# Biomarkers in Cervical Cancer

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**Abstract:** Cervical cancer, a potentially preventable disease, remains the second most common malignancy in women worldwide. Human papillomavirus (HPV) is the single most important etiological agent in cervical cancer, contributing to neoplastic progression through the action of viral oncoproteins, mainly E6 and E7. Cervical screening programs using Pap smear testing have dramatically improved cervical cancer incidence and reduced deaths, but cervical cancer still remains a global health burden. The biomarker discovery for accurate detection and diagnosis of cervical carcinoma and its malignant precursors (collectively referred to as high-grade cervical disease) represents one of the current challenges in clinical medicine and cytopathology.

**Keywords:** Cervical cancer, Human papillomavirus (HPV), biomarker.

## Introduction

Worldwide, cervical cancer is the second most common cancer in women; and is estimated to cause over 470,000 new cases and 233,000 deaths each year. Based on strong epidemiological evidence, supported by basic experimental findings, there is no doubt that persistent infections with high-risk types of human papillomavirus (HPV) represent a necessary cause of cervical cancer (Walboomers et al. 1999). HPVs infect epithelial cells and cause a variety of lesions ranging from common warts to cervical neoplasia and cancer. Over 100 different HPV types have been identified so far, with a subset of these being classified as high risk. High-risk HPV DNA is found in almost all cervical cancers (>99.7%), with HPV16 being the most prevalent type in both low-grade disease and cervical neoplasia. Productive infection by high-risk HPV types is manifest as cervical flat warts or condyloma that shed infectious virions from their surface. Viral genomes are maintained as episomes in the basal layer, with viral gene expression being tightly controlled as the infected cells move towards the epithelial surface. The pattern of viral gene expression in low-grade cervical lesions resembles that seen in productive warts caused by other HPV types. High-grade neoplasia represents an abortive infection in which viral gene expression becomes deregulated, and the normal life cycle of the virus cannot be completed. Most cervical cancers arise within the cervical transformation zone at the squamous/columnar junction, and it has been suggested that this is a site where productive infection may be inefficiently supported (Doorbar, 2006).

Although HPV infection is widespread, few people even know they are infected as the symptoms are seldom noticeable. It is even less well known is that nearly all cervical cancers (99.7%) are directly linked to previous infection with one or more of the oncogenic types of HPV (Walboomers et al. 1999). It is estimated that for every 1 million women infected, a hundred thousand (about 10%) will develop precancerous changes in their cervical tissue. Of these, about 8% of them will develop early carcinoma limited to cervical epithelium (carcinoma *in situ*; CIS) and a few of them will develop invasive cancer unless the precancerous lesions are detected and treated with such cases having been found to carry the oncogenic HPVs (e.g. types 16 and 18) that cause cervical cancer.

The HPV genome consists of 8 kb, and is a double-stranded DNA molecule. The relative arrangement of the 8–10 open reading frames (ORFs) within the genome is the same in all papillomavirus types, and a particular characteristic of papilloma viruses is that the partly overlapping ORFs are arranged on only one DNA strand. The genome can be divided into three regions: the long control region (LCR) without coding potential; the region of early proteins (E1–E8); and the region of late proteins (L1 and L2) (Walter and Philip, 2004). E6 and E7 are the most important oncogenic proteins. These proteins have pleiotropic functions, such as transmembrane signaling, regulation of the cell cycle, transformation of

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established cell lines, immortalization of primary cell line and regulation of chromosomal stability. Both E6 and E7 proteins can bind to multiple cellular targets. The interactions that are thought to be most relevant for their transforming functions are E6 binding, via the cellular protein E6-AP, to the tumor suppressor gene product p53, and E7 binding to the retinoblastoma tumor suppressor gene product pRb and its related pocket proteins, p107 and p130 (Dyson et al. 1989; Davies, 1993). The first interaction results in rapid ubiquitin-dependent proteolytic degradation of p53, which prevents cells from undergoing p53-mediated apoptosis (Thomas, 1999). A consequence of E7-pRb interaction is interfering with cell cycle control. In combination, the E6-p53 and E7-pRb interactions seem to compromise the accuracy of mitosis. In addition, HPV E6 can activate the telomere lengthening enzyme telomerase independent of p53 binding, and E7 can induce abnormal centrosome duplication through a mechanism independent of inactivation of pRb and its family member. It is likely that these latter properties also contribute to the transforming characteristic of these viral oncoproteins.

