CHAPTER II

REVIEW OF RELATED LITERATURES

1. Cervical Cancer

Worldwide, cervical cancer comprises approximately 12% of all cancers in women. It is the second most common cancer in women worldwide, but the commonest in developing countries. Annual global estimates around the year 2000 are for 470,600 new cases and 233,400 deaths from cervical cancer annually (21). Eighty percent of these cases occur in developing countries. The data from Surveillance Epidemiology and End Results (SEER) form 1998-2002 displays incidence rate of cervical cancer and death rate in asian /pacific islander people was 8.9 and 2.7 per 100,000 women per year respectively. (22)

Squamous cell carcinoma of the cervix, which accounts for about 85% of cervical cancers, has all the characteristics of a sexually transmitted disease. The sexual history, especially the number of sexual partners, is the predominant risk factor for cervical cancer. The wide differences in cervical cancer incidence world-wide can be explained on the basis of three factors: sexual practices of the women, sexual practices of the men, and the availability of health care in the form of pap smear screening programs and treatment of preinvasive disease (23).

Nearly all cervical cancers originate in the "transformation zone" which is located at the lower and of the cervix where the columnar cells of the endocervix form a junction

with the stratified squamous epithelium of the vagina. Cells of the transformation zone undergo a rapid turnover and appear to be particularly vulnerable to the action of carcinogens. The high incidence of cervical cancer as compared to the low incidence of cancer at other sited in the female lower genital tract (vagina, vulva, perineum) is ascribed to the high susceptibility of the transformation zone to carcinogens.

Invasive cervical cancer is preceded by a progressive spectrum of abnormalities or the cervical epithelium (24) (Figure 1) . These abnormalities are classified, most recently, as low-grade and high-grade squamous intraepithelial lesions (SILs). (25). Previously, the lesions of the preinvasive disease were classified as cervical intraepithelial neoplasia (CIN) grades 1,2, and 3; or as mild dysplasia, moderate dysplasia and carcinoma in situ (CIS). The category low-grade SIL encompasses both CIN 1 and HPV infection. The severity of the lesion is graded by the extent to which the normally differentiating cervical epithelium is replaced by the non differentiating basal-like cells. In high-grade SIL almost the entire thickness of the epithelium is replaced by abnormal cells. In invasive cervical carcinoma, the abnormal cells breach the basement membrane, invade the stromal tissue, and eventually metastasize to lymph nodes and to other sites in the body. Most of the preinvasive cytologic abnormalities resolve on their own, and only a small fraction progresses to invasive cancer (26). The time interval between the early cervical abnormalities and invasive carcinoma may span several decades. During this long interval, cytological abnormalities are detectable by Pap smear and can be readily treated. Low – grade SIL is most frequent in the early 20s,

high-grade SIL in the late 20s and early 30s, and invasive cervical a of the cervix has been derived from many different sources which include clinical and epidemiological investigations, pathogenesis studies, examination of the molecular basis of transformation by HPVs.

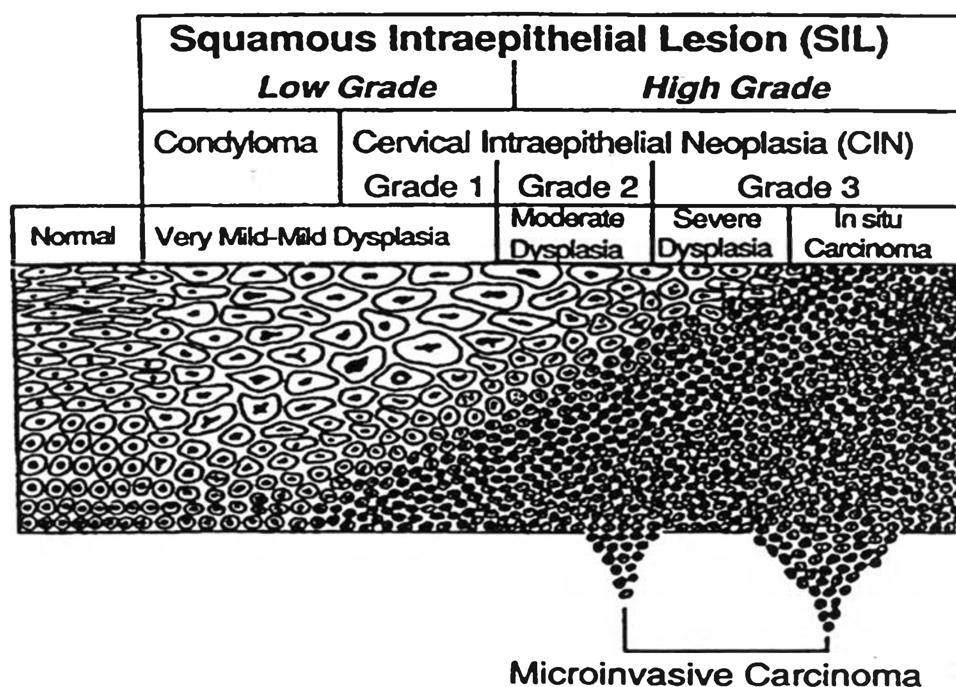

Figure1. cervical squamous carcinoma precursors. Schematic representation of cervical cancer precursors and the different terminologies that have been used to refer to them. (Figure from Fields Virology, Third edition, Lippincott-Raven publishers , Philadelphia 1996)

