

Understanding Chromosomal Aberrations in Cervical Cancer: Key to Potential Biomarkers

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Abstract: *Cancer continues to be one of the leading causes of death worldwide. In 2020, it accounted for 10 million deaths worldwide with lung and colorectal cancers among the most common ones. Cervical cancer is the fourth leading gynaecological cancer in the world. Research strongly suggests the presence of chromosomal abnormalities in the form of chromosomal instability (CI) and chromosome structural rearrangements in almost all cancers. This review paper explores the genetics and chromosomal aberrations in cancer cells with a specific emphasis on cervical cancer. The paper also discusses several genetic and chromosomal biomarkers in cervical cancer like gain of 3q26 and Loss of Heterozygosity (LOH) at 6p21.2 and 18q21.2 and proposes the possibility of 8q24 translocation breaks as a convenient and quick diagnostic method to detect cervical cancer caused by HPV-18.*

Keywords: aneuploidy; biomarkers; cervical cancer; chromosomal aberrations; chromosome instability

1. Introduction

Although the prevalence of cancer across various countries has remained relatively constant or, in some countries, declining as well, (1) the rates are still quite high. Cancer still remains one of the leading causes of deaths in the world with 19.29 million new cases of cancer diagnosed in 2020 and 9.958 million deaths due to cancers of trachea, bronchus, lung, skin and cervix (2). Most human tumours are known to have clonal chromosomal abnormalities (3). These abnormalities are capable of regulating protein and RNA expression as well as function (4-6) Chromosomal abnormalities found in cancer cells chiefly comprise of aneuploidy (loss or gain of complete chromosomes) and chromosomal structural rearrangement. Chromosomal Instability (CI) occurs when chromosomes do not segregate properly during the process of cell division. CI leads to aneuploidy thus causing abnormal number of chromosomes per cell. Disturbance in mitotic fidelity leads to CI and aneuploidy, which have correlation with poor prognosis, therapeutic resistance and metastasis (7). Aneuploidy in terms of loss or gain of at least 1 chromosome has been detected in almost 90% of tumours (8). Most solid tumours and almost half of lymphomas and leukaemia, show aneuploidy (9).

Chromosomal rearrangements are a result of chromosome structure instability caused due to inefficient DNA damage repair (9). This damaged DNA differs from the original DNA as during the structural change, some genes are repressed or lost. Both solid tumours and blood cancers show structural defects in chromosomes.

1.1 Chromosomal Instability and Aneuploidy

In case of stable, diploid cell lines, 1 chromosome gets mis-segregated per 100 cell divisions, which does not happen with cancer cells (9). During division of cells in cancer cell line showing CI, mis-segregation of chromosome occurs

once every 1 to 5 cell divisions (10). CI can be achieved through changes in proteins involved in mitotic spindle checkpoint and cohesion of sister chromatids. It is worth mentioning that in case of human cancers, CI generally does not occur due to improper checkpoint regulation, but due to an error in attachment of kinetochore microtubules (kMT), merotelic (9). Merotelic condition is the condition in which kinetochore microtubules from opposite poles simultaneously attach to a single kinetochore and hence hinder proper segregation process. Moreover, they are able to surpass the regulatory effects of proteins (Mad2) involved in spindle assembly checkpoint. This happens since the spindle assembly checkpoint is based upon the fact that whether all kinetochores are attached to the MT or not, they do not take into consideration the number of MT attached per kinetochore. In merotelic attachment, the microtubule attaches to the kinetochore and hence the Mad2 is inactivated, further activating CDC20 and APC/C ubiquitin ligase, which degrades securin and hence promotes anaphase. The rate of merotelic present during the anaphase stage depends on the overall turnover, i.e., the attachment/detachment dynamics. Merotelic errors are corrected by detaching the mis-attached microtubule from the kinetochore (9). Studies demonstrate that cancer cells are less capable of correcting any errors in attachment of kMTs and thus lead to frequent CI in tumours (11). Increase in merotelic rates may be as a result of depletion or upregulation of kinetochore proteins like MCAK, Kif2b, Aurora B, adenomatous polyposis coli, CENP-E, CLASP, NDC80 complex, Mps1, and Mad2 (9).

