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# MOLECULAR BASIS OF CERVICAL CANCER: A REVIEW

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## ABSTRACT

Cervical cancer is sexually transmitted disease resulting from the high risk oncogene infection by Human papillomavirus of type 16 and 18. In cervical cancer cells the two viral oncogene E6 and E7 are found to be variably expressed. These genes modulate the molecule involved in innate and adaptive immunity development and also inhibit the major tumor suppressor gene p53 and pRb respectively. Molecular biology had become an encouraging structure for exploring the process of development and progression of cervical cancer. There are many fields of biomedical sciences which had investigated the oncogene and tumor suppressor gene expression from various fields one is genomics which determines the correlation between genetic expressions and pathological features by DNA Microarray technique. There are also some genetic changes which lead to cancer without any alteration in DNA sequence called Epigenetics. It comprises DNA methylation, hypermethylation and hypomethylation beside DNA there is also a major involvement of RNA in developing cancer both coding and non-coding RNA respectively. Majorly non-coding RNA is found to be upregulated in cervical cancer this whole mechanism is studied by the help of transcriptomics. Genes are translated into proteins and any alteration in genes will cause defective translation. Proteomics provides understanding of proteins in large scale and also deregulation in the proteins. Metabolomics is an analytical technique helpful for the patients of cervical cancer in predicting neoadjuvant therapy encompasses all treatments involved before primary treatment of cervical cancer. These defects in molecular mechanism ultimately cause effect in cell signaling and cell cycle. These molecular and biomedical studies could be helpful in understanding the mechanism associated with cervical cancer and also in diagnosis and prognosis of cervical cancer.

**KEYWORDS**: Molecular biology, HPV, Pathogenesis, Transcriptomic, Proteomics, Metabolomics.

## INTRODUCTION

Among several types of cancer cervical cancer has being one of the major concerns arising globally with 57,000 diagnosed cases and in which 311,000 deaths were registered in 2018 by Bray et al This cancer is shown to be declined in many developed countries but still is a main reason of mortality in women around less advanced countries as shown by Torr et al. More than 85% of cervical cancers cases arise from the country have low income. This incompatibility of death rate in between developed and underdeveloped countries is due lack of awareness regarding hygiene screening programs and exposure to risk factors. Cervical cancer begins when healthy cells in the cervix develop changes (mutation in their DNA). Healthy cells grow and multiply accordingly at fixed rate and regulated by cell cycle. Mutation causes cell to grow out of control and they don't die. These abnormal cells accumulate and form mass of abnormal cells called tumor. These abnormal cancerous cells invade nearby cells through metastasis and form cancer. All of the pre-malignant changes that occur at squamocolumnar junction which is a transition zone between ectocervix and endocervix, these changes are associated mainly with HPV16 and 18 high risks HPV. The premalignant changes in squamous cells of cervical epithelium are named as cervical intraepithelial neoplasia (CIN). These CIN if not properly treated initially, HPV associated infection will leads to destructive host cell immune system causes carcinoma in situ or metastasize to form cancer. However, there have been many evidences that HPV associated viral proteins leads to abnormal changes in HPV infected cells they also form premalignant cells which transformed into cancerous cells, CIN are graded into CINI, CINII, and CINIII on the basis of its severity. The epithelial cells of normal cervix are organized very well but the cells of cervix with CIN and HPV infection become abnormal. The mild dysplasia in cervix leads to slight abnormalities on surface of cervix named as low grade CIN1 and the precancerous cells present on cervix with two third of epithelial part gets infected leads to moderate dysplasia CIN2.[1],[2] CIN 2 and 3 associated lesions are grouped into high grade CIN.[3],[4]The major risk factors of cervical cancer beside HPV are smoking, age, parity, oral conceptive use, age at first sexual intercourse exposure to

estrogen diethylstilbestrol and lower socioeconomic status.

