Cervical cancer, is a disease that has the potential to be prevented, and is still the second most common cancer that becomes progressively worse in women worldwide. Human papillomavirus is the single most important disease causing agent in cervical cancer. HPV contributes to abnormal progression of cells through the action of two viral oncoproteins E6 and E7, which interfere with critical cell cycle pathways, p53, and retinoblastoma. The p53 gene like the Rb gene, is a tumor suppressor gene, i.e., its activity stops the formation of tumors. However, mutations in p53 are found in most tumor types, and so leads to the complex network of molecular events leading to tumor formation. Primary HPV positive anogenital cancers normally develop without somatic mutation within the p53 gene. In this study, however, we have identified p53 point mutations in metastases arising from HPV positive cervical carcinomas. The association of p53 mutation with metastases may explain the poor prognosis reported for HPV negative primary cancers, many of which already contain mutant p53. A high proportion of p53 mutations detected in both primary and metastatic cancers are GC-->TA transversions, strongly suggesting a role for external carcinogens in the development of these cancers. The present article discusses about the mutation in p53, which can cause metastases in primary cervical cancer.
uneffective regulation of cell growth and cell division also by apoptosis i.e. programmed cell death. Hence there is continuous division of cells causing aggregates of DNA damaged cells called Tumors. Further we are about to study the etiology, epidemiology, mutation and metastases in cervical cancer.

CERVICAL CANCER

The cervix is the narrow neck like passage forming lower end of the uterus, where it contacts the vagina. Each year in US around 12,000 women are affected by cancer of the uterine cervix. An infectious agent, the human papillomavirus (HPV) is the cause of most cases of the cervical cancer. Detection in the early stages is highly curable. [3].

There are usually no symptoms or signs observed at the early stages. As the cancer grows, symptom like abnormal vaginal bleeding can be seen. Abnormal vaginal bleeding is bleeding that occurs between periods, during sex, or after menopause. Pain during sex and vaginal discharge are other possible symptoms of cervical cancer [3].

HPV: Top Cause of Cervical Cancer

The human papillomaviruses (HPVs) are a large group of viruses, about 40 of which can infect the human genital tract. Some HPVs are known to cause cervical cancers, while others cause genital warts. Most genital HPV infections go away on their own, but chronic stages can cause precancerous and cancerous changes in the cells that line the uterine cervix. Over 90% of cervical cancers are caused by HPV infection [3].

Fig.1- General structure of HPV [3].

HPV Cause Cervical Cancer

The changes in the cells of the cervix of high-risk HPVs can thus cause cancer. These are initially precancerous changes that can be recognized with screening tests. With time, the precancerous cells can develop into cancer cells. After cancer has developed it spreads within the cervix and eventually to the surrounding areas [3].

HPV Symptoms

The types of HPVs that cause genital warts are different from those that cause cervical cancer. Genital warts are not precancerous lesions and will not develop into cervical cancer. The "high-risk" or potentially cancer causing types of HPV can stay in the body for years without causing symptoms. Most infections, however, go away on their own and do not cause cellular changes [3].

Peoples at Risk for HPV

HPV infection is extremely common in most men and women who had sex, they will contract the infection at some point in life. Also in some people, the infection persists for years, even if they are not sexually active. HPV is also known to cause cancers in other areas of the body, including the penis, anal area, vulva, vagina, and oral cavity [3].

Other Risk Factors for Cervical Cancer

Women of African, American ethnicity have a higher risk of cervical cancer than European, Asian women. Other factors that increase the risk of cervical cancer include: smoking, long term use of oral contraceptive pills, having many children, having HIV or a weakened immune system [3].

Early detection tests for cervical cancer are Pap Test and HPV DNA Test [3].

Pap Test for Early Detection

A Pap smear (also known as the Pap test) is a medical procedure in which a sample of cells from a woman’s cervix (the end of the uterus that extends into the vagina) is collected and spread (smear) on a microscope slide. The cells are examined under a microscope in order to look for pre-malignant (before-cancer) or malignant (cancer) changes. A Pap smear is a simple, quick, and relatively painless screening test [4].

The observed results are classified as follows

- Ascus or agus: This result means there are atypical cells of uncertain significance caused by HPV that may lead to cancer [5].
- LSIL (low-grade dysplasia) or HSIL (high-grade dysplasia): This means precancerous changes are likely to be present. The risk of cervical cancer is greater with HSIL [5].
- Carcinoma in situ (CIS): This result usually means the abnormal changes are likely to lead to cervical cancer [5].
- Atypical squamous cells (ASC–H): Abnormal changes have been found and may be HSIL [5].
- Atypical glandular cells (AGC): Cell changes that may lead to cancer are seen in the upper part of the cervical canal or inside the uterus [5].
- When a Pap smear shows abnormal changes, further testing or follow-up is needed. The next step depends on the results of the Pap smear, your previous history of Pap smears, and risk factors you may have for cervical cancer [5]. Follow-up testing includes Colposcopy-directed biopsy. For minor cell changes, a repeat Pap smear in 3-6 months is recommended [5].