Other factor which may lead to CI and merotelic is the increase in the number of centrosomes per cell (9). It is well documented that ideally two centrosomes are found in each cell during the process of cell division which give rise to the spindle fibres. Hence increasing number of centrosomes per cell may lead to two things: firstly, it will enhance the chances of more than one microtubule attaching to a kinetochore from each pole; secondly, the attachment may also raise geometric constraints. Although this is a very rare

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case, and this error is corrected very quickly, the inter-transition state is what needs to be tackled.

Furthermore, RNA sequence analysis showed that these CI-high cells enriched for mesenchymal genes including the ones associated with metastasis and epithelial-to-mesenchymal transition (EMT) (12). This reveals that high CI is directly or indirectly associated with tumour metastasis (12).

1.2 Chromosomal Aberrations in Cancer Cells

Chromosomal aberrations refer to the rearrangements/changes in the genomic sequence. In case of cancer, these rearrangements inhibit the regular mechanisms of cell regulation in terms of cell division, cell death, metabolism etc. and simultaneously may even promote the uncontrolled division of cells. These rearrangements may occur in numerous ways like reciprocal translocations, inversions and insertions (13). A probe-hybridisation based method, spectral karyotyping (SKY) allows the investigation of chromosomal rearrangements, translocations and identification of marker chromosomes which are unresolvable by G-band karyotypic

analysis (14). The method of SKY allows the characterization of chromosomal aberrations in solid tumours, if the deletion in the chromosome is more than 20 MB (14). Smaller chromosomal rearrangements can be observed with the help of array comparative genomic hybridization (9).

Translocations are a result of improper DNA repair. While developing, tumours often go through a breakage-fusion-bridge cycle (15). This happens when the length of telomeric sequence becomes less than a critical threshold, and hence they are no longer capable of preventing chromosomes from fusing into each other, thus resulting in the formation of dicentric chromosomes. These then form bridges between daughter cells and most often, break during abscission. The breakage is repaired using DNA repair mechanisms, resulting in generation of chromosomal translocations. Cancer cells possess the ability to overcome this breakage-fusion-bridge cycle, via the activation of telomerase, but this happens only after a couple of cycles and hence cannot control the development of CSI. Some of the chromosomal aberration and their effects on cancer are listed in Table 1.

Table 1: List of chromosomal aberration and their effects on cancer

Chromosome arm	Chromosomal Aberrations	Effect	References
8p	Deletion	Deletion is higher in case of stage 3 cancers. It mostly leads to poor prognosis in case of breast cancer.	(15)
17p	Deletion	It leads to chronic lymphocytic leukemia. Results in rapid progression and poor response to chemotherapeutics.	(58)
3p	Deletion	Found commonly in uterine cervical carcinoma	(59)
8q	Amplification	Seen often during later stages of pancreatic cancer	(60)
1q	Amplification	High risk of myeloma. It is also associated with Hepatocellular carcinoma.	(61)
11q, 17q, 20q	Amplification	Associated with pancreatic cancer	(60)
7p, 8q, 13q, and 20q	Amplification	Found in colorectal cancer	(62)

2. Chromosomal Abnormalities in Cervical Cancer

As per WHO report (2020), cervical cancer is the fourth leading cancer in women worldwide. 604,127 new cases were detected in 2020 (3.1% of total cancer cases in 2020) (2). Cervical cancer affects the cells of the lowermost part of the uterus, i.e. the cervix. It is caused because of Human Papillomavirus (HPV) and is transmitted through sexual

contact. Out of the 200 types of HPV strains, approximately 40 types infect the genitals and around 15 strains cause abnormal cell changes in the cervix. While most strains are responsible for low grade HPV infections (HPV 6, 11, 40, 42, 43, 44, 54), types 16 and 18 are considered to be high-risk types. Other high risk HPV types include: HPV 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 and 70. They are responsible for cervical cell lesions. The HPV genome can be divided into three regions as shown in Table 2.