The HPV is a major etiological factor for progression of cervical cancer, formation of cervical cancer is initiated by the persistent infection of mainly high risk HPV of type 16 and 18.[5] High risk type perform key role in cause of cervical tumors and low risk cause benign lesions such as warts associated with genital types.[6]

The HPV consist of oncogenes E6 and E7 expression of both genes together greatly enhanced cell transformation and both of these viral genes also modulate molecules involved in immune defense mechanism mainly innate and adaptive immunity to invade immune system, mainly in early stage of E6 and E7 oncogenes replication both inhibit the ability of interferon which is made in response to viral infection. The viral gene E6 cause inhibition of IRF-3 for inducing interferon-beta activation and E7 binds interferon regulatory factor 1 for preventing interferon α and β activation of because activation of interferon will leads to the interference in viral infection which will help in preventing cervical cancer. The oncogenes of HPV E6 and E7 of high risk are important for cellular transformation while HPV of low risk type not interact efficiently. Oncogene E7 of high risk HPV interact with tumor suppressor genes (pRb) and degrade them and also their family proteins (p107, p130) and inactivates Histone deacetylase (HDAC) protein, TATA binding protein. The oncogene E6 targets p53 tumor suppressor gene responsible apoptosis and cell cycle regulation**.**

The HPV infects to host cell leads to activation of innate and adaptive immune system by the Major histocompatibility complex I or II. The MHCI presents antigen to cytotoxic T- cells with CD8+ marker and MHCII present antigen to Helper T-cell with CD4+. This mechanism of HPV infection starts when it invades to epithelial layer which is a primary physical barrier of innate immunity invaded in response of against any type of infection. The immune defensive cells macrophage, Langerhans, Natural Killer cells inhibits the expression of Toll like receptors which caused due to the HPV infection.[7] These TLR receptors recognize the viral component and activate the (NFK-B and IRF-3)for producing cytokine which regulates immunity and inflammation as in response of viral attack.[8] The E5 viral oncogenic protein has ability of inhibiting MHCI and E6 has ability to inhibit TLR which further activates IRF-3.[9] In initial stages of cervical carcinogenesis epithelium cells of cervix undergoing differentiation triggers the TLR receptors regulation which results in antiviral response by IFN regulatory factor.[10] Ghosh et al have also found that the expression of TLR-9 were not same in different stage of cervical cancer development, they have observed lower expression of TLR in CIN-1 as in comparison of CIN2/3 and highest in squamous carcinoma cells group of samples.[11] Therefore constituently expression of oncoproteins E6 and E7 will downregulate TLR-9 and interferon response will be impaired and leads to evasiveness of immune system, which will form persistent HPV infection.[12]

The innate immune system is escaped by HPV and it invades the cells of epithelium and in response dendritic cells engulfs the HPV antigen and starts maturation. The antigen by phagolysosome send to MHC class I or II after that activation of antigen presenting cell (APC) promote the inflammation response and cytokine activation an antiviral response such as TNF-α and IFN-**γ.** This will stimulate macrophage and promotes tumor immunity, also in response of extracellular pathogen interleukins are produced. Therefore, APC provide stimulus for producing regulatory T-cell it will lead in activation ofIL-10 and TGF beta which will further inhibit the APC function. In cervical cancer the transformation of normal to precancerous and cancerous lesions are involved with quantity of regulatory T-cell production which was reported in women with persistent HPV infection.[13]

**Genomics**

Genomics is a branch of Molecular biology which is concern with structure function variation and mapping of gene. Genomics mainly deals with changes in cancer related genes it measures the variation or changes in the oncogene and tumor suppressor gene at DNA level or RNA level. Also it provides the view regarding the relationship between gene expression and pathological features associated with it. Though it gives a more extensive understanding regarding the mechanism of cervical cancer development and progression all of these changes can be visualize by DNA microarray technique.