2. Human Papillomavirus

Papillomaviruses infect many vertebrate species but are highly species specific. All the papillomaviruses, regardless of host specificity, are quite similar in physical structure and genome organization. The virion is composed of a double – stranded, circular, 7,904 base pair DNA genome encased in a naked iscosachedral capsid about 55 nm in diameter. All papillomaviruses have a similar genetic organization (Figure 2). Currently, there are at least 100 different genotypes of HPV that have been identified based on DNA sequence relatedness in the L1 gene, at which encodes the major capsid protein. Of these, at least 30 types exhibit a tropism for anogenital mucosa (27).

The HPVs can be broadly grouped into cutaneous types and mucosotropic types based on their preferred tissue tropism (Table 1). The cutaneous types are typically found in the general population and cause common warts. Other cutaneous types are found in individuals who are immunosuppressed. The mucosotropic HPVs are further classified into high-risk and low-risk types, referring to their association with cervical cancer. The most common low-risk types are HPV 6 and 11, detected most often in benign gernital warts. HPV 16, 18, 31, and 45 are the predominant types found in cervical SCC, accountion for nearly 80% for cases (28), with HPV 16 alone accounting for about half the cases worldwide (29). HPV 18 is the most prevalent type in cervical adenocarcinomas (55%) , followed by PHV 16 (32%) and HPV 45 (10%) (30) .

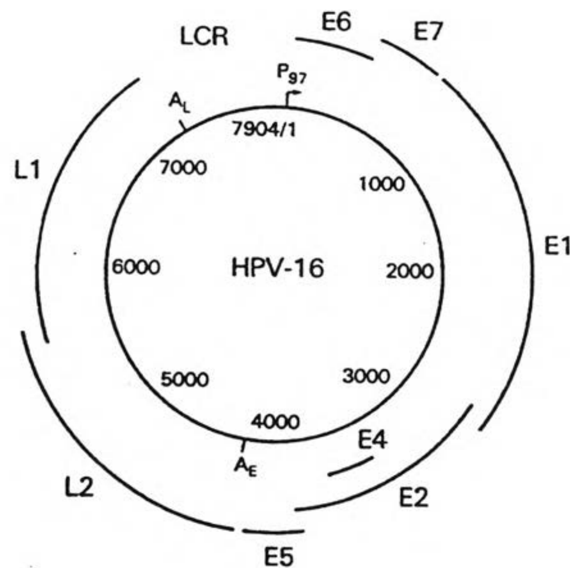

Figure 2. Genomic map of HPV-16. The genome is a double strand circular DNA molecule of bp. Transcription occurs in a clockwise manner; the only transcription promoter presently mapped for HPV-16 is designated P97. The ORFs deduced from the DNA sequence are designated designated E1 to E7, L1 and L2 are indicated outside the circular genome. A_E and A_L are represent early and late polyadenylation sites. The viral long control region (LCR) contains transcription and replication regulatory elements.(Figure from Fields Virology, Third edition, Lippincott-Raven publishers , Philadelphia 1996)

Table 1. HPV classification by tissue tropism

Group	Prototypes	Site	Acute disease	Chronic disease
Cutaneous	HPV 1, HPV 2	Skin	Warts	None
Cutaneous-high risk	HPV 5, HPV 8	Skin	Flat lesions or warts	SCC
Mucosal-low risk	HPV 6, HPV 11	Anogenital mucosa	Warts	None
Mucosal-high risk	HPV 16, HPV 18, HPV 31,HPV 33, HPV 45	Anogenital mucosa, oral mucosa	Flat lesions	SCC

HPV , human papillomavirus; SCC, squamous cell carcinoma

The viral genome is divided into an early region (about 4.5kb) which encodes the genes required for viral DNA replication and cellular transformation, a late region (about 2.5 kb) that codes for capsid proteins and a regulatory region (about 1kb) that contains the origin of replication and many of the control elements for transcription and replication. The regulatory elements in noncoding region has binding sites for numerous cellular factors, some that are common to all types and some that are unique. The unique factor-binding sites likely explain the variability in the tissue tropism of the virus types (31). The functions of the eight designated ORFs in the early regions (E1 to E8) and of the two ORFs (L1,L2) are described in Table 2.