HPV infection causes changes in expression of host cervical cell cycle regulatory proteins. Such differentially expressed host proteins and nucleic acids may have a role as 'biomarker' of dysplastic cells. Investigation of potential biomarkers may also help to unravel new pathways involved in the HPV-mediated pathogenesis of cervical dyskaryosis.

## Cervical Cancer Screening

For more than 50 years the Pap smear has been the mainstay of cervical screening resulting in a dramatic decrease in death from cervical cancer. However, the Pap smear has certain disadvantages (Table 1). It has a low sensitivity and high false negative rate. The data reveals that some of the false negative Pap smears rarely contain any abnormal cells on the slide (DeMay, 1996; Spitzer, 2002). So far, an effort to seek an explanation for this matter has been focused on either the incomplete transfer of cells from collection devices to the slide or inadequate sampling. This results in the development of liquid-based cytology technique (DeMay, 1996; McGoogan et al. 1998). Additionally, one of the emerging explanations is the lack of exfoliation of dysplastic cell (shedder and nonshedder hypothesis) (Felix et al. 2002; Felix, 2003). Data from some studies have been shown to the effect that there is an abnormal expression of the adhesion molecules in a subset of dysplastic lesions of the cervix (et al. 2002; Felix, 2003). It can prevent detection by any test requiring exfoliated abnormal cell, including liquid-based technique. Despite the nonshedding behavior, those lesions can be identified by visual test (Felix, 2003). There have been a number of visual tests which investigated for primary screening or used as adjunctive test of cytology method. These tests include cervicography, visual inspection with acetic acid (VIA), speculoscopy. At the present time, cervicography has a limited role as a primary screening or an adjunct to Pap smear (Schneider et al. 1999; Autier et al. 1999; Costa et al. 2000). However, as a triaging strategy for patients with ASCUS Pap smear, it is still a promising technique (Ferris et al. 2001; Brotzman, 2002). Direct inspection is the other method based on applying acetic acid to the cervix and then visualizing it. It can be done under incandescent light with or without magnification or the chemiluminescent light (Wright et al. 2002; Parham, 2003). This chemiluminescent light is of low intensity. It is diffuse and produces minimal reflective glare from normal tissue. There are studies showing that the use of chemiluminescent light allows the examiner to identify acetowhitening better than the incandescent light does (Lonky and Edwards, 1992; Mann et al. 1993; Lonky, 2002; Parham, 2003). Speculoscopy is developed for cervical screening by using chemiluminescence and low-power magnification to examine the cervix after applying an acetic acid. It can detect acetowhite dysplastic lesions and has been reported to be effective in detecting cervical intraepithelial lesions when combined with the Pap smear (Lonky, 2002; Wright et al. 2002; Parham, 2003).

**Table 1.** The single Pap smear test has limited sensitivity and specificity.

| Limitations of Pap smear screening |
|---|
| • For a high grade lesion, the sensitivity of a single pap smear is only 60–80% |
| • Errors in sampling, slide preparation and interpretation are inherent in cytology |
| • Sampling for atypical glandular cells is exceptionally difficult |
| • False-positive rates range from 15–50% |
| • False-negative rates may reach 30% |

Presently, new technologies such as liquid-based cytology, HPV DNA test have been introduced. This test is used to detect the HPVs, which is considered the primary cause of virtually all cervical cancers. There are at least 30 different types of HPV strains that target the genital area, and are transmitted through sexual, skin-to-skin contact. Of these, approximately 13 are considered to be 'high risk' because they can trigger the development of abnormal cells associated with cervical cancer. The remaining 'low-risk' types can cause genital warts. Although the Pap smear can pick up the cellular changes caused by high-risk types of HPV, it's not as sensitive as the HPV test, which specifically detects the viral DNA. The HPV test is not yet routinely used by the majority of doctors, in part because it is more expensive than a regular Pap test. Therefore, it would be important to improve the cost-effectiveness of screening and reduce the psychologic burden of benign positive test results.

