CHAPTER 1

INTRODUCTION

Anatomy of uterine cervix

The cervix is actually the lower, narrow portion of the uterus. It protrudes into the upper part of vagina. The portion projecting into the vagina is referred to as the portio vaginalis or exocervix.[3] The passageway between the uterine cavity and the vagina is referred to as the endocervical canal. The exocervix is lined by non-keratinizing stratified squamous epithelium which is composed of several layers divided into basal, parabasal, intermediate and superficial (Figure 1). The endocervix is covered by mucous-secreting cell layer sprinkled with ciliated cells, simple columnar epithelium (Figure 2), which lines the surface and endocervical glands. These so-called glands are actually not true glands but they come from cleft-like infoldings of the surface epithelium. The border between the stratified squamous epithelium of the exocervix and the columnar epithelium of endocervix is called the squamocolumnar junction. The original squamocolumnar junction is the point where the original exocervical squamous epithelium joins the endocervical glandular epithelium and is out on the exocervix during the reproductive years. This junction is usually sharply defined. The functional squamocolumnar junction is the point of active replacement of columnar endocervical epithelium by squamous cells (Figure 3). This junction is often irregular and patchy, and it changes its contours and its locations during reproductive life. After squamous metaplasia has replaced endocervical tissue, the original squamocolumnar junction is the fusion point between the new squamous epithelium laid down in the original squamous epithelium and the transformation zone, the area between the two squamocolumnar junctions. This zone can be visualized with the aid of a colposcope. This is fortunate because neoplastic change begins most commonly in the transformation zone and neoplastic transformation is accompanied by structural alterations that can be recognized using the colposcope. The combination of cytologic preparation, colposcopic examination, biopsy, and

local destruction of intraepithelial abnormalities in the transformation zone under colposcopic visualization is a powerful tool for the early detection and successful treatment of in situ neoplastic processes involving the cervix. The stroma of the cervix is composed of dense fibromuscular tissue containing relatively little smooth muscle and ground substance. Through the stroma course the vascular, lymphatic, and nervous supply of the cervix. Beneath the squamocolumnar junction the cervical stroma is often infiltrated with leukocytes forming part of the defence against ingress of microorganisms. The function of the cervix is to admit spermatozoa to the genital tract at the time when fertilization is possible, i.e. around the time of ovulation, but at other times, including pregnancy, its function is to protect the uterus and upper tract from bacterial invasion. In addition, the cervix must be capable of great dilatation to permit the passage of the fetus during parturition.[64]

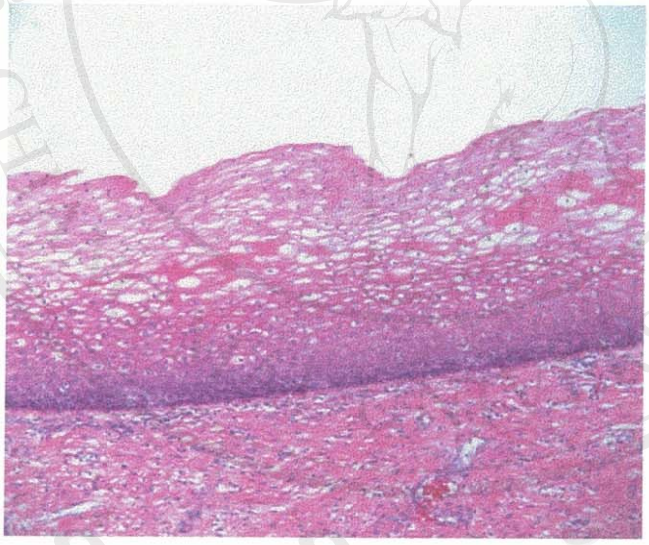

Figure 1 Mature squamous epithelium of the exocervix demonstrating a normal maturation sequence from basal cells to superficial cells. (H&E, 10x)