Table 2. Functions assigned to the papillomavirus open reading frames

ORF	Function
L1	L1 protein, major capsid protein
L2	L2 protein, minor capsid protein
E1	Initiation of viral DNA replication
E2	Transcriptional regulatory protein with an auxillary role in viral DNA replication
E3	No known function
E4	Late protein; disrupts cytokeratins
E5	Membrane transforming protein of HPVs; interacts with growth factor receptors
E6	Transforming protein of HPVs; target degradation of p53
E7	Transforming protein of HPVs; binds to the retinoblastoma protein
E8	No known function

The expression of the viral proteins is very tightly regulated and dependent on cell differentiation (32). Papillomaviruses infect the basal cells of the epithelium when the integrity of the epithelium is compromised by a microabrasion or other traumas.The viral genome then becomes established in the basal cells as an episome. A low copy number of episomes is replicated in tandem with the cell's chromosomes, establishing a continuous supply of cells chromosomes, establishing a continuous supply of cells the ultimately generate progeny virions. Infected daughter cells then begin to migrate up and differentiate in the outer layers of the epithelium. These highly differentiated cells support vegetative viral DNA replication and expression of the structural proteins. Virions are assembled in the outermost cells of the eqithelium and are spread as these cells are sloughed off.

The HPV E1 ORF encodes proteins required for the maintenance of the viral genome and its replication. The HPV E2 ORF encodes the major transcriptional regulatory proteins that interact with specific binding sites in the noncoding region. The E2-encoded proteins have both positive and negative effects on transcription. The functions of the E4 ORF-encoded proteins are not completely elucidated but include disruption of the cytokeratin networks, resulting in the characteristic koilocytotic appearance of HPV-infected cells and possible regulation of mRNA stability (33). The E5 protein is a hydrophobic membrane-associated protein that appears to activate the epidermal growth factor receptor, resulting in stimulation of cell growth (34) . The E6 and E7 proteins are the major oncoproteins of HPV , and they also have been shown to play

a role in viral episome maintenance during productive infection (35). Recently, E7 has been suggested to play a role in disrupting the keratinocyte differentiation program (36). The L1 and L2 ORFs encode the major and minor capsid proteins of the virus, respectively.

The HPV E6 and E7 ORFs have been studied extensively since the first demonstration that they encoded proteins with properties similar to those of the oncoprotins of other DNA tumor viruses. Specifically, the E6 protein from high – risk HPV types was shown to target the p53 tumor suppressor protein for degradation, and the E7 protein, also from the high-risk HPV types, was shown to bind to the active form of the retinoblastoma (Rb) protein (37). The ability of HPV proteins to alter control of the cell cycle provides biochemical evidence of their role in oncogenesis.

The HPV 16 E6 protein is a DNA-binding protein. Although first demonstrated to target p53 for degradation, many subsequent studies have shown that HPV 16 E6 interacts with many cellular factors , resulting in the blocking of apoptosis, alterations in cellular transcription, disturbance of intercellular signaling, and an increase in the life span of cells, all of which may contribute to malignant transformation. The E6 protein form the low-risk HPV types binds p53 with much lower affinity and does not target p53 for degradation (38).

The HPV E7 proteins bind to Rb and inhibit its function; however, the binding affinity of the low-risk E7 proteins much lower than the high-risk E7 proteins (39). The ability to bind to and inhibit Rb functions is also characteristic of the SV -40 large T

antigen and the adenovirus E1A protein, and there is considerable sequence homology among these Rb-binding proteins. HPV E7 also interacts with other proteins involved in cell cycle regulation .

Infection with HPV is an early step in cervical carcinogenesis. The physical state of the HPV DNA within the infected cell has been shown to predict the pathologic course of the HPV-induced cervical lesion. The viral DNA is mostly in an episomal form in low-grade lesion, but it is integrated into the host cell chromosome in high-grade lesions and cancer (40) . Integration disrupts the E2 ORF, eliminating the transcriptional regulatory proteins and leading to the continued expression of the E6 and E7 oncoproteins (41) .

In low-grade cervical lesions, E6 and E7 transcripts are expressed at very low levels in basal cells with an increase in expression in the more differentiated upper layers of the epithelium. High-grade lesions and cancers show high levels of E6 and E7 transcripts throughout all layers of the epithelium (42) . This high-levels expression of E6 and E7 imparts growth advantage on the cells, leaving them vulnerable to other genetic changes that ultimately result in malignant transformation. Additionally, the breakdown of intracellular surveillance systems in controlling E6 and E7 expression in basal cells contributes to malignant progression (4) .

Many of the histologic features of both low-grade and high-grade cervical lesions and cancer have been attributed to HPV infection. Nuclear enlargement and hyperchromasia characteristic of atypia are likely due to activation of host DNA synthesis by E6 and E7 (43). In addition, if E4 is expressed and thus disrupts the

cytokeratin filaments, then characteristic koilocytes are seen. In high-grade lesions, the deregulation of E6 and E7 expression in basal cells gives rise to cells that proliferate uncontrolled, eventually making up the majority of the cells in the epithelium. This is a recognized as abnormal squamous maturation with numerous mitotic cells.