## Molecular Biomarkers in Cervical Cancer

### HPV E6

The E6 oncoproteins of high risk HPV interfere with the function of the cellular tumor suppressor protein p53 through the induction of increased proteasome-dependent p53 degradation. High risk HPV E6 proteins target the cellular E3 ubiquitin ligase E6-AP to p53, resulting in the transfer of ubiquitin peptides from E6-AP to p53, which marks p53 for degradation by the 26S proteasome. Low risk and cutaneous epithelia-infecting HPV E6 proteins are unable to target the cellular p53 protein for degradation through the proteasome pathway. Although E6-induced loss of p53 is an important element of E6-induced cellular transformation, recent studies have identified a number of additional cellular targets of E6 that may also play an important role. These included the following (Filippova et al. 2004; Yim et al. 2004): proteins involved in the regulation of transcription and DNA replication, such as p300/CBP (Huang and McCance, 2002), Gps2 (Degenhardt and Silverstein, 2001), IRF-3 (Ronco et al. 1998), hMcm7 (Kukimoto et al. 1998), E6TP1 (Gao et al. 1999) and ADA3 (Kumar et al. 1999); proteins, involved in apoptosis and immune evasion, such as Bak (Thomas and Banks, 1998), Bax (Bemard et al. 2003), TNF receptor 1 (TNF R1), FADD (Filippova et al. 2002) and c-Myc (Chen and Defendi, 1992); proteins involved with epithelial organization and differentiation, such as paxillin (Tong and Howley, 1997), E6BP/ERC-55 (Chen et al. 1995), zyxin (Degenhardt and Silverstein, 2001) and fibulin-1 (Du et al. 2002); proteins involved in cell-cell adhesion, polarity and proliferation control, which contain a PDZ-binding motif, such as hDLG (Kiyono et al. 1997), hScrib (Nakagawa and Huibregtse, 2000), PKN (Gao et al. 2000), MAGI-1 (Glaunsinger et al. 2000), MAGI-2, MAGI-3 (Thomas et al. 2002) or MUPP1 (Lee et al. 2000); and proteins involved in DNA repair, such as XRCC1 (Iftner et al. 2002) and 6-*O*-methylguanine-DNA methyltransferase (MGMT) (Strivenugopal and Ali-Osman, 2002) (Figure 1).

### HPV E7

HPV E7 proteins interact with the so-called 'pRb-associated pocket proteins,' including the retinoblastoma protein pRb, which are negative cell cycle regulators involved in the G1/S and G2/M transitions. The interaction between high-risk E7 and pRb results in enhanced phosphorylation and degradation. pRb destruction leads to the release of E2F family of transcription factors and the subsequent activation of genes promoting cell proliferation. However, the stimulatory effect of E7 upon cell proliferation not only depends on its association with pRb, since E7 targets the function of a plethora of cell cycle regulators, including cyclin A (Dyson et al. 1992), E (McIntyre et al. 1996) and cyclin-dependent kinase inhibitor $p21^{Cip1}$ (Jones et al. 1997) and $p27^{kip1}$ (Zerfass-Thome et al. 1996) together with the metabolic regulators, acid-α-glucosidase (Zwerschke et al. 2000) and M2 pyruvate kinase (Zwerschke et al. 1999). HPV E7 also interferes with the activity of a variety of cellular transcription factors, such as AP-1 (Antinore et al. 1996), p48 (Bamard and McMillan, 1999), interferon regulatory factor-1 (IRF-1) (Part et al. 2000), forkhead transcription factor MPP2 (Luscher-Firzlaff et al. 1999), TATA- box binding protein (TBP) and TATA-box binding protein-associated factor (TAF110) (Mazzarelli et al. 1995), as well as with the Mi2 histone deacetylase (Brehm et al. 1999). Also, E7 interacts with the S4 subunit of the 26S proteasome (Duensing and Munger, 2003), a human homolog of the