**DNA microarray**

The DNA Microarray is a technique which involves microscopic DNA spot collection attached to the solid surface, measures the huge number of gene expression simultaneously or genotype of multiple regions of genomes. It is a powerful technique for investigating the changes of gene expression worldwide in cells and tissue. The assembling procedure of this technique is automated and helps in detecting and analyzing polymorphism and gene expression which allows finding the accurate treatment. This technique was used by Wong et al. containing 11,000 features approx. for visualizing the normal and cervical cancer gene expression profile, they reported there were 40 genes which were different between normal and cancerous tissue and were capable in segregating completely between normal and cervical cancer tissue. All these observation by him concluded that this technique is beneficial in regulating the stages of disease and also response of radiotherapy in cervical cancer cell.[14] Further Song et.al conducted a study in which they analyzed the expression of gene pattern of normal and carcinoma in situ cervix by the help of DNA microarray technique[15] Zhu et al. also studied several genes in which they found few genes were unregulated few were

down regulated among normal and cancerous cell of cervix. The upregulated genes were related with apoptosis pathway, B-cell lymphoma-2 and cellular inhibitor of apoptosis protein-1.[16]

The HPV associated cervical cancer mechanism was detected by DNA microarray technique which reveals that as HPV 16 and 18 are found in cervical cancer cells but type 18 is more aggressively correlated with cervical cancer. This also shows that various genes involved in cell signaling pathways play distinct role in HPV16 and 18 mediated cancerous cells. The HPV mediated or induced cervical cancer is a convoluted mechanism, as DNA damage response (DDR) is mainly responsible for repair of cellular system and gives signal to cell for dividing. If this DDR function get deregulated, when the oncoproteins of HPV virus effects the consequences sequence of DDR in different ways and this damage will remain unrepaired and became helpful for virus in causing integration at break points of DNA.[17] The HPV cause primarily interferon response which will help in collapse of episomal HPV and E2 inhibition and then selection of cells with elevated level of oncogenes E6 and E7, with integrated HPV genome. The TLR9 downregulation causes impairment in interferon response causing persistent HPV infection. The elevated level of expression of oncogenes E6 and E7 helps in causing genetic instability chromosomal rearrangement and finally promotes the integration risk of viral DNA.

**Transcriptomics**

It involves the study of RNA transcription and gene expression. It has been widely utilized in determining molecular mechanism of cervical cancer by providing the better understanding for diagnosis and prognosis of cervical cancer. Transcriptomic involve the process called transcription which involve DNA transcribed into RNA and further RNA translate into protein mainly mRNA called coding RNA. There are also RNA present those do not translate into protein called non-coding RNA. These non- coding RNA are responsible for diagnosis of cancers because they have emerged as vital element in various cancers.

The integration of all this information will not only lead us to understand cervical cancer by a worldwide point of view but also beneficial in identification of factors which provide help in diagnose and prevention of cervical cancer and mortality rate. The molecule and phenotypes of cells had provided basis of molecular mechanism in several disease mainly cervical cancer. Several techniques based on gene amplification like PCR and sequencing is associated with transcriptomic which enable to analyze different expression and transcripts of cancerous and normal cells and it can be found in both of the tumor suppressor gene and proto-oncogene the level of expression of oncogenic will be high and tumor suppressor gene will be low.

Non coding RNAs are classified into regulatory and housekeeping ncRNAs.[18] According to different transcript sizes they were divided into small ncRNA and long ncRNA. Long non-coding RNA was shown with gene modulating activity in response to external stimulus DNA damage. IncRNA are overlapped between various multiple coding and non-coding transcripts.[19] Derrun et al. have found that Inc RNAs are generated by pathways similar to coding gene of cancer initiation progression protein.[20] Several Inc RNA were reported differently expressed in variety of disease even in cancer.[21] The role of IncRNA and their mechanism in cervical cancer are-