3. DNA Double Strand Breaks

DNA double strand breaks (DSBs) are the result of two simultaneous nicks in opposite strands of the DNA helix. A requirement for DSB formation is that the two nicks are in sufficiently close proximity to one another (<12bp) that base pairing and chromatin structure are unable to maintain the broken DNA ends juxtaposed. It is known that DSBs can be generated following exposure of cells to exogenous agents such as ionizing radiation radiomimetic drugs and topoisomerase inhibitors (7,8). Another type of DSBs arises from endogenous processes, called pathological DSBs. For example, oxidative free redicals [reactive oxygen species (ROS)] form inhaled oxygen such as hydrogen peroxide and superoxide anions that have the potential to cause DSBs (2). Furthermore, DSBs may occur when the replication machinery meets nicks or other types of lesions in the template DNA (7). DSBs may result form incorrect resolution by normal DNA metabolic proteins such as RAG1 and RAG2 (7,44). Although they represent a major danger to the stability of the genome, DSBs are created intentionally be specific nucleases during regulated chromosomal rearrangements. A well-characterized example of this is V(D)J recombination, in which immunoglobulin (Ig)

heavy chain and light chain variable region genes and T-cell-receptor genes are assembled from germline variable (V), diversity (D) and joining (J) sites in B- and T-lymphocytes, respectively (44,45). These recombination pathways are initiated by DSBs that are generated at target loci by the site-specific RAG1 and RAG2 nuclease complex. Furthermore, physiological DSBs are produced during meiosis to initiate recombination between homologous chromosomes and are also generated during Ig class-switch gene rearrangement pathways(7) . It has been suggested that cells are able to adapt to low levels of irreparable DNA damage(7). However , only a single DNA DSB is potentially cytotoxic or under certain circumstances can trigger programmed cell death (apoptosis) (46). DSBs are considered to be particularly dangerous lesions because they are more difficult to repair than other types of DNA damage. For example , single-strand nicks are more easily repaired by any for the excision-repair pathways that involve using the undamaged anti-parallel strand as a template (47). In contrast, DSBs result in loss of integrity of both DNA strands. Furthermore, the biochemical configurations of the broken DNA ends are typically incompatible, and rejoining by a simple ligation step is not usually possible to repair the lesion (8).

There is comprehensive experimental evidence supporting an causal relationship between DSBs and genomic in stability. Erroneous rejoining of DNA DSBs can induce deletion or insertion mutations and chromosomal translocations that are potentially carcinogenic (9). Indeed, chromosomal translocations involving the Ig and T-cell-receptor loci are evident in many cancers of lymphoid origin, suggesting that they

are the result of defective resolution of DSBs related to V(D)J recombination. For example , in the B-cell malignancy, Burkitt's lymphoma, the *c-MYC* gene is often juxtaposed to the Ig heavy-chain genes as result of incorrect chromosomal recombination (44,45). More generally, the loss or amplification of chromosomal regions that is a feature of many cancer cells may be associated with inappropriate DSB repair. It has been suggested that errornous DSB repair can lead to carcinogenesis in cases where the deleted genes are associated with tumor suppressor loci or amplified genes encode an oncogene (7,9). Moreover, defects in cellular reponses to DSBs, including mutations in factors involved in signaling and repair, may increase cancer predisposition or could frequently be the initiation events of carcinogenesis (7,9). After DSB formation, the cell cycle is arrested, followed by DSB repaired. In cases where the extent of DNA damage is irreparable, certain cell types can enter programme cell death (9). The intensity of DNA damage is critical factor in directing the signaling cascade toward reversible arrest or apoptosis (48)

DSB repair mechanisms

DSBs in mammalian cells are repaired by one of two distinct and complimentary pathways homologous recombination (HR) and non-homologous end joining (NHEJ) . HR relies on extensive sequence homology between the recombining ends and essentially involves copying the missing information from and undamaged homologous chromosome (7,9). Thus, it is typically error free and occurs without the loss of genetic

information. This pathway is the one predominantly used by simple eukaryotes, such as the yeasts *S.cerevisiae* and *S.pombe*, to repair DSBs (49). In contrast to HR , NHEJ does not require an undamaged DNA molecule and does not rely on extensive sequence homology. Instead, NHEJ involves processing of the broken DNA termini to make them compatible, followed by a ligation step (8). In most cases, this pathway results in the loss of a few nucleotides at each broken and (8). Hence, DSB repair by NHEJ is typically error-prone and is not a perfect process with respect to preserving genomic information. NHEJ is the more common DSB pathway in higher eukaryotes, and predominates in most stages of the cell cycle, particularly in G_0 an G_1 (7-9). Furthermore, the NHEJ pathway is essential for repair of DSBs the occur during V(D)J recombination and Ig class-switch recombination in lymphocytes (8). However, HR is also important for DSB repair in multicellular eukaryotes, particularly during the late S and G_2 phases of the cell cycle (7-9). NHEJ has been studied extensively in mammalian cells using genetic and biochemical techniques . It is proposed that the Ku70/Ku86 (Ku 86 is also called Ku80) heterodimer is the frist protein to bind to broken DNA termini at the site of DSBs (8). Ku is abundant in cells and binds to DNA ends with high affinity in a non-sequence-dependent manner (50-52). Binding of Ku to DNA recruits another PIKK family member, know as the DNA-dependent protein kinase catalytic subunit (DNA – PKcs)(53). The precise contribution of DNA-PKcs results in dissociation of the DNA-Ku-DNA-PKcs complex. Following assembly of the DNA-Ku-DNA-PKcs complex and DNA end processing, the DNA termini are ligated by the XRCC4-DNA-ligase-IV