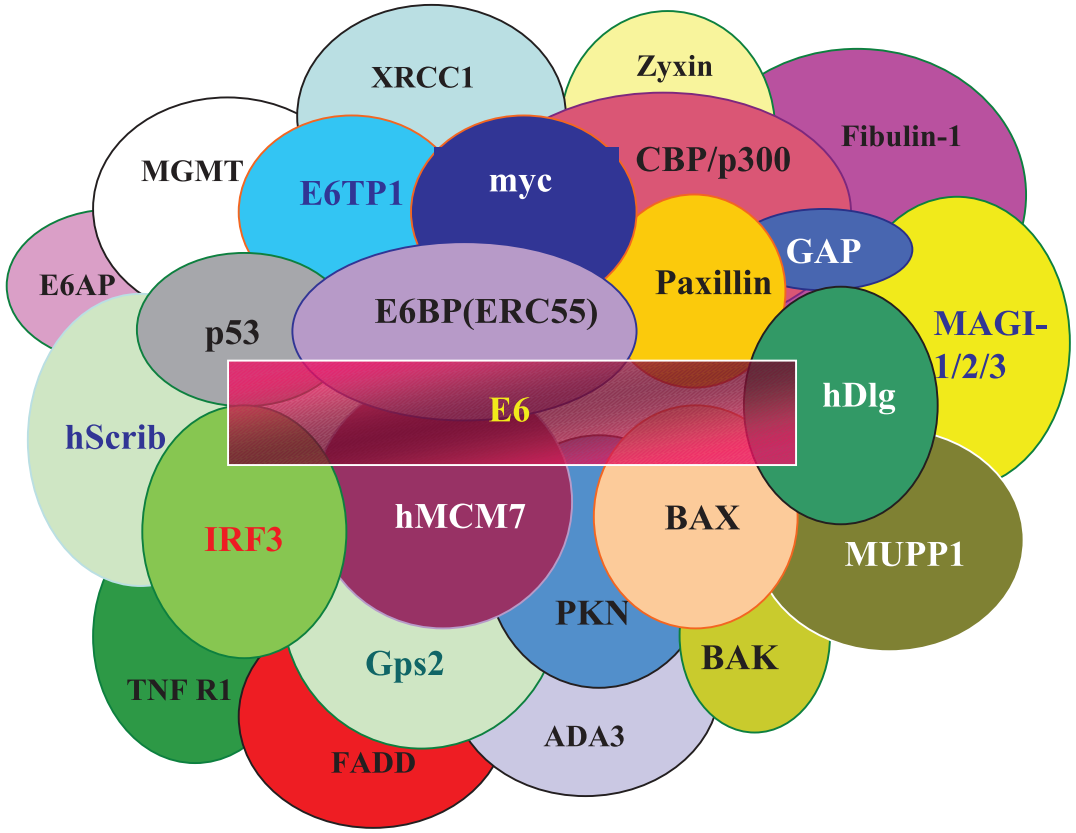



**Figure 1.** Cellular binding partners for HPV E6.

***P53 :*** **Tumor suppressor protein:regulation of cell response to mitogenic events**
***E6AP*** **: Regulation of signal transduction in proliferation**
***IRF-3*** **: Induction of interferon-β mRNA**
***Myc*** **: Transcriprion factor**
***hDlg*** **: human homologue of *Drosophila* Discs Large tumour suppressor**
***E6BP(ERC55)*** **: a calcium binding protein**
***hMCM7*** **: human minichromosome maintenance 7 protein (a component of replication licensing factors)**
***E6TP1*** **: GTPase-activating protein**
***XRCC1*** **: DNA repair protein**
***CBP/p300*** **: p53 coactivator**
***Paxillin*** **: Focal adhesion molecule**
***PKN*** **: protein kinase**
***MUPP1*** **: Multi-PDZ scaffold protein**
***Bak and Bax*****: a proapoptotic protein**
***Gps2*** **: G-protein pathway suppressor 2**
***MAGI-1/2/3*** **: Tight-junction protein**
***hScrib*** **: the human homologue of the *Drosophila* Scribble**
***ADA3*** **: Transcriptional coactivator**
***MGMT*** **: DNA repair protein**
***Zyxin*** **: Focal adhesion molecule**
***Fibulin-1*** **: Extracellular matrix protein**
***FADD*****: Fas-associated death domain**
***TNF R1*****: Tumor necrosis factor receptor 1**

*Drosophila* tumor suppressor protein Tid56 (hTid-1) (Schilling et al. 1998), interferon regulatory factor-9 (IRF-9) (Antonsson et al. 2006), Smad protein (Habig et al. 2006), insulin-like growth factor binding protein (IGFBP-3) (Mannhardt et al. 2000) and histone H1 kinase (Davies et al. 1993) (Figure 2).