**1. HOX transcript intergenic (HOTAIR)**

HOTAIR is a well-studied non-coding RNA, it interacts with the polycomb repressive complex2 composed of EZH2, SUZI2 and EFD. This leads to H3K27 trimethylation and silencing of HOXD.[22] HOTAIR is known to upregulates in several cancers and correlated with tumor progression and metastasis. Its overexpression would lead tumor recurrence and overall survival by cell cycle progression and apoptosis but this is not the case in all cancer. The HPV16 infected cervical cancer sample have shown deregulated level of HOTAIR and it was also found that HPV16 expression has a correlation with HOTAIR but it does not involve in viral status. However, the latest investigations shown that cervical cancer with HPV-16 infection has deregulated level of HOTAIR and was correlated with type16 HPV and association by oncogene E7 without any kind of dependence of HPV virus status. The oncogenic E7 effects the HOTAIR expression and further cause inability of HOTAIR to its target HPV16 and HOXD10 and E7interact directly with HOTAIR. So HOTAIR work as early detection marker for finding out HPV16 positive women.[23] The negative correlation was found between HOTAIR and p21 down regulation by radio resistance induced HOTAIR. The invasion /migration and reverse epithelial-mesenchymal transition (EMT) process is promoted by HOTAIR.[24] It was found that it inhibits tumor suppressor (PTEN) phosphate and tension homolog and RNA binding motif protein 38(RBM38)[25] and activate oncogenes (HER2 and MMP3) . All these factors provided information which will help us in novel therapeutic target for treatment of cervical cancer in future.

**2. Metastasis associated Lung adenocarcinoma transcript 1**

MALT-1 plays numerous role in many cancers and is found in elevated level in cervical cancer tissue than in normal tissues was correlated with tumor size.[26] MALT-1 a non-coding RNA mediate apoptotic pathway in cervical cancer and proliferation. TheCaSKi cell line silencingMALAT1results in elevated level of expression of Caspase8, 3 Baxand lower level of antiapoptotic factor Bcl-2 and BclXl, were observed which decrease the migration capacity of cells. It was found thatHPV infection was associated with increase of MALAT1

expression. In cervical cancer cell higher level of initiator and executioner caspase were found and inhibition of MALT-1 in cervical cancer cells leads to downregulation of Bcl-2 and upregulation of Bax. In CaSki cell line MALAT1 was knockdown which shown reduced cyclin and CDK expression (cyclin D1, E and CDK6) of cell cycle causing G1 arrest.[27] The HeLa cells of cervical cell line were found to be suppressed cells proliferation by inhibition of MALAT1, so it has importance in development and metastasize of cervical cancer.[28] All these facts reveal that blockage of MALT-1 can leads to therapeutic value in prevention of cervical cancer.

**Epigenetics**

It is a study of alteration in expression of gene without any changes in DNA sequence. The main aim of epigenetic is to understand molecular pattern and flow of genetic information.[29] Epigenetics had been majorly responsible for diversity of organism, cancer, immune system response. In epigenetics DNA methylation and histone acetylation are the two most important modifications and both of this modification is involved closely with each other so it has been expected that they both processes in many disease such as cancer therefore in majority of tumors the epigenetic could be the major cell alteration forming transformed phenotype.[30]

**1- DNA Methylation**

This modification is responsible for proper replication of the DNA at cytosine nitrogenous base by adding methyl group at C5 position. If DNA methylation is not correctly regulated will leads to uncontrolled DNA replication and further cause cancer, in human genome methylation is not uniformly present it contain unmethylated segment with methylated region interspersed. The remaining genome is smaller region of DNA called CpG islands, they contribute half portion of human genome and mainly found in constitutively expressed gene like housekeeping gene.[31],[32] There are at least 3 DNA methyltransferases (DNMTs) recognized which preferentially methylates hemi-methylated DNA. Among all these three DNMTs most abundant is DNMT1 which is localized to replication foci, regulates methylation during replication and DNA repair mechanism. Abnormalities associated with methylation during DNA repair or replication is associated with cancer for long time and both hypomethylation and hypermethylation can leads to carcinogenesis.