Table 2: Regions of HPV genome

Upstream regulatory region (URR)/ long control region (LCR)	It comprises of 1/8 th of the entire viral genome. Aids in viral replication and transcription.
Early region (E) comprising of E1, E2, E4, E5, E6, E7	Involved in crucial functions life replication, gene expression, immune evasion and persistence of the genome.
E3, E8	Found in only a limited number of HPV strains like HPV 1, 11, 16, 31 and 33 (21)
Late region (L)	L1- codes for minor capsid L2- codes for major capsid

It has been observed that 99.7% cervical squamous cell cancer cases involve the presence of HPV(16). Once the host gets infected with the papillomavirus, initially, random integration of linear viral genome into the cellular genome takes place. However, integration may also occur at specific recurrent chromosomal locations within the human genome, like 8q24 and 12q15 (17). Genome sequences studies

indicate that the integration sometimes takes place near the c-myc oncogene, which is located on chromosome 8 (18). The integration event partially or totally inactivates the E1 and E2 proteins of the early region. One of the functions of the E2 is to repress the E6 and E7 expression, hence its inactivation is a crucial marker which upregulates onco-proteins E6 and E7. The early region E6 is responsible for binding to and

degrading the tumour-suppressor protein p53, disturbing the DNA repair pathway and terminating the apoptotic process (19). The E7 protein causes the degradation of retinoblastoma protein pRb via the ubiquitin-mediated proteolytic pathway (20) along with the cells' re-entry into S-phase of the cell cycle and upregulation of p16 expression. The interaction of HPV-16/E7 with the dephosphorylated pRb (21) leads to release of various growth factors including cyclins (example: cyclin-E) (19), cyclin dependant kinases

and E2F-1 (22). Therefore, making it quite evident that the expression of E6 and E7 viral proteins gives a survival benefit to HPV and promotes its replication in the suprabasal epithelial layer (23).

The stages of cervical cancer development are depicted in Figure 1.