## Mini chromosome maintenance (MCM)

DNA replication occurs only once in a single normal cell cycle, due to a mechanism known as 'licensing' of DNA replication. This process requires the assembly of a protein complex which includes the mini chromosome maintenance (MCM) proteins and the cell division cycle protein 6 (CDC6) (Cook et al. 2002; Shin et al. 2003). Disassembly of this complex prevents repetitive replication during the same cell cycle (Lei and Tye, 2001). Changes in the expression pattern of DNA 'licensing' proteins are frequently observed in dysplastic cells. In comparison with be present only during the cell cycle in normal cells, MCM proteins and CDC6 have been demonstrated to be overexpressed in dysplastic cells.

In normal cervical epithelium, MCM protein staining is limited to the basal proliferating layer and is absent in differentiated and quiescent cells. In cervical glandular and squamous dysplasia, however, MCM expression is dramatically increased, suggesting its potential as a biomarker of cervical dysplasia (Ohta et al. 2001; Stoeber et al. 2002; Going et al. 2002; Alison et al. 2002; Davies et al. 2002; Davidson et al. 2003). MCM5 has been the focus of much of this research, but MCM7 is also a highly informative marker of cervical cancer. The number of nuclei positive for MCM5 at the surface of dysplastic epithelium correlates with the severity of dysplasia (Williams et al. 1998; Freeman et al. 1999) (Table 2).

**Table 2.** MCM5 and HPV oncoprotein expression.

| MCM5 |
|---|
| • MCM5 overexpression may be due to the release of Rb inhibition on transcription factor E2F due to binding of HPV E7 oncoproteins |
| • E2F may bind to the MCM5 promoter to increase transcription of MCM5 |
| • MCM5 mRNA expression increase significantly with increasing severity of dysplasia |

## Cell division cycle protein 6 (CDC6)

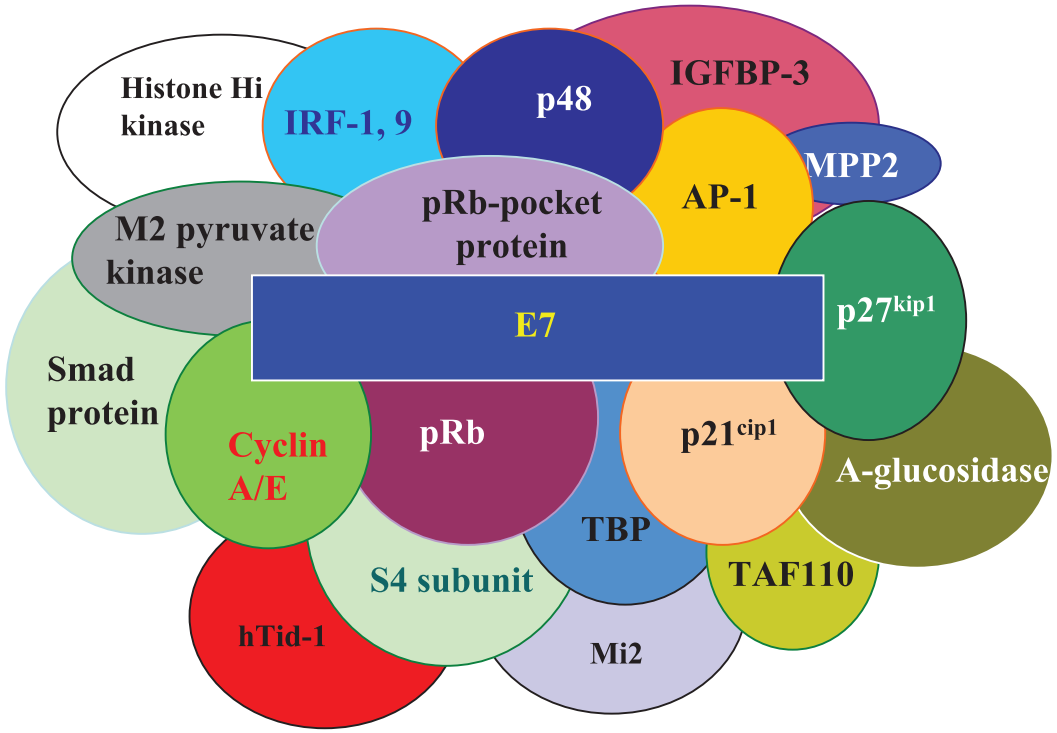
Both MCM5 and CDC6 play essential roles in the regulation of eukaryotic DNA replication. CDC6 was first identified in 1998 as a marker of cervical dysplastic cells in cervical biopsies and in smears using polyclonal antibodies. Not only MCM5 but also CDC6 protein expression are present in proliferating cells and absent in differentiated or quiescent cells. In normal cervical epithelium, CDC6 staining is absent or limited to the basal proliferative layer. However, CDC6 protein expression is dramatically up-regulated in squamous and glandular cervical carcinomas. Several studies have illustrated a linear increase in CDC6 expression observed in normal cervix, preinvasive neoplasia and invasive cervical carcinoma. CDC6 was preferentially expressed in areas exhibiting histological HPV changes. Interestingly, the expression pattern of CDC6 closely mirrors that of the high-risk HPV E6 oncoprotein, which is mainly expressed in higher grade lesions and invasive carcinomas (Table 3).