**a) Hypermethylation**

It is an epigenetic control important for gene inactivation in cancer cells and found in almost every type of tumors. Tumor suppressor genes are not only inactivated by the structural modification but also due to lack of gene expression because of hypermethylation of promoter of the tumor suppressor position.[33] The pRb was found to be first tumor suppressor gene to get hypermethylated and silenced.[34] Afterwards many studies were done which revealed the similar facts for multiple tumor suppressor gene p16, E-cadherin, MLHI.[35] Therefore, the hypermethylation of promoter region of gene is whether a cause or result of tumor suppressor gene inactivation is yet not known. The methylation at lysine is associated with histone modification which occurred along with re-silencing of p16 in cells, it was earlier activated by knocking out DNA methyltransferase enzyme responsible for catalyzing DNA methylation.[36]

**a- Hypomethylation**

It refers to loss in methyl group in nucleotide at 5-methylcytosine, mainly because chromosomal instability and various other process deregulation. This has been known that DNA hypomethylation can occur worldwide in cancerous cells. This methylation mainly occurs at coding regions, introns and at chromosome pericentromeric regions of chromosomes. Hypomethylation have capability of progression from premalignant conditions to malignancy. The main cause of cancer through hypomethylation is instability of chromosomal, reactivation of transposable elements or activation of improper gene.[37] Hypomethylation leads to several disorders like co-genial disorder caused by mutation in DNMT3b and several other . This disorder not only cause cancer but also somatic tumors like breast, ovarian, and other epithelial tumor have unbalanced chromosomal translocation at pericentromeric DNA of chromosome 1 and 16.[38]

**Proteomics**

Proteomics refers to study of large scale proteins basically a proteome is set of proteins produced in any organism. The proteomic study of cervical cancer was performed by laser capture and mass spectrometry techniques in the tissue sample. This revealed increase protein levels of tumor tissue than in normal tissue. The most noticeable feature was increase in MCM and its associated proteins. MCM helps during DNA replication initiation in unwinding DNA and fork progression, its process start at S-phase by the recruitment of cdc6 and cdt-1 at origin of replication and then they both recruit MCM at origin of replication forming pre-replicative complex for licensing the DNA for replication.[39],[40] Due to its important role in replication if any deregulation occurs in MCM it contributes to carcinogenesis in humans.[41]

There are also several hypotheses that MCM proteins are upregulated in many tumors is crucial for progression of tumor and it can serve as valuable target in anticancer therapy and molecular marker for detecting the cervical cancer. The data analysis of gene expression of proteins revealed that cervical cancer is related and correlated with biological interaction and DNA replication, repair, and recombination pathway respectively. Several methods of hypothesis testing shown that mainly cervical cancer specific proteins are lost in initial stage of cancer during tumorgenesis and for detection perspective increase level of protein marker are more relevant than down-regulated proteins.

While investigating proteomics 19 protein associate with cervical cancer were upregulated in both early and late stage. Ojesina et al found that protein CEACAM5 has HPV integrating site was highly expressed, this protein has also function as cell-adhesion molecule.[42] The MAPK1 and S100P gene were detected in cervical cancer its function in cell growth, adhesion, survival and differentiation and cell cycle progression and cell survival respectively but function S100P in cervical cancer is not clear.[43] It conclude that Laser Capture combined with mass spectrometry is reliable for detecting individual proteins and with high-resolution mass spectrometry with the approach of detecting difference in individual protein levels in tissue in initial stage of cervical cancer of patients in comparison of healthy tissue. The two significant differential networks were also found, down regulated network which probably show the loss of proteins associated with cervical cancer specifically and upregulated network shows cancer gain of protein associated with cervical cancer. Proteomic emerged by the gradual completion of Human Genome Project and requires well founded technology of mass spectrometry and tissue microarray.[44] As all kind of genetic changes will lead to change in protein expression and these defects of proteins are identified and predicted by proteomics.