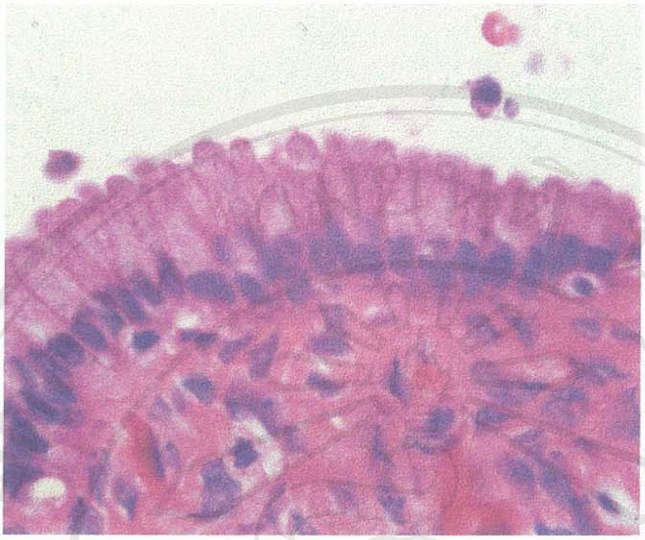

Figure 2 Histology of endocervix (H&E, 100x)

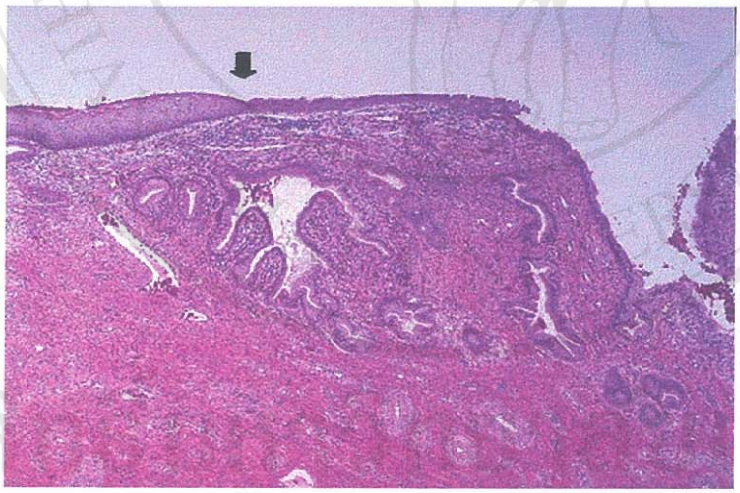

Figure 3 Histology of cervix (H&E, 50x)[45] :
functional squamocolumnar junction(arrow)

Cervical carcinoma

Cervical carcinoma is the second most common cancer in women worldwide and the second cause of cancer death.[2,58] In Thailand, the number of patients with cervical cancer have increased. From 1987 to 2002, the number of cervical cancer patients are about 8500 or 78.1% of all different cancer type in female genital tract (Table 1). Among these, 81.2% are squamous cell carcinoma, followed by adenocarcinoma (14.4%) and adenosquamous carcinoma (2.3%), respectively (Table 2). The cervical cancer patients who are in the early stage of disease can be treated successfully with either radical hysterectomy(RDH) with pelvic lymphadenectomy(PLD) or pelvic radiation.[40,47] Most patients consider surgery because this treatment method has useful for preserving hormonal function, less complications, and better tolerable.[18]

Table 1 The number of patients with different cancer type in female genital tract in Maharaj Nakhon Chiang Mai Hospital.[1]

Cancer	Number(%)
Cervix	8,560(78.1)
Ovary	1,192(10.9)
Uterus	757(6.9)
Vulva	301(2.7)
Vagina	133(1.2)
Uterine tube	22(0.2)
Total	10,965(100)

Table 2 Pathology of the cervical cancer patients in Maharaj Nakhon Chiang Mai Hospital.[1]

Pathology	Number(%)
Squamous cell carcinoma	2,471(81.2)
Adenocarcinoma	437(14.4)
Adenosquamous carcinoma	71(2.3)
Neuroendocrine tumor	56(1.8)
Other	8(0.3)
Total	3,043(100)