## $p16^{INK4A}$

$p16^{INK4A}$ is a tumor supressor gene and a key regulator of the cell cycle. The expression pattern of $p16^{INK4A}$ in dysplastic squamous and glandular cervical cells in tissue sections and in cervical smears has been extensively investigated (Sano et al. 1998; Klaes et al. 2001; Bibbo et al. 2002). In all normal cervical tissues examined, no $p16^{INK4A}$ staining is evident. Additionally, all normal regions adjacent to cervical intraepithelial neoplasia (CIN) lesions do not show any detectable expression of $p16^{INK4A}$. While $p16^{INK4A}$ identified dysplastic squamous and glandular lesions with a sensitivity rate of 99.9% and a specificity rate of 100% in

**Table 3.** CDC6 and HPV oncoprotein expression.

| CDC6 |
|---|
| • Inactivation of Rb by HPV E7<br>- Release inhibitor of E2F<br>- May transcriptionally up-regulate CDC6 |
| • CDC6 mRNA expression is significantly increased in high-grade dysplastic cells |
| • Overexpressionof CDC6 promotes re-replication, genomic instability and DNA damage in human cancer cells with inactive p53, but not in cells with functional p53 |
| • High-risk HPV E6 oncoprotein targets p53 for proteolytic degradation, allowing re-replication to occur in the presence of CDC6 overpression |




***pRb*** **: Tumor suppressor protein; regulation of cell cycle control**
***pRb-pocket proteins*** **: Regulation of cell cycle control**
***Cyclin A,E*** **: Kinase activity**
***P21$^{cip1}$***: Cyclin-dependent kinase inhibitor
***p27$^{kip1}$*** :Cyclin-dependent kinase inhibitor
***α-glucosidase***: Glycotic control enzyme
***M2 pyruvate kinase***: Modulation of the activity of glycotic enzyme 2
***AP-1*** : Transcription factors
***p48*** : IFN regulatory protein
***IRF-1***: Regulates expression of IFN-β
***IRF-9*** : IFN-α-responsive transcription factor subunit
***Mpp2*** : Forkhead transcription factor
***TBP*** : TATA box-binding protein; initiator of transcription
***TAF110*** : Initiator of transcription
***Mi2***: Histone deacetylase
***S4 subunit*** : S4 subunit of the 26S proteosome
***hTid-1*** : Human homolog of the *Drosopila* tumor suppressor protein Tid56
***IGFBP-3*** : Insulin-like growth factor-binding protein
***Histone H1 kinase***: Kinase activity
***Smad proteins 1-4***: Regulators of transcription

**Figure 2.** Cellular binding partners for HPV E7.

cervical biopsy sections, only a few studies have examined the possible prognostic value of $p16^{INK4A}$ in cervical lesions (Murphy et al. 2003). It is now widely accepted that $p16^{INK4A}$ is a sensitive and specific marker of squamous and glandular dysplastic cells of the cervix and also a surrogate marker of high risk human papillomavirus, suggesting a valuable adjunctive test in cervical cancer screening (Table 4).