**Metabonomics**

Metabolomics is a modern analytical method for studying the product of small nuclear endogenous substance such as homogenate tissue and cells which reveal the metabolic importance in life activities.[45] This technique is used in measuring metabolism of cancer, altered pathways and metabolites. In past decades, research of cancer metabolism reveals mainly two characteristics, it has got major attention- a) Warburg effect: It states that even in the presence of oxygen there is elevated level of glucose consumption and lactate secretion. b) Glutamine addiction: It states that glutamine is essential for cell growth.[46] The cervical cancer associated Metabolomicsis performed by nuclear magnetic resonance (1H NMR), the main metabolite present in cervical cancer are formate, β-glucose, alanine, valine, isoleucine, inositol, glutamine and low density cholesterol was found by Ye et al in serum profile of cervical cancer which could contribute in distinctions.[47] Other investigator Yin et al utilize another technique of spectrometry Ultra performance liquid chromatography for detecting molecular metabolite in Squamous cells of cervical cancer in plasma and they found two important metabolite which play important role in pathogenesis of cervical cancer phosphatidylcholine and lysophosphatidylcholine.[48]

**Cell signaling**

Cells interact to their surrounding signals and responds by many different ways depending on cell types is called cell Signaling. The single celled organisms need to detect nutrients in their environment, and multicellular organisms are involved in a complex system of communication with each other. If any kind of deregulation or inhibition in the intracellular signal transduction occurs will lead the regulation of cancer cell growth and viability. The cervical cancer caused by HPV leads to damage the tumor suppressor gene by integration of HPV oncogenes E6 and E7. As if any kind of damage happens the cell has a specialized mechanism of repairing it and if it does not do so then cell undergo apoptosis. In HPV associated cancer HPV oncogenes deregulate the cell cycle checkpoints by inhibiting CDK and tumor suppressor genes. Degradation of p53 by E6 allows continuous replication of cells and by taking this advantage HPV make numerous copies of its episomes and E7 degrades pRb and cause unorganized entry in cell cycle . This will leads to proliferation of cells, as HPV viral for replicating requires its entry in cell cycle S-phase and this occurs by inactivation of tumor suppressor gene pRb and by releasing E2F (transcription factor)which allows progression of HPV virus from G1 checkpoint.

Abnormality in cell signaling pathway is primarily involved in causing several cancers. The major pathway which gets affected in cervical cancer is Wnt pathway. The genetic and epigenetic changes leads to mutation, it further inhibits Wnt pathway components like Wnt ligand, receptors, and intracellular mediators. This pathway has emerged for regulation of cell development, adhesion.[49] Wnt proteins are family of highly conserved secretory glycoprotein consist of 19 Wnt ligands and these ligands combined with multiple receptors for activating various downstream pathways. Wnt pathway consist frizzled receptor and co-receptor is LRP5/6 which received the signal. Further both ligand and receptor activate destruction complex adenomatous polyposis coli (APC), GSK-3β, Axin and phosphoralyate β catenin.[50] In absence of wnt ligand β catenin is located at cellular junction and when Wnt signaling is initiated it causes disruption in destruction complex by phosphoryalating GSK-3β to inactive form. Hence cytoplasmic β catenin is transported to nucleus where it binds to promoter region of target gene.

The human papalliomavirus plays major role in initiating cervical cancer, the component of Wnt pathway β catenin enhance HPV16 mediated cervical carcinogenesis, therefore transformation of HPV expressed cells requires activation of Wnt pathway.[51] The various studies conducted revealing the effect of Wnt signaling in HPV associated oncogenic pathway. Both the genes E6 and E7 upregulates the β catenin expression and stimulates TCF-mediate transcription, it decrease the ubiqutin ligase type-3 protein and induces β catenin degradation.[52] SFRP gene silencing cause activation of oncogenic Wnt pathway which leads to cervical cancer progression this was reported by Chung et al.[53] similarly epigenetic silencing of WIFI gene also leads to cervical carcinogenesis. The non-canonical Wnt pathway play a major oncogenic role in cervical cancer. Planar cell polarity pathway ass*ociated with Wnt pathway plays* role

of differentiation in cervical cancer pathogenesis as reported by Carlos et al. All of this concludes that HPV associated transformation and carcinogenesis require Wnt pathway activation both of the Wnt pathway participates in tumor progression, invasion either individually or with the help of several other means.