In patients with early stage of cervical carcinoma who underwent RDH with PLD, the overall 5-year survival rate is 80-98%.[4,13,23,28,32,42] In these patients, the presence of lymph node metastases is the most significance prognostic factor.[17,26,28,37] Cervical cancer frequently metastasizes to regional lymph nodes in the pelvis, followed by metastasizing to paraaortic nodes[17](Figure 4). Patients who had retroperitoneal node metastases or positive surgical margin or parametrial involvement should receive postoperative adjuvant therapy. Despite the adjuvant treatment, the recurrence occurred in 28-40% of patients with pelvic node metastases[32,59,60] whereas the recurrence was still seen in 7-14% of those without pelvic node metastases.[32,60] The prognostic factors related with an increased risk of recurrence in node negative patients include parametrial invasion[24], histology of pure adenocarcinoma[53], lymphovascular space invasion (LVSI)[39,48], clinical stage[37], tumor size and histologic grade.[14,30,39] However, these clinicopathologic prognostic factors do not always correspond with the recurrence of disease.[60] At present, there is no consensus about the criteria to select high risk group in these node negative patients, who need adjuvant post operative treatment. Therefore, new biomolecular prognostic factor should be investigated in order to find out the real aggressiveness of cervical cancer.

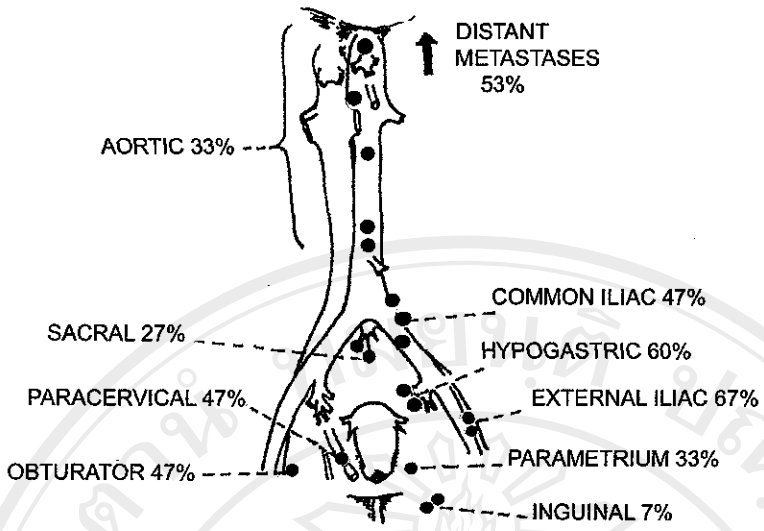

Figure 4 Diagram showing the frequency of lymphatic spread of cervical carcinoma[46]

Immunohistochemistry and tumor prognostication

Immunohistochemistry is an invaluable adjunct to morphological diagnosis in the area of oncological pathology.[34] In addition to diagnostic goal, a major purpose of histological examination of tumors is the prediction of prognosis and clinical outcome. Increasing efficiency of cancer treatment system demands that a variety of biological parameters should be assessed to predict tumor behavior and respond to specific therapies. Among various useful tumor assessment methods, immunohistochemistry is the one simple method of analysing many of prognostic parameters.[34] At present, the identification of oncogenes, tumor suppressor genes and enzymes that may more accurately predict the behavior of tumors are mainly focused.[57,62] Up to date, there have been many studied about the role of a tumor suppressor gene in carcinogenesis.[21,22,35,43,55] This gene normally regulates cell growth and differentiation and plays an important role in the deterrent of carcinogenesis in normal cells.[34] Genetic alteration of tumor suppressor gene leads to the loss of tumor suppressor function thereby contributing to carcinogenesis.[34]

The p53

The p53 protein was first identified as a host cells protein complexed with large T antigen, the major transforming protein of DNA tumor virus SV40.[22] The p53 gene residues in 20 kb DNA on the 17p13.1 locus.(Figure 5)

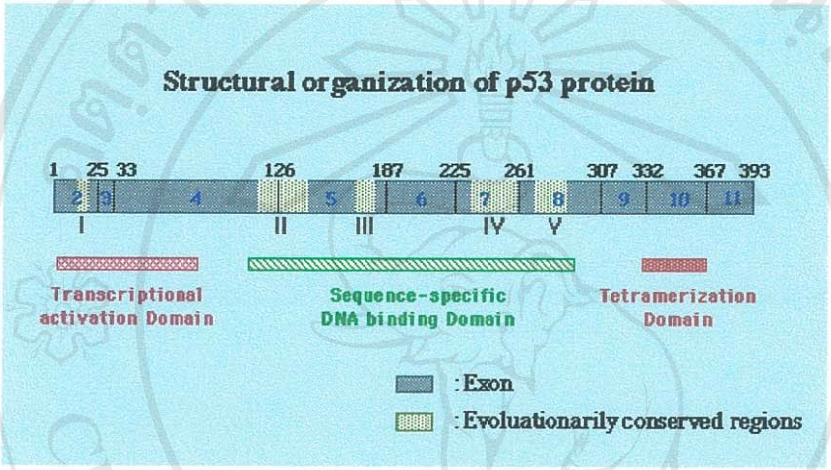

Figure 5 Structure of p53 protein[36]