## Squamous cell carcinoma antigen (SCC)

SCC belongs to the family of serine and cysteine protease inhibitors (Suminami et al. 1991). This antigen is present in normal cervix epithelium with an increased expression in proportion to dyspalstic lesion and cervical squamous cell carcinoma. Though SCC is not sufficient for use in screening, pretreatment serum SCC values works as an independent prognostic factor. Approximately 60% of patients with cervical cancer are detected with elevated levels of serum SCC at initial diagnosis, when all stages are included (Farghaly, 1992). Besides, serum SSC -> SCC levels correlate significantly with tumor stage (Crombach et al. 1989; Duk et al. 1990). More specifically -> If split with stage, serum SCC is elevated in 24–53% of patients with Stage IB or IIA squamous cell cervical cancer, and in 75–90% of patients with advanced stage (FIGO IIB and higher) disease (Gaarenstroom et al. 1995; Duk et al. 1996). Several studies have concluded that serum SCC is useful in monitoring the course of squamous cell cervical cancer following primary therapy (Bolli et al. 1994; Bonfrer et al. 1997). Persistently elevated and/or increasing serum SCC levels after and/or during treatment suggest tumor persistence or progressive disease (Brioschi et al. 1991). Patients with plateau SCC level revealed higher incidence of treatment failure after radiotherapy, indicating SCC levels provide useful information for the need of further work-up and management (Hong et al. 1998). In view of a strong correlation with the clinical course, SCC is suitable for monitoring the early detection of recurrent or progressive disease after primary treatment, and may therefore be useful in the management of patients. However, there is as yet no evidence that earlier detection of recurrent disease influences treatment outcome (Table 5).

**Table 4.** $p16^{INK4A}$ and HPV oncoprotein expression.

| $p16^{INK4A}$ |
|---|
| • Inactivation of Rb by HPV E7 protein may up-regulate $p16^{INK4A}$ |
| • $p16^{INK4A}$ may be directly induced by the transcription factor E2F released from pRb after binding of HPV E7 |
| • An HPV-independent pathway for $p16^{INK4A}$ up-regulation many also exist |

## Cell proliferation markers

The rate of cell proliferation in a tumor is generally thought to be of prognostic importance, and until recently the only means available to the pathologist to assess this was to count the number of mitotic figures, a technique fraught with difficulties and pitfalls. A number of antigens have now been described, which are expressed specifically by proliferating cells and which, with the use of monoclonal antibodies, can be demonstrated immunocytochemically: demonstration of these antigens affords, in theory at least, a much more accurate estimate of the number of proliferating cells than does a mitotic count. The two proliferation antigens which have been most widely studied are proliferating cell nuclear antigen (PCNA), which is expressed during the G1 and early S phases of the proliferative cycle, and Ki-67, which is expressed during the G2 and mitotic phases of the cycle. Ki-67 is the more reliable indicator of the growth fraction of a tumor, largely because PCNA has a long half-life and may still be demonstrable in post-mitotic cells (Scott et al. 1991). The study of Ki-67 was originally, however, limited by the necessity to use fresh or snap frozen tissue (Hall and Levison, 1990), but the recently introduced antibody MIB-1 can be used to detect the antigen in fixed paraffin-embedded tissue (McCormick et al. 1993). The number of cell nuclei staining positively for these markers of proliferation can be estimated by simple counting or can be measured in an image analysis system. In cervical intraepithelial neoplasia both PCNA and Ki-67 expressions are, as compared to normal cervices, increased in the upper levels of the cervical epithelium (Konishi et al. 1991; Shurbaji et al. 1993; Mittal et al. 1993; Raju, 1994; McLuggage et al. 1996), and it is thought that this staining pattern, particularly that for Ki-67, may be of considerable value in distinguishing CIN from non-neoplastic