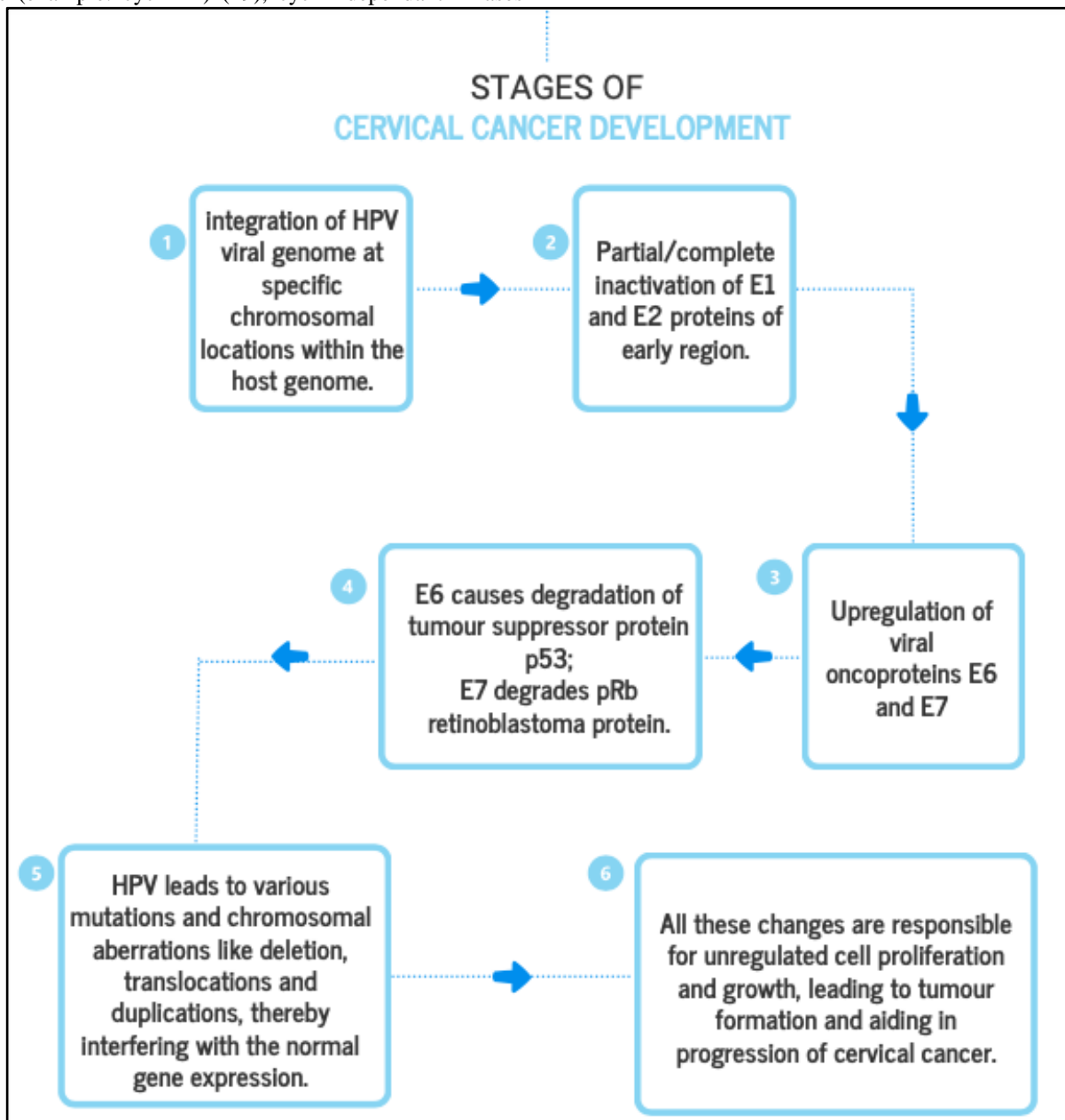


Figure 1: Stages of cervical cancer development. The figure depicts various stages of cervical cancer development

Multiple studies are indicative of the fact that HPV proteins have an interaction with human proteins involved in DNA damage repair. Also, the HPV-16/E2 interaction with topoisomerase 2-binding protein 1, TopBP1, promotes viral DNA replication as well as transcription (24). Furthermore, E2 is responsible for activating the death domain receptor (DDR) which helps in viral replication and amplification. The loss of E2 has been correlated to poor survival and reduced patient response to chemotherapeutics.

Other effects of E6 include interfering with DNA lesion repair by targeting X-ray repair cross-complementing protein 1 (XRCC1) (25); causing accumulation of centrosomes etc. In addition to this, the oncoprotein E6 delays senescence by upregulating the expression of telomerase reverse transcriptase (hTERT) (23). The human papillomavirus E7 protein reduces the activity of p21 and p27 cyclin dependant kinase inhibitors and hence leads to progression of the cell cycle. Moreover, HPV-16/E7 restrains duplication of the centrosome. It also leads to the weakening of the DNA damage checkpoint by claspin degradation (26). Therefore, it

is apparent that the high levels of E6 and E7 viral oncoproteins causes impairment of regulatory checkpoints, reduced apoptotic rates, increase in DNA damage (by effecting damage repair). These result in abnormal number of centrosomes, multipolar mitosis, misalignment of chromosomes and anaphase bridges thus leading to genomic instability.

Cervical cancer cells display numerous chromosomal abnormalities as shown in Table 3. Loss of heterozygosity (LOH) is an early event that occurs frequently during cervical squamous cell carcinoma development (27). Allelotype analysis of cervical cancer cells suggest that LOH frequently occurs in the following chromosome locations: 3p14-22, 4p16, 4q21-25, 5p13-15, 6p21.2, 6p21.3, 6p22, 6p25, 11p15.5, 11q23.3, 11q25, 17p13.3, 1812.2-22

(17, 28, 29).3q+ arm, i.e. the region 3q24-28, has found to be amplified in almost 90% of invasive carcinomas (17).A study found that 6 out of 8 markers have greater than 20% LOH frequency at locations 1p36.1 to 1p36.33 on chromosome 1 (27). Only about 7% of the cervical cancer cells show the presence of microsatellite instability (17).Frequent point mutations seen in cells with cervical carcinoma include mutations in H-Ras codon 12 (G → A; guanine to adenine transition), (30) epidermal growth factor receptor EGFR (in exon 18) (31) and p16^{INK4a}. p16^{INK4a}, a tumour suppressor protein, is induced in cells when HPV-E7 interacts with pRb. And its overexpression was reported to mark a better prognosis in cervical cancer (32).It has been reported that H-Ras mutation in cervical cancer does not have much prognostic significance (30).