The gene is composed of 11 exons that to produce a massenger RNA. Alterations in the p53 gene are most commonly missense mutations with a resultant faulty protein. The p53 protein was first discovered in 1979 during studies of SV40 (simian virus) transformed cells, when it was found to complex with the SV40 large tumor antigen. It contains 393 amino acid residues with 4 specific binding domain: N-terminus (1-43), sequence specific DNA binding domain (110-286), tetramerization domain (326-355) and C-terminus (363-393). The N-terminus is a transcription activation domain that regulates gene expression. Certain amino acids within the N-terminus appear critical for positive and negative regulation of target genes by p53. The sequence specific DNA binding domain is an antiparallel [beta] sheet that serves as a scaffold for 2 [alpha] helix loops that physically bind with DNA. More than 90% of the missense mutations occur in this domain, resulting in defective contact with DNA or loss of structural integrity of the protein.

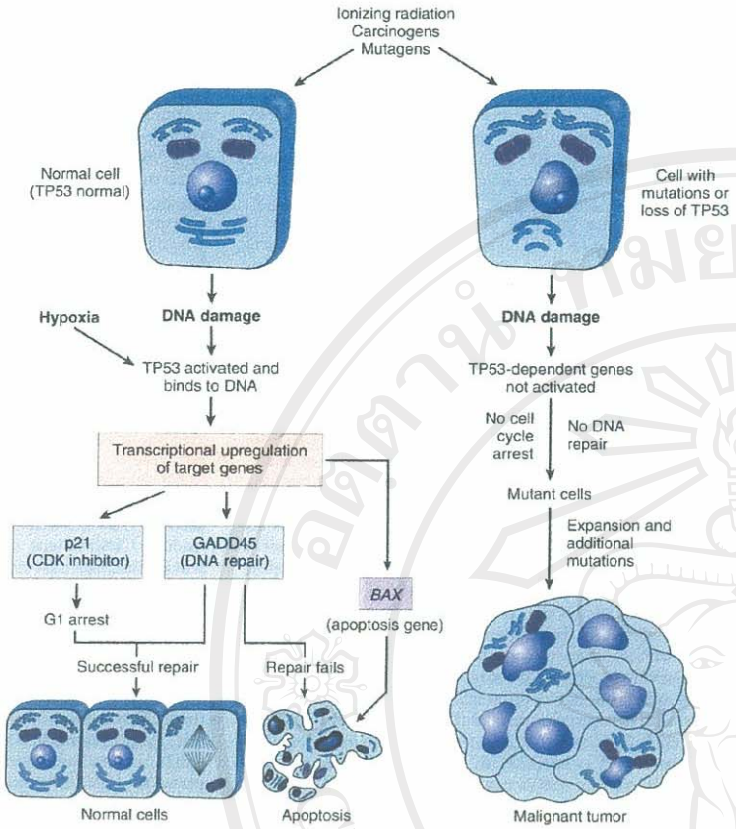

Figure 6 The role of p53 in maintaining the integrity of the genome. Activation of normal p53 by DNA-damaging agents or by hypoxia leads to cell cycle arrest in G_1 and induction of DNA repair, by transcriptional up-regulation of the cyclin-dependent kinase inhibitor CDKNIA (p21) and the GADD45 genes. Successful repair of DNA allows cells to proceed with the cell cycle; if DNA repair fails, p53 induced activation of the BAX gene promotes apoptosis. In cells with loss or mutations of p53, DNA damage dose not induce cell cycle arrest or DNA repair, and genetically damaged cells proliferate, giving rise eventually to malignant neoplasms.[52]