**Table 5.** Currently available and potentially useful serum marker squamous cell carcinoma (SCC).

| Squamous cell carcinoma (SCC) |
|---|
| • Pre-treatment identification of high risk group with lymph node metastases in squamous cell cervical cancer |
| • Pre-treatment prediction of prognosis in squamous cell cervical cancer |
| • Prediction of response to treatment in squamous cell cervical cancer |
| • Monitoring disease and detecting recurrent disease in squamous cell cervical cancer |

lesions that may mimic CIN. Two studies of PCNA expression in cervical carcinoma have yielded conflicting results, one finding the PCNA index to be of considerable import (Oka et al. 1992) and another being unable to show that this index is of any prognostic value (Al-Nafussi et al. 1993). Investigations of Ki-67 expression in cervical carcinoma have generally failed to show any relationship between the number of positively stained cells and prognosis (Cole et al. 1992; Levine et al. 1995; Oka and Arai, 1996), though in one study the Ki-67 index was significantly related to tumor size, lymphatic spread, and disease-free interval in patients with stage I disease (Garzetti et al. 1995). In endometrial adenocarcinomas the PCNA index has been found to correlate with tumor grade, depth of myometrial invasion, and recurrence risk (Garzetti et al. 1996a), and it has been suggested that PCNA staining can be used as a method of pre-operative identification of high risk patients (Garzetti et al. 1996b). Ki-67expression in endometrial carcinomas was found to be correlated with grade but not with stage or depth of myometrial invasion in one study (Nielsen et al. 1994), but it emerged as a highly significant indicator of tumor recurrence in another (Geislet et al. 1996) (Table 6). By contrast, others have found neither staining for PCNA nor Ki-67 to be of any prognostic value in endometrial neoplasm (Hamel et al. 1996; Nordstrom et al. 1996).

Although few new markers have reached the clinic in recent years, several reported cancer biomarkers have been found to have low sensitivity in that they are found only in a small subset of patients with a particular type of cancer.

## Needs of Biomarker Discovery

The future of clinical cancer management belongs to the prognostic and predictive biomarkers of cancer. These markers are of utmost importance as they will be the used to make clinical decisions that will eventually save lives. In the future, biomarkers will guide decision making during cancer management. Biomarkers that correctly predict outcome in a specific disease and allow physicians and patients to make informed treatment decisions need to be developed. Biomarkers will not only help screen, detect, diagnose, help in prognostic evaluation, monitor treatment and predict recurrence, but also play a major role in clinical decision making.

## New Biomarker Development

Concern remains as to whether the tools available are well suited to provide the technological support to meet the demands of new biomarker development. Until recently, the discovery of cancer biomarkers has been a slow approach to identify proteins that are dysregulated as a consequence of the disease and shed into the body fluids such as serum, urine or saliva. Unfortunately, this approach is arduous and prolonged as each candidate markers must be identified among thousands of proteins. The recent advancements in genomic and proteomic technologies including gene array technology, serial analysis of gene expression (SAGE) improved 2-DE and new mass spectrometric techniques coupled with advancements in bioinformatic tools, shows great promise of meeting the demand for the discovery of a variety of new biomarkers that are both sensitive and specific

**Table 6.** Cell proliferation markers PCNA and Ki-67.

| | Type | Limitation |
|---|---|---|
| PCNA | Proliferation marker | Multiple factors affect staining intensity |
| Ki-67 | Proliferation marker | Multiple factors affect expression levels |

(Chatterjee and Zetter, 2005). Like these, high-throughput approaches are useful in cancer biomarker discovery and clinical diagnostics. The combined use of proteomics, genomics and bioinformatics tools may hold promise for early detection of disease by proteomic patterns, diagnosis based on proteomic signatures as a complement to histopathology, individualized selection of therapeutic combinations that best target the entire disease-specific protein network, rational modulation of therapy based on changes in the diseased protein network associated with drug resistance and understanding of carcinogenesis.

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