Considerations: Most of the time, cervical cancer develops very slowly and follow-up Pap smears should identify worrisome changes in time for treatment hence, pap smears are not 100% accurate [5].
**HPV DNA TEST:** Cervical cancers are associated with persistent infection with oncogenic human papillomavirus (high risk HPV). A high risk HPV DNA test detect the presence of oncogenic strains of HPV in cervical cells. It will only detect an HPV infection that is present at the time of the test. HPV DNA testing does not detect abnormal changes in the cervix.

**Cervical Cancer Stages:**

The stage of cervical cancer refers to the extent to which it has spread. Stage 0 means that the cancer cells are found on the surface of the cervix, and stage I means the cancer is localized to the cervix. Spread to the upper part of the vagina signals the stage II cancer. Stage III tumors extend to the lower vagina, and in Stage IV, the tumor has spread to the bladder or rectum, or to distant sites in the body [4].

There are different types of treatment available for different stages of cervical cancers:

- **Surgery (only till stage II this can be done):** The uterus is removed.
- **Radiation therapy:** There are two types of radiation therapy; external radiation therapy (used to destroy cancer cells that may remain after surgery) and internal radiation therapy (placement of radioactive material inside the tumor itself to destroy cancer cells).
- **Chemotherapy:** This treatment is used mainly if cervical cancer is spread to different sites of the body. In this treatment toxic drugs are used to kill cancer cells. (Chemotherapy as well as radiation therapy has many side effects) [4].

**TUMOR PROTEIN P53**

The structure of the core domain of the p53 protein (light blue) bound to DNA (dark blue). The six most frequently mutated amino acids in human cancers are shown in yellow—all are residues important for p53 binding to DNA. The red ball is the zinc atom [6].

The p53 gene, like the Rbgene, is a tumor suppressor gene, i.e., its activity stops the formation of tumors. p53 (also known as protein 53 or tumor protein 53), is a tumor suppressor protein that in humans is encoded by the TP53 gene. p53 is crucial in multicellular organisms, where it regulates the cell cycle and, thus, functions as a tumor suppressor that is involved in preventing cancer. As such, p53 has been described as "the guardian of the genome" because of its role in conserving stability by preventing genome mutation [7].

**LOCATION OF P53**

Cytogenetic Location: 17p13.1

Molecular Location on chromosome 17: base pairs 7,571,719 to 7,590,867

**FIG.4- CHROMOSOMAL STRUCTURE OF P53 [7].**
The TP53 gene is located on the short (p) arm of chromosome 17 at position 13.1.

More precisely, the TP53 gene is located from base pair 7,571,719 to base pair 7,590,867 on chromosome 17, this can be seen in fig. 4. It is estimated that p53 mutations are the most frequent genetic events in human cancers, accounting for more than 50% of the human cancers [7].

FUNCTION OF P53: p53 has many mechanisms of anticancer function, and plays a role in apoptosis (programmed cell death), genomic stability, and inhibition of angiogenesis. In its anti-cancer role, p53 works through several mechanisms (refer to fig. 5):

- It can activate DNA repair proteins when DNA is damaged.
- It can arrest growth by holding the cell cycle at the G1/S regulation point on DNA damage recognition (if it holds the cell here for long enough, the DNA repair proteins will have time to fix the damage and the cell will be allowed to continue the cell cycle).
- It can initiate apoptosis, the programmed cell death, if DNA damage proves to be irreparable [7].

p53 pathway: In a normal cell p53 is inactivated by its negative regulator, mdm2. Upon DNA damage or other stresses, various pathways will lead to the dissociation of the p53 and mdm2 complex. Once activated, p53 will induce a cell cycle arrest to allow either repair and survival of the cell or apoptosis to discard the damaged cell. How p53 makes this choice is currently unknown [7].

HPV E6 proteins

HPV contains E6 proteins that are approximately 150 amino acid polypeptides having an apparent molecular mass of approx. 18 kD. The most characteristic feature of all HPV E6 proteins is the presence of four Cys-X-X-Cys motifs which permit the formation of two zinc fingers [9][10][11]. In the case of the high-risk mucosal HPV types contains a PDZ-binding motif [12] that, in turn, contains an overlapping protein kinase A (PKA) site [13].

CORRELATION AND DEGRADATION BETWEEN HPV PROTEIN AND P53:

Degradation and inactivation of tumor suppressor p53 and pRb HPV E6 and E7 [14].