Mutations in the sequence specific DNA binding domain alter the tumor suppressor activity of p53 and are common on cancer. Normal p53 function also relies on proper conformation and oligomerization of the protein, a process that is regulated by the tetramerization domain. Two monomers associate to form an antiparallel double stranded sheet, known as a dimer. The tetrameric form of p53, a dimer of dimers, has enhanced ability to interact with either DNA or various proteins. Although relatively uncommon, mutations in the tetramerization domian prevent tetramerization, DNA binding and tumor cell growth inhibition. Finally, the C-terminus contains 9 basic amino acids that specially bind with DNA and RNA , and appear important in p53 regulation of DNA repair processes(Figure 6).

p53 protein expression and tumor prognosis

There are many studies demonstrated that the correlation between prognostic factors and p53 expression in various tumors. Abnormalities of p53 protein are probably the most common genetic alteration in human cancer. The p53 gene, coding for a 53 kDa with 393 amino acid nuclear phosphoprotein[7,9,11], is located in the short arm of chromosome 17 and plays an important

role in regulating cell cycle, programmed cell death or apoptosis, DNA synthesis, cellular proliferation, and co-ordinates a complex system of response to DNA damage. The wild-type p53 protein is a nuclear phosphoprotein with a very short half-life and is expressed in low levels in normal cells.[9,34] As a result, it is undetectable in normal cells by immunohistological methods. The alteration of p53 gene results in various mutant p53 proteins, which have a change in conformation and exhibit their accumulation that can be demonstrated by the immunohistological method. The p53 immunostaining permits not only the identification of tumors with aberrant accumulation of the protein, but also the subclassification of p53 positive lesions according to their characteristic staining patterns (nuclear versus cytoplasm) and the proportion of positive tumor cells within each lesion.[5] Most of studies using immunohistochemical technique indicate that the mutation of p53 gene is an independent prognostic parameter in various cancers such as breast cancer[51], lung cancer[16], gastric cancer[33] and ovarian cancer.[12]

LITERATURE REVIEW

The immunohistochemical studies of p53 protein expression in various human cancers have been reported. Kerns et al.[27]demonstrated the overexpression of p53 protein in paraffin-embedded tissue from 50 epithelial ovarian cancers and 25 primary breast cancers using monoclonal antibody Pab1801. They found that immunohistochemical technique could accurately reflect the level of p53 expression in human tumors. In cervix uteri, the alteration of p53 may involve in the progression of premalignant and malignant tissue in cervix uteri.[19] Increased expression of p53 in dysplasic and malignant lesions compared to non-dysplasic lesions suggests that p53 protein accumulation may be an early event in carcinogenesis.[30] Studies on the correlation between expression of p53 protein and prognostic parameters in cervical cancer are controversial. Howayda et al.[2] studied the significance of immunohistochemical expression of p53 in invasive cervical carcinoma in the Egyptian population and to correlated its expression with clinical staging of the International Federation of Obstetrics and Gynecology (FIGO), the histologic type and tumor grade. They found that p53 expression was only significantly associated with early clinical stages. However, more than half of patients were lost to the follow up and the investigators could not find the significance of such expression on the clinical outcome of the patients. Chen et al.[10] evaluated the pattern of p53 expression in stage IB squamous cell carcinoma of uterine cervix underwent RDH and PLD with immunohistochemical technique. They found that the expression of p53 may be indicative of an unfavorable prognosis

