest that a putative tumor suppressor gene for endometrial cancer may locate on chromosome 18q.

Oncogenes in endometrial cancer

Ki-ras point mutations have been implicated in the development of atypical endometrial hyperplasias and endometrial carcinomas. This findings suggest that Ki-ras mutation may be an early oncogenic event in endometrial cancer (48).

Overexpression of HER-2/neu has been observed in tumor with advanced stage, deep myometrial invasion, and poor survival in endometrial cancers in several studies. Gene amplification did not underlie all cases of HER-2/neu overexpression, although both gene amplification and overexpression were each associated with poor outcomes (49). Recently, another new chromosomal region which has putative tumor suppressor gene for endometrial cancer was identified in chromosome 10q 25-26 (50).

CONCLUSION

Recent progress in molecular biology has permitted us to better understand the mechanisms of carcinogenesis, tumor progression, and host antitumor immune response. Two decades ago, cancer research was dramatically changed by the identification of cellular oncogenes and subsequent recognition that cancer is largely a genetic disease. This advance shifted the focus of experimentation from carcinogenesis and mutagens to oncogenes and tumor suppressor genes, which we now know regulate cell division, differentiation, and development. These developments have laid the conceptual and technical foundations of alternative therapeutic strategies for cancer treatment including gene therapy. Gene therapy for

cancer represents one of the most important novel therapeutic approaches. But it is difficult to predict, which gene therapy strategies will be clinically most effective in gynecologic cancer. More researches are needed to improve the technologies of gene transfer to cancer cells and therefore better be able to correct tumor specific genetic changes. Together with other endeavors, the gene therapy for cancer should not be considered dream in the near future.

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