Table 3: Chromosomal abnormalities frequently observed in cervical cancer cells

Chromosomes	Chromosomal abnormality	References
3q, 1q, 5p, 6p, 7p, 7q, 8q, 9q, 16q, 20p, 20q, 1p	Gain	(17, 28, 41)
4p, 2q, 3p, 4q, 5q, 6q, 11q, 13q and 18q	Loss	(17, 28, 41)

When HPV attacks the epithelial host cells, an immune response is activated. Toll-like receptors (TLR) belong to the family of pattern recognition receptors, well known for their involvement in defending the body against infection (33).TLR signalling pathway eventually activates transcription factors (like NF-κB), interferon response factor-3, along with MHC-I and II (34).HPV-E5 and E7 are capable of repressing MHC-I response (35). Higher the expression of TLR, higher is the level of carcinoma infection. E6 and E7 help in downregulating the expression of TLR, and hence can survive host immune response.

The most important feature of cancer cells is the uncontrolled cell division. Mini-chromosome maintenance

proteins assist in initiation of DNA replication (36). These MCM proteins (MCM2, 4, 5, 6, 10) are found to be overexpressed in case of cervical cancer. The occurrence of over-expression of MCM2, 4 and 10, increases with increased tumour stage (37). Microtubule nucleation factor TPX2 helps in the development of spindle fibres. Therefore, its overexpression results in increased number of centrosomes, aneuploidy and malignancy in tumours (38).Also, upregulation of CCNA2 and CCNB1 cyclin proteins and associated kinases (CHEK1 and CDK1) is reported in cervical cancer cells, which help in the G1-S as well as G2-M transition (39).Some other chromosomal abnormalities observed in the cervical cancer cells are summarised Table 4.

Table 4: Various other chromosomal abnormalities observed in the cervical cancer cells are summarised below

Genes involved	Abnormality	Function of gene	Effect of abnormality	References
LKB1	Homozygous deletion that affects part of or the entire LKB1 gene.	Codes for Serine/ threonine kinase; acts as a cervical tumour suppressor	Homozygous deletion may also result in down regulation of neighbouring genes SBNO2 and c19orf26, and leads to formation of LKB1 fusion transcript.	(63, 64)
FGFR3-TACC3	FGFR3-TACC3 fusion	FGFR plays an important role in cellular processes like cell growth. Need a fibroblast growth factor (FGF) ligand for activation. TACC3 is required of mitotic spindle stability.	Leads to up regulation of PI3K/AKT, RAS/MAPK and STAT pathways along with other pathways responsible for chaperon activation and stress response.	(65-67)
FGFR	Translocation	FGFR belongs to the family of Receptor Tyrosine Kinase; helps in cell proliferation and differentiation.	FGRF signalling via overexpression, point mutations, chromosomal translocations plays a role in increasing cell proliferation, angiogenesis and differentiation.	(68)

2.1. Chromosomal and Gene Biomarkers for Cervical Cancer Diagnosis

Cervical Intraepithelial Neoplasia (CIN) is a precancerous condition in which abnormal cells grow on the surface of the cervix. Usually, these changes in CIN grade 1 (CIN1) revert to normal cells in young women because of immune response and rapid turnover of cells on the cervix (40). However, women with CIN-2 and CIN-3 are susceptible to the development of invasive cancer although the progression

takes several years (40). The chromosomal and gene biomarkers help in the prognosis and recognising the stages of cervical cancer progression. It has been observed that various regions are amplified in cervical cancer cells (1q, 3q, 5p and 8q) and gain of 3q has been reported in various cases (41, 42). Telomerase RNA component (TERC) within this region at 3q26 is associated with development of CIN3+ with initial diagnosis of CIN1/2 (43). Therefore this can be used as an efficient marker for determining the patients who need to be referred for further treatment (like colposcopy).