The p53 gene has been mapped to chromosome 17. In the cell, p53 protein binds DNA, which in turn stimulates the expression of p21 that interacts with a cell division-stimulating protein (cdk2). When p21 is complexed with cdk2 the cell cannot pass through to the next stage of cell division. Mutant p53 can no longer bind DNA in an effective way, and as a consequence the p21 protein is not made available to act as the 'stop signal' for cell division. Thus cells divide uncontrollably, and form tumors (refer to fig. 6).
HPV E6 and E7 binding to P53 and pRb interfering with the normal functioning of the tumor suppressors.

Initially p53 was identified through its association with another DNA tumor virus protein, SV40 Tag [15][16]. The interactions by their association result in the formation of stable complexes between the viral proteins and p53. On the other hand, initial detection of p53 HPV-transformed cell lines, such as HeLa cells was unsuccessful, although high levels of p53 mRNA were detected [17][18]. Werness et al., (1990) [19] showed that the E6 proteins from HPV-16 and HPV-18 are able to bind to p53, and it was further promotes the degradation of p53 (refer to fig.7) via the ubiquitin pathway [20]. And this E6-mediated degradation of p53 is dependent upon a cellular protein, E6-associated protein (E6-AP), also known as UBE3A, [21][22][23]. It has been assumed that the degradation of p53 contributes to the oncogenic potential of the high risk HPVs, although several studies have shown that mutants of E6, defective in their ability to induce the degradation of p53, can still immortalize human embryonic cells[24][25]. Thus, although it seems improbable that E6-induced degradation of p53 has no role in the development of HPV-associated malignancies, other functions of E6 must also be involved[14].

Viral DNA replication and p53

It is not yet clear that whether p53 is present in viral replication complexes inhibiting replication or the virus has recruited p53 to assist replication. However, HPV requires DNA polymerase for its own DNA replication [26][27][28], and the intrinsic 3' - 5' exonuclease activity of p53 is able to enhance the replicative fidelity of polymerase [29]. Hence p53 may have been recruited by the virus in a proofreading capacity. It also appears that stimuli which activate p53’s sequence-specific DNA binding activity may inhibit the 3' - 5' exonuclease activity [30] suggesting that, when ‘non-activated’, p53 may be active in cooperating with polymerase to effect DNA repair [31]. So, p53 appears to involve with a large number of different DNA tumor viruses at the level of viral DNA replication and may be layers of complexity in the E6-p53 relationship[14].

CAUSES OF MUTATION IN P53

HPVs have circular, double-stranded DNA genomes that are approximately 8 kb in size and encode eight genes, of which E6 and E7 have transforming properties. These proteins have pleiotropic functions, such as transmembrane signaling, regulation of the cell cycle, transformation of established cell lines, immortalization of primary cell line and regulation of chromosomal stability. The viral E6 and E7 oncoproteins are necessary for malignant conversion. The abilities of high-risk HPV E6 and E7 proteins to associate with the tumor suppressors p53 and pRB, respectively, have been suggested as a mechanism by which these viral proteins induce tumors[32].

1. BY E6 ONCOPROTEINS

The E6 protein consists of 158 amino acid residues and contains two zinc-finger binding motifs [32]. The E6 protein is thought to promote cell proliferation by stimulating degradation of the tumor suppressor p53 protein via the formation of a trimeric complex comprising E6, p53 and the cellular ubiquitination enzyme E6-AP. E6-stimulated degradation interferes with such biological functions of p53; thus perturbing the control of cell cycle progression, leading finally to increased tumor cell growth [33]. Although it is commonly accepted that the ability of high-risk type HPV E6 to target p53 for degradation contributes to virus-induced MUTATION, it is also clear that the E6 protein has oncogenic activities that are independent of p53[14].

2. BY E7 ONCOPROTEINS:

E7 binds to a region of the Rb protein commonly referred to as the 'pocket domains' [34]. The 'pocket domain' sequences of Rb are essential for its tumor suppressor function, with many naturally occurring loss-of-function mutations of Rb appearing to cluster in these 'pocket domains'. One of the major biochemical functions of Rb is to bind E2F-family transcription factors and repress the expressions of replication enzyme genes [35]. The ability to repress the expressions of replication enzyme genes correlates with the tumor suppression function of Rb. E7 disrupts the interaction between Rb and E2F, resulting in the release of E2F factors in their transcriptionally active forms [36]This E7-mediated conversion of E2Fs to their activator forms stimulates replication and cell division, which is consistent [14].

HPV E6 and E7 binding to P53, with Rb interfering with the normal functions of the tumor suppressors.