complex . The finding that the XRCC4-DNA-ligase-IV complex does not form a stable complex with DNA in the absence of the Ku complex has prompted the suggestion that Ku may recruit and load the ligase complex at the sites of DSBs (8).

The repair of DSBs by HR was characterized in bacteria and yeast. Genetic analysis of *S.cerevisiae* indicated that proteins coded by the RAD52 epistasis group of genes – Rad50, Rad51, Rad52, Rad54, Rad55, Rad57, Rad59, Mrel1 and Xrs2 – are important in HR (54). In *S.cerevisiae* , an early event in HR is nucleolytic resection of the broken ends in the 5' to 3' direction (7,9). This process involves the Rad50-Merl 1-Xrs2 complex. (9) The resulting 3' single – stranded DNA overhangs are then bound by Rad51 (55). This process is facilitated by replication protein A (RPA), Rad54 and Rad52, which is known to bind to DNA termini (D7). Following binding of Rad51 to the 3' end , the RPA-coated Rad51 nucleoprotein filament mediated a search for homologous sequences on an undamaged DNA duplex. Once a homologous region is located, Rad51 initiates strand exchange in which one of the damaged DNA strands invades the homologous duplex, forming a D-loop (D7). This process is facilitated by Rad55 and Rad57 in yeast (55). The 3' termini then prime new DNA synthesis, resulting in the extension of the damaged DNA ends are ligated by DNA polymerases that copy information from the homologous undamaged partner . Identified of mammalian homologs exist for all of the known gene products that are in involved in HR in *S.cerevisiae* are studied, indicating that the basic HR pathway is conserved in higher eukaryotes. (56)

4. Epigenetics and DNA Methylation

Epigenetics

Epigenetics can be defined as the study of genome function that is contained outside of DNA itself and by means of which stable alterations in gene expression are set. Epigenetics is a well-estabished phenomenon that plays a major role in a diversity of biological processes such as embryonic development, cancer biology,and immune system response,among many others. The two most widely studied epigenetic changes are DNA methylation and histone deacetylation; however, others complicated situation such as the RNA interferences phenomenon, which has proven to be implicated in transcriptional silencing through small duplex molecules that recruit silencing complexes to the chromation was reported (57,58)

DNA Methylation

DNA methylation, the addition of a methyl group to the 5′carbon of deoxycytosine, is one such epigenetic modification found in DNA (59) . It is heritable and involved in regulation of gene transcription . In mammals, the major target for DNA methylation is a found in CpG –dinucleotide. CpG dinucleotide is cytosine located next to a guanine(5′-CpG-3′) (60). These targets of methylation are not equally distributed in the genome, but found in long CG-rich sequences present in satellite repeat sequences, middle repetitive rDNA sequences, centromeric repeat sequences and CpG islands.

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CpG islands are sequences longer than 200 bp with a GC content of over 50% (in contrast to a genome-wide average of about 40%) and an observed over expected ratio of CpG of 0.6 or greater (61). Interestingly, CpG islands are found mainly in the 5′ - regions of house-keeping genes as well as some other specifically tissue expressed genes and usually extend from the promoter region into the first exon and sometimes into intron 1(62). Most CpG islands are unmethylated in normal cells; however, there are certain conditions where these sequences become methylated and form part of gene regulation (63). The majority of CpG islands on the inactive X-chromosome in a female cell are methylated (64), and certain CpG island-like sequences in the vicinity of imprinted genes have been found to be methylated in an allele-specific manner (65). Furthermore, it has been found that some CpG islands become methylated with age (66). While CpG islands are usually unmethylated, other GC-rich sequences, e.g. the centromeric repeat sequences and satellite sequences, are highly methylated in normal cells.