Methylated CDKN2A serves as an important potential biomarker in cervical cancer. Li et al. suggested that a link might be present between abnormal methylation of CDKN2A and cervical cancer carcinogenesis, and thus this may serve as an early biomarker of pathogenesis (44).

Moreover, a potential chromosomal biomarker is focally amplified long non-coding RNA (lncRNA) on chromosome 1 (FALEC). It is found to be up-regulated in cervical cancer cells and is positively correlated with tumour size, metastasis of the lymph node as well as the FIGO stage (45). Koenen *et al.* (46) suggested that gain of 3q26 may be a prognostic marker for high grade Cervical Intraepithelial Neoplasia (CIN). Its absence in CIN indicates a high probability of disease regression. In addition to these, it has been observed that the Loss of Heterozygosity (LOH) at 6p21.2 and 18q21.2 is associated with poor survival rates, both in terms of overall survival and disease free survival after radiotherapy (47). Yang *et al.* (2018) proposed that Colorectal Neoplasia Differentially Expressed (CRNDE) lncRNA may be a novel prognostic predictor as it promotes proliferation and inhibits apoptosis of cervical cancer cells by targeting PI3K/AKT and therefore may be associated with poor prognosis (48). Furthermore, small nucleolar RNA host gene 1 (SNHG1), located on chromosome 11, has found to be upregulated in cervical cancers. It plays a role proliferation, migration and invasion of cancer and therefore it is likely to serve as a prognostic biomarker as well as a target for therapy (49).

A well-established fact is that the integration of HPV genome to the host human genome in case of cervical cancer occurs near the c-Myc oncogene (18). This oncogene is located at chromosome 8q24.21. It has been seen that cervical cancer cells infected by HPV-18 are more likely to have breakpoints in 8q24.21 ($p=7.68 \times 10^{-4}$) (17, 50). Also, these translocation breakpoints in 8q24 due to HPV-18 integration in cervical carcinoma can be located in the distal end of FRA8C (8q24.13) (51). Further, viral integration occurs in 100% of HPV-18 infected cervical cancer cells (52, 53). Although, this integration event happens in late stages of pre-cancer and invasive carcinoma, (54) 8q24 translocation breakpoints may be proposed as a novel biomarker used for diagnosis of HPV-18 infected late cervical cancer progression stages which require immediate action. Study performed by Vodenkova *et al.* indicate that chromosomal aberrations as well as chromatid-type aberrations can act as potential risk markers in the assessment of cancers like lung, breast and colorectal cancers respectively (55). Also, Fanconi anaemia (FA), a rare syndrome, also uses chromosome breakage test for its diagnosis based on metaphasic karyotype analysis (56). The use of chromosome break analysis for diagnosis of FA and various cancers proves that a similar karyotypic analysis to check for 8q24 translocation breaks may serve as a convenient and quick diagnostic method in case of cervical cancer as well.

3. Conclusion

All cancer cells share a common aspect, which is the presence of chromosomal abnormalities. Therefore, an in-depth study of the chromosomal aberrations taking place in cancer cells can give an insight into the relation between

chromosomal changes and tumour initiation/ progression. According to the American Cancer Society, although the 5-year survival rates in case of localised cervical cancer are high (92%), the survival rate decreases significantly in case of distant metastasis (17%) (57). Hence, it is important to diagnose and target cervical cancer at the earliest. Chromosomal and gene biomarkers can help in early detection and diagnosis, leading to improved survival outcomes. We emphasise the need for more research in the identification of genetic and chromosomal biomarkers of cervical cancer. Considering this, the paper proposes identifying 8q24 translocation breaks as a convenient and quick diagnostic method for cervical cancer. However this needs to be further explored and studied.

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Abbreviations

CI, chromosomal instability; LOH, loss of heterozygosity; kMT, kinetochore microtubules; MT, microtubules; TP53, tumour protein p53; HPV, Human Papillomavirus; MCM, Minichromosome maintenance protein; CIN, Cervical Intraepithelial Neoplasia.

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