Fig.8- Indirect inactivation of p53. [9]
The E6 viral protein expressed by HPV specifically binds to the p53 protein and induces its degradation [20]. This observation explains the rarity of p53 mutations in cervical cancers [41]. p53 inactivation by a viral protein has not been formally demonstrated in other human cancers associated with viral infection, such as HCC (associated with HBV) or Burkitt lymphoma (associated with EBV).

**p53 Mutation:**

Fig.9- The worldwide distribution of cancers and p53 mutations. [9]

Impact on Protein Structure

The p53 DBD is made of an immunoglobulin-like β-sandwich of two antiparallel β-sheets, providing a scaffold for a flexible DNA-binding surface [6]. Mutant proteins have thus been classified as "contact" (e.g., R248 and R273) or "structural" (e.g., R175, G245, R249, and R282) [38]. DNA-contact mutants retain the overall architecture of the DBD with loss of a critical DNA contact. Structural mutants cause distortions that create internal cavities or surface crevices in the protein scaffold, inducing conformational changes in the DNA binding surface.

Impact on Transcriptional Activities

p53 regulates transcription through specific binding to response elements in the promoters or introns of target genes [39].

**Dominant–Negative and Gain-of-Function Effects**

Mutant p53 proteins often accumulate in the nucleus of in situ and metastatic cancer cells, suggesting an oncogenic effect in addition to loss of wild-type suppressor function. Experimentally, several hotspot mutants have been shown to cooperate with oncoproteins for cellular transformation [40], setting the concept of gain-of-function (GOF). Alternatively, mutant p53 proteins may exert dominant–negative effects (DNE) over wild-type p53. Missense mutations in p53 DBD are only slightly overrepresented in cancer, whereas nonsense mutations are overrepresented by a few factors and silent mutations underrepresented by some factors. Loss of function is the critical factor for the selection of mutations in cancer.

Fig.10- The invasion and metastasis in cervical cancer (www.cancer.gov)

The E6 protein consists of 158 amino acid residues and contains two zinc-finger binding motifs [32]. The E6 protein is thought to promote cell proliferation by stimulating degradation of the tumor suppressor p53 protein via the formation of a trimeric complex comprising E6, p53 and the cellular ubiquitination enzyme E6-AP. E6-stimulated degradation interferes with such biological functions of p53; thus perturbing the control of cell cycle progression, leading finally to increased tumor cell growth [33]. Although it is commonly accepted that the ability of high-risk type HPV E6 to target p53 for degradation contributes to virus-induced MUTATION, it is also clear that the E6 protein has oncogenic activities that are independent of p53 [14].

Invasion and Metastasis

The normal cells are invaded by cancerous cells whereas metastasis is caused by the inactivation or the degradation of p53. Invasion refers to the direct migration and penetration by cancer cells into neighboring tissues. Metastasis refers to the ability of cancer cells to penetrate into lymphatic and blood vessels, circulate through the bloodstream, and then invade normal tissues elsewhere in the body.

**METASTASIS**

P53 mutant detected in metastasis from HPV positive cervical cancer

The metastatic tumor cells of cervix contains only mutant p53 sequence and are either homozygous or hemizygous for the mutation. Detection of mutant p53 in small foci of cells arising within a wild type p53 tumor has been known [37]. In a study, the fourth metastasis contained point mutation of the p53 sequence resulting in single amino acid substitution codons 173 (val → leu), 175 (arg → pro) and 181 (arg → leu). In all three cases multiple sequencing strand revealed a strong band at the position of the mutant nucleotide with a weaker band corresponding to the wild type p53 sequence at the same location. The metastatic progression of
HPV positive primary cervical cancers is frequently accompanied by mutation within p53 sequences.[41] Certain point mutations within p53 activate a dominant transforming activity leading to the inactivation of wild-type suppressor function.

Mutation within the p53 gene in an HPV positive primary cancer might confer a growth advantage and contribute to the acquisition of metastatic potential in these cells. One might have important prognostic value for predicting metastatic progression is for identification of such cells in primary cancers [42].

Experimental analysis of p53 mutations

Most p53 mutations are detected by DNA sequencing, immune histochemical analysis, NMR spectroscopy etc. However; it is known that single missense mutations can have a large spectrum from rather mild to very severe functional effects [35].

Conclusion: The major role is of the E6 oncoprotein, which in conjunction with the hijacked E6-AP restricts the activities of p53 and acquires a successful replication of HPV and unfortunately to tumour progression. Hence the E6-P53 associated complex is a target to control and eradicate dangerous human cancers.

Small mutations like the missense, nonsense mutation on insertion deletion of several nucleotides is essential to inactivate the function of p53 and thus can lead to the expression of the mutant protein that causes tumor or can lead to the absence of protein. Thereby we conclude that mutation in p53 can lead to primary, metastatic cervical cancer. Imbalance in the levels of p53 by the virus can make the cells immortal leading to the formation of tumors.

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