Mechanisms of DNA methylation

DNA methylation is mediated by a family of DNA methyltransferase(Mtases) that includes Dnmt1, Dnmt3a, and Dnmt3b. Dnmt1 is a maintenance Mtase that primarily replicates methylation patterns, while Dnmt3a and Dnmt3b are capable of methylating previously unmethylated DNA, referred to as de novo methylation (67). DNA methylation patterns are established during differentiation, and serve to suppress genes

unnecessary for the function of the mature cell. This involves de novo methylation of DNA, and requires Dnmt3a and Dnmt3b (68).Their role in mature cells is less clear, but Dnmt3b appears to be necessary for maintaining methylation of pericentromeric heterochromatin (69). Following differentiation the patterns are replicated during mitosis by the maintenance DNA Mtase Dnmt1 (67). During mitosis, Dnmt1 recognizes hemimethylated CG dinucleotides in the parent DNA strand, and catalyzes the transfer of the methyl group from S-adenosylmethionine (SAM) to the cytosine residues in the unmethylated daughter DNA strand, producing symmetrically methylated sites and maintaining methylation patterns (67) (Figure 3).

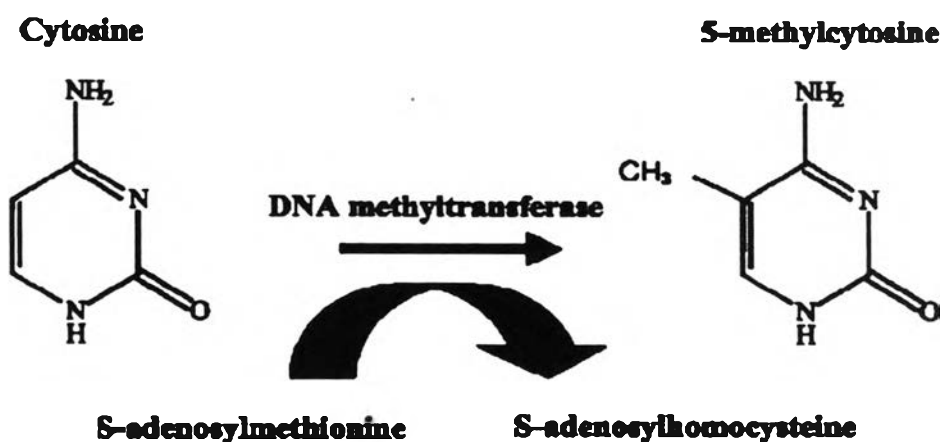

Figure 3. Cytosine methylation. The DNA methyltransferases catalyze the transfer of the methyl group from S-adenosylmethione to cytosine, producing 5-methylcytosine and S-adenosylhomocysteine. (Figure from Doerfler, W. DNA methylation and gene activity. Annu Rev Biochem 1983;52:93–124.)

Mechanisms of gene suppression

The methylation of CG sequences can affect nearby gene expression. Hypomethylation of regulatory sequences usually correlates with gene expression, while methylation results in transcriptional suppression. In contrast, methylation of coding sequences generally has little effect on gene expression (70). Methylation suppresses transcription by at least three mechanisms. Methylation of recognition sequences prevents the binding of some transcription factors such as AP-2 (71). A family of methylcytosine binding proteins has been described, which inhibit binding of transcription factors to promoters (72) in Figure 4,Interestingly, all three DNA Mtases can also suppress gene expression directly independent of their methylation activity (19). Finally, some methylcytosine binding proteins such as MeCP2 and MBD2 can promote chromatin condensation into an inactive configuration through interactions with chromatin inactivation complexes containing histone deacetylases (72). This can affect gene expression at a distance from the methylated region.

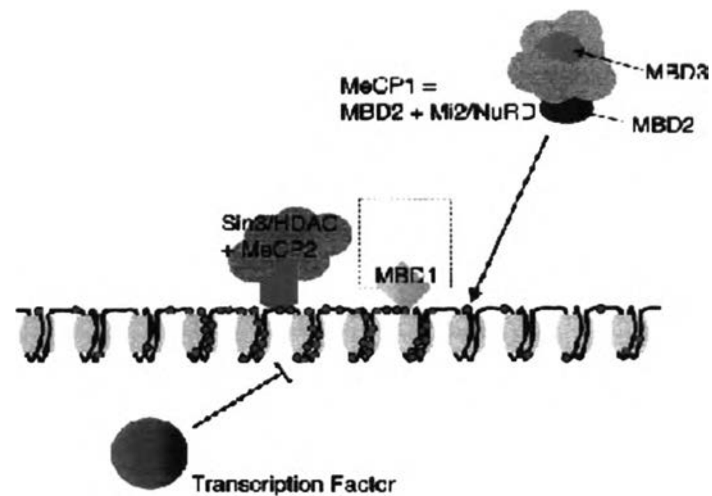

Figure 4 Mechanisms of transcriptional repression by DNA methylation. A stretch of nucleosomal DNA is shown with all CpGs methylated (red circles). Below the diagram is a transcription factor that is unable to bind its recognition site when a methylated CpG is within it. Many transcription factors are repelled by methylation. Above the line are protein complexes that can be attracted by methylation, including the methyl-CpG-binding protein MeCP2 (plus the Sin3A histone deacetylase complex)and the MeCP1complex comprising MBD2 plus the NuRD corepressor complex. MeCP2 and MBD1 are chromosome bound proteins, whereas MeCP1 may be less tightly bound. (Figure from Baylin S. Mechanisms underlying epigenetically mediated gene silencing in cancer. Semin Cancer Biol 2002;12:331.)