in patients with stage IB squamous cell carcinoma of the uterine cervix. Lakshmi et al.[30] showed a good correlation between expression of p53 protein and histologic grade of cervical carcinoma and found that p53 protein accumulation may be an early event in carcinogenesis, while for others, p53 protein expression did not seem to be the prognostic significance in cervical cancer.[9,11,21] These conflicing reports may be due to different methods of evaluating the results(the definition of positive staining), the difference of immunohistochemical detection and statistic analysis in these studies.[3,11,20,25,44] Some authors did not clarify whether they had blinded the interpretators about the clinical outcome of patients.[25] Furthermore, there are the limitation of these studies[2,44], for example, the limited number of the patients[2,20,44] and limited follow up time.[2] Despite the advances in the management of early stage disease, recurrent carcinoma of the uterine cervix remains incurable malignant disease. Thus, many investigators have focused on various histopathologic factors that have been related to recurrence in cervical cancer that underwent RDH and PLD. These include FIGO stage[13], histologic type[13], depth of invasion[23,29], tumor size[29,39], and parametrial extension.[24] Lymph node metastasis has been one of the most important factors in estimating the prognosis of the patients with invasive cervical cancer. Kobierski et al.[28] studied lymph node metastasis in 499 patients with cervical carcinoma at stage I and IIA after radical hysterectomy. They found that the 5-year survival was estimated at 82.2% and 50.8% in their group without or with lymph node metastasis respectively. In addition, lymph node metastasis has associated with an increased risk of recurrence, clinical stage and histologic type.[37] Based on the theory of metastasis cascade, the invasion of tumor cells into blood vessels or lymph vessels is one of the critical steps for the establishment of metastasis. Lymphovascular space invasion (LVSI), which implies both blood vessel and lymph vessel invasion, is one of the important histopathologic prognostic indicator for cervical carcinoma.[48] Takeda et al.[53] identified the independent prognostic factors for 187 patients with stage IB to IIB cervical carcinomas treated with RDH and systemic retroperitoneal lymphadenectomy. The histopathologic parametrial invasion, LVSI, and histology of pure adenocarcinoma were found to be important prognostic factors. Memarzadeh et al.[39] estimated the impact of parametrial lymphovascular and perineural involvement on nodal metastasis and the failure pattern of 93 patients with early stage cervical cancer treated with RDH and PLD with or without paraaortic lymphadenectomy. They concluded that the presence of parametrial lymphovascular space invasion correlated significantly with the risk of nodal metastasis in women with early stage cervical cancer. Graflund et al.[13] studied the value of p53, bcl-2, and p21 immunoreactivity as the predictors of pelvic lymph node

metastasis, recurrence, and death due to the disease in the early stage (FIGO stage I-II) of cervical carcinomas. They evaluated FIGO stage, histologic type, and tumor grade in this series of patients treated by RDH and found that the presence of lymph node metastasis, tumor recurrence, and death from disease were only significantly associated with the FIGO stage. However, the investigators did not find any significant association between p53 overexpression and lymph node metastasis, tumor recurrence, or survival rate. Manusirivithaya et al.[37] studied in 685 cervical cancer patients who underwent RDH as their primary treatment and had follow up for three years. They found that 97 cases had tumor recurrence. Patients with nodal metastasis had 42.1% risk of recurrence compared to 11.6% in those without nodal metastasis. This result revealed that lymph node metastasis was the most significant prognostic factor for tumor recurrence. Moreover, the work of Manusirivithaya and colleauges[37] also showed that other factors associated with a higher risk of recurrence in cervical cancer were clinical stage and histologic type. In Thailand, however, few data concerning the correlation between p53 protein expression and tumor recurrence in cervical carcinoma is available. Thus further study is still required to clarify the correlation between p53 protein expression and tumor recurrence in cervical carcinoma. The purpose of this study is to examine the association between p53 protein expression and tumor recurrence in Thai patients with cervical cancer undergoing RDH and PLD.

HYPOTHESES

Null hypothesis

H_0 : The proportion of p53 protein expression in cervical cancer patients who underwent RDH and PLD in those with tumor recurrence is equal to the proportion in those without tumor recurrence.

H_0 : proportion of p53 expression$_{recurrence}$ = proportion of p53 expression$_{non\text{-}recurrence}$

Alternative hypothesis

H_1 : The proportion of p53 protein expression in cervical cancer patients who underwent RDH and PLD in those with tumor recurrence is different from the proportion in those without tumor recurrence.

H_1 : proportion of p53 expression$_{recurrence}$ ≠ proportion of p53 expression$_{non\text{-}recurrence}$

Purposes of the study

1. To compare the proportion of p53 protein expression in cervical cancer patients, who underwent RDH and PLD between those with tumor recurrence and those without tumor recurrence
2. To examine the correlation between prognostic factors and expression of p53 protein in cervical carcinoma by using immunohistochemistry technique
3. To study the pattern of p53 protein expression in cervical cancer

Application advantage

The results of this study may be useful for treatment system of cervical cancer in patients with early stage cervical cancer underwent RDH and PLD. If the result of this study shows that the p53 protein expression significantly correlates with tumor recurrence, these patients should receive some forms of adjuvant post-operative treatment or pelvic radiotherapy in order to decrease risk of tumor recurrence.