Important of DNA methylation in normal cells

The importance of DNA methylation in the function of normal cells is evidenced by its role in differentiation of development, X chromosome inactivation, genomic imprinting, maintenance of chromatin structure, and suppression of "parasitic" DNA (74).

DNA methylation in cancer

Tumorigenesis is known to be a multistep process in which defects in various cancer genes accumulate . Virtually every tumor type has revealed an enormous complexity of altered gene functions, including activation of growth-promoting genes as well as silencing of genes with tumor growth-suppressing functions, all contributing to uncontrolled growth. Cancer gene functions can be classified into six essential alterations in cell physiology, including self-sufficiency in growth signals, insensitivity to growth inhibitory signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis . It is now clear that the genetic abnormalities found in cancers will not provide the complete picture of genomic alterations. Epigenetic changes, mainly DNA methylation and, more recently, modification of histones, are now recognized as additional mechanisms contributing to the malignant phenotype. The study of these epigenetic changes on a genome-wide scale is referred to as epigenomics (75).

Hypomethylation and hypermethylation are most often used to describe relative states, best understood in relative to an expected, "normal" setting or degree of methylation (i.e. the level of methylation that in seen in nonmalignant, nonaging cells). Hypomethylation and hypermethylation of DNA are relative terms and denote less or more methylation than in some standard DNA. When applied to cancer epigenetic, that standard is normal tissue. However, there are considerable differences in the amounts and distribution of DNA methylation among different vertebrate tissues because DNA methylation is not only species specific but also tissue-specifc (76) .

The discovery of extensive cancer associated DNA hypomethylation in the human genome (77) preceded that of cancer-linked DNA hypermethylation. DNA hypomethylation in cancer often affects more of the genome than does hypermethylation so that net losses of genomic 5-methylcytosine are seen in many human cancers. The biological significance of DNA hypomethylation in cancer is less understood. However, the role in carcinogenesis of cancer linked hypermethylation of transcription control regions is clear because of the consequent transcriptional silencing of genes important for prevention of cancer (tumor suppressor gene).

5. Cyclin A1

The cyclins form a large protein family involved in the regulation of eukaryotic cell cycle. Cyclin binding is a key event required for activation of cyclin-dependent protein kinases (CDKs), which regulate progression between phases of the cell cycle. Several CDKs function at different stages of the cell cycle and the activities of CDKs are regulated by various cofactors and modifying enzymes. The D cyclin-associated CDK4 and CDK 6 are the earliest CDKs, being activated in G1 phase. CDK2 binding to cyclin E and A is then activated before S phase. CDK1 (also known as CDC2) in association with cyclins A and B function at the G_2/M transition (78). Human cyclin A forms complexes with both CDK2 and CDK1 . The activities of CDK2-cyclin A and CDK1-cyclin A are required for entry into S and M phases, respectively (79).

Human cyclin A1 is the second cyclin A type. It was firstly isolated and characterized by Rong Yang, Roberta Morsoetti and H. Phillip Koeffler in 1997. Cyclin A1 has 48% identity with human cyclin A and is located at chromosome 13q12.3-q13, approximately 1000 kb from the sequence-tagged site marker *WI*-3374(12)

Cyclin A1 differs from the cylin A2 (also known as cyclin A), Its expression increases at the entry into S phase of previously synchronized leukemic cells (12). In G_2M, cyclin A1 expression and cyclin A1-CDK2 kinases activity reach their maximum levels, but cyclin A1 is detectable throughout the cell cycle in contrast to cyclin A2 (80).

Cyclin A2 is a key regulator of the cell cycle in mammalian cells. It is ubiquitously expressed and essential for progression through the cell cycle. Cyclin A2 involved in both S phase and G_2M transition through its association with CDKs. Cyclin A2 associated with CDK2 at the onset of DNA replicationin S phase (81) and with CDK1 mainly at G_2M transition (79).

Cyclin A1 expression is tissue-specific, and high levels of expression are restricted to testis in the healthy organism in humans (10) to eggs and early embryos in Xenopus (82), and to the germline in mice (10). Cyclin A1 is expressed shortly before or during the first meiotic division in spermatogenesis (83) , and male cyclin A1 knockout mice are infertile (11). Spermatogenesis is arrested prior to entry into metaphase I associated with inactive cyclin B-CDK1 complexes and therefore loss of M-phase factor activity (84). Cyclin A1 expression is also diminished in patients suffering from infertility (86) . It interacts with the cell cycle regulators E2F and pRB in SAOS-2 cell (80), which indicates a tissue specific role in mitosis. However, the expression throughout the cell cycle rules out a major regulatory role for cyclin A1 in the mitotic cell cycle. The promoter of cyclin A1 is dependent on four Sp1 transactivation sites in a CpG island upstream of the transcriptional start site (87)

Cyclin A1 is supposed to play a role in the pathogenesis of myeloid leukemia, since it is highly expressed in leukemias of myeloid origin (88). Upon induction of myeloid differentiation, cyclin A1 expression decreases (88). Overexpression of murine cyclin A1 in transgenic mice leads to abnormal myelopoiesis in the first months of the

birth as well as to the development of myeloid leukemia at a low frequency. This indicates that cyclin A1 alone is not sufficient to induce transformation but contributes to leukemogenesis (89).

In cancer tissue, elevated levels of cyclin A1 expression have been implicated in acute myeloid leukemia (11), acute lymphoblastic leukemia (90), biphenotypic acute leukemia (91) and also found in male germ cell tumor (92). In solid tumor tissue, cyclin A1 was demonstrated expression high level in poorly differentiated prostate cancer (90) and p53-apoptosis sensitive bladder cancer cell line (93). In HeLa cell which is known HPV 18 infected cervical cancer cell lines was reported expression of cyclin A1 by RTPCR technique but not express by northern blot technique (12)

In normal tissue, the methylation of cyclin A1 promoter was methylated in kidney, colon, spleen, testis, colon and small intestine, but not in brain, liver, pancreas or heart. (Figure 5). Expression of cyclin A1 was present in spleen, prostate, leukocyte, colon and thymus. (Figure 6) by real time RTPCR technique (94).

The interesting function of cyclin A1 is that its involved in DNA double strand break repair. After irradiation, cyclin A1 was induced by p53 on the transcriptional level. In addition, they identified the Ku70 DNA repair protein as a binding partner and substrate of the cyclin A1-CDK2 complex. DNA double strand break repair was deficient in *CCNA1*$^{-/-}$ cells. They found that both cyclin A1 and cyclin A2 enhance DNA double strand break repair by homologous recombination, but only cyclin A1 significantly activated nonhomologous end joining.(16)

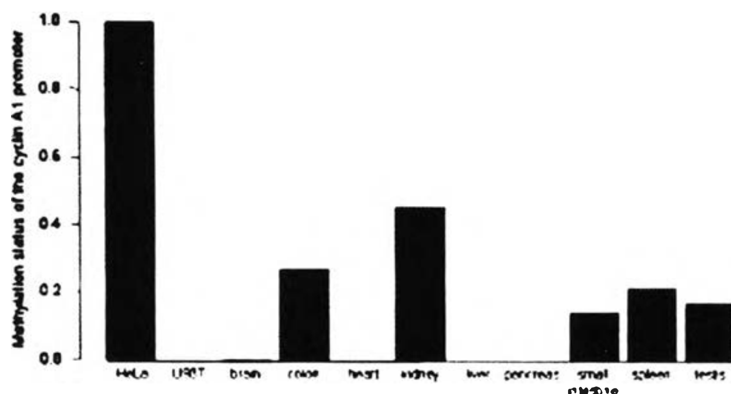

Figure 5. *CCNA1* promoter methylation status in human organs. The methylation status of the CCNA1 promoter was analyses in the different human organ by real-time PCR technique. The degree of CpG methylation was calculated according to the formula Fraction of methylated molecules= 2(threshold non-specific primers minus threshold methylation sqecific primers)

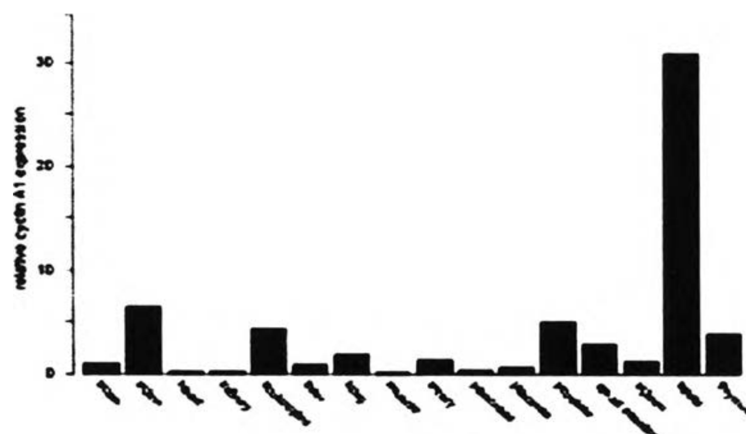

Figure 6. Cyclin A1 expression in human organs. Expression levels of cyclin A1 were analyzed in a panel of different human cDNAs by real-time quantitative PCR. Expression levels were standardized using expression of the house keeping gene GAPDH. (Figure 5 and 6 from Muller-Tidow C, Bornemann C, Diederichs S, Westermann A, Klumpen S, Zuo P, Wang W, Berdel WE, Serve H. Analyses of the genomic methylation status of the human cyclin A1 promoter by a novel real-time PCR-based methodology. FEBS Lett. 2001;490(1-2):75-8.)