Regular cytological screening and molecular screening in these women to detect early cases of carcinoma cervix, the treatment of which will check any progression to cervical cancer and will thus reduce their miseries.[54]

## CONCLUSION

There are several disease and among them cervical cancer is unique in terms of its capability of performing as a disease model because it is caused by pathogen with well-established life cycle and mechanism. The cytology, HPV screening and surgical removal of premalignant disease has being very successful in reducing the rate of cancer.

The effective combination of genomics, proteomics, transcriptomics, metabonomics explore the defective mechanism of genes, proteins, RNA and metabolic product by which prognosis and treatment of cervical cancer become possible. Cell signaling also place a major part in all of these process, without it not a single cell would respond. Hence, will lead to uncontrolled cell division and cell cycle leading to finally causation of cervical cancer.

## REFERENCES


1. Holowaty, P.; Miller, A.B.; Rohan, T.; To, T. Natural History of Dysplasia of the Uterine Cervix. Obstet. Gynecol. Surv, 1999; 54: 375–377.
2. Schiffman, M.; Kjaer, S.K. Chapter 2: Natural History of Anogenital Human Papillomavirus Infection and Neoplasia. J. Natl. Cancer Inst. Monogr, 2003; 14–19.
3. Anderson, M.C.; Brown, C.L.; Buckley, C.H.; Fox, H.; Jenkins, D.; Lowe, D.G.; Manners, B.T.; Melcher, D.H.; Robertson, A.J.; Wells, M. Current views on cervical intraepithelial neoplasia. J. Clin. Pathol, 1991; 44: 969–978.
4. Arends, M.J.; Buckley, C.H.; Wells, M. Aetiology, pathogenesis, and pathology of cervical neoplasia. J. Clin. Pathol, 1998; 51: 96–103.
5. Pappa K I, Kontostathi G, Lygirou V, Zoidakis J, Anagnou N P. Novel structural approaches concerning HPV proteins: Insight intotargeted therapies for cervical cancer.Oncol. Rep, 2018; 39: 1547-1554.
6. Hausen H Z. Viruses as tumor initiator and tumor promoter. Hameatol. Blood Transfus, 1985; 29: 306-307.
7. Deligeoroglou E, Giannouli A, Athanasopoulos N, Karountzos V, Vatopoulou A, Dimopoulos K, et al. HPV Infection: Immunological Aspects and Their Utility in Future Therapy. Infect. Dis. Obstet. Gynecol, 2013; 1–9.
8. Carmody, R.J.; Chen, Y.H. Nuclear factor-kappaB: Activation and regulation during toll-like receptor signaling. Cell. Mol. Immunol, 2007; 4: 31–41.
9. Park, J.S.; Kim, E.J.; Kwon, H.J.; Hwang, E.S.; Namkoong, S.E.; Um, S.J. Inactivation of interferon regulatory factor-1 tumor suppressor protein by HPV E7 oncoprotein. Implication for the E7-mediated immune evasion mechanism in cervical carcinogenesis. J. Boil. Chem, 2000; 275: 6764–6769.
10. Decarlo, C.A.; Rosa, B.; Jackson, R.; Niccoli, S.; Escott, N.G.; Zehbe, I. Toll-Like Receptor Transcriptome in the HPV-Positive Cervical Cancer Microenvironment. Clin. Dev. Immunol, 2011; 1–9.
11. Ghosh, A.; Dasgupta, A.; Bandyopadhyay, A.; Ghosh, T.; Dalui, R.; Biswas, S.; Banerjee, U.; Basu, A. A study of the expression and localization of toll-like receptors 2 and 9 in different grades of cervical intraepithelial neoplasia and squamous cell carcinoma. Exp. Mol. Pathol, 2015; 99: 720–724.
12. Hasan U A, Bates E, Takeshita F, Biliato A, Accardi R, Bouvard Vet al. TLR9 Expression and Function Is Abolished by the Cervical Cancer-Associated Human Papillomavirus Type 16. J. Immunol, 2007; 178: 3186–3197.
13. Torres PK, Bahena-Román, M.; Madrid-González, C.; Burguete-García, A.I.; Bermúdez-Morales, V.H.; Peralta-Zaragoza, O.; Madrid-Marina, V. Role of IL-10 and TGF-β1 in local immunosuppression in HPV-associated cervical neoplasia. World J. Clin. Oncol, 2014; 5: 753–763.
14. Wang X, Huang X, Zhang Y. Involvement of Human Papillomaviruses in Cervical Cancer. Front Microbiol, 2018; 9: 2896.
15. Song J.Y. Microarray analysis of normal cervix, carcinoma in situ, and invasive cervical cancer: identification of candidate genes in pathogenesis of invasion in cervical cancer. Int J Gynecol Cancer, 2008; 18(5): 1051–1059.
16. Zhu M.Y. Variation in apoptotic gene expression in cervical cancer through oligonucleotide microarray profiling. J Low Genit Tract Dis, 2015; 19(1): 46–54.
17. Tornesello M L, Annuziata C, Buonagluro L, Buonagluro F M. Human Oncoviruses p53 tumor suppressor pathway deregulation at the origin of human cancers. Cancers (Basel), 2018; 10: 213.
18. Bharadwaj M, Hussaian S, Thakur N. Genetic basis of HPV mediated cervical cancer in Indian women. Int. Conf on System In Med and Bio, 2010; 57: 35-335.
19. Guttman M, Amit I, Garber M, French C, Lin MF, Feldser D, *et al*. Chromatin signature reveals over a thousand highly conserved large non‐coding RNAs in mammals. Nature, 2009; 458: 223‐7.
20. Derrien T, Johnson R, Bussotti G, Tanzer A, Djebali S, Tilgner H, *et al*.The GENCODE v7 catalog of human long noncoding RNAs: Analysis of their gene structure, evolution, and expression. Genome Res, 2012; 22: 1775‐89.

21. Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, *et al*. Long non‐coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature, 2010; 464: 1071‐6.
22. Prensner J R, Chinnaiyan A M. The emergence of Inc RNAs in cancer biology. Cancer Discov, 2011; 391-407.
23. Sharma S, Mandal P, Sadhukhan T,Chowdhury R, Mondal R N, Chakravarty B, et al . Bridging links between long non-coding RNA HOTAIR and HPV oncoprotein E7 in cervical cancer pathogenesis. Sci. Rep, 2015; 5: 11724.
24. Tsai MC, Manor O, Wan Y, Mosammaparast N, Wang JK, Lan F, *et al*.Long noncoding RNA as modular scaffold of histone modificationcomplexes. Science, 2010; 329: 689‐93.
25. Li J, Wang Y, Yu J, Dong R, Qiu H. A high level of circulating HOTAIR is associated with progression and poor prognosis of cervical cancer. Tumour Biol, 2015; 36: 1661‐5.
26. Jing L, Yuan W, Ruofan D, Jinjin Y, Haifeng Q. HOTAIR enhanced aggressive biological behaviors and induced radio‐resistance via inhibiting p21 in cervical cancer. Tumour Biol, 2014; 36: 3611‐9.
27. Guo F, Li Y, Liu Y, Wang J, Li Y, Li G, *et al.* Inhibition of metastasisassociated lung adenocarcinoma transcript 1 in caSki human cervical cancer cells suppresses cell proliferation and invasion. Acta Biochim Biophys Sin (Shanghai), 2010; 42: 224‐9.
28. Lin R, Maeda S, Liu C, Karin M, Edgington TS. A large noncoding RNA is a marker for murine hepatocellular carcinomas and a spectrum of human carcinomas. Oncogene, 2007; 26: 851‐8.
29. Parkin DM, Bray F, Ferlay J, Pisani P: Estimating the world cancer burden: Globocan. *Int J Cancer*, 2001; 94: 153-156.
30. Kawasaki H, Taira K: Transcriptional gene silencing by short interfering RNAs. *Curr Opin Mol Ther*, 2005; 7: 125-131.
31. Bird AP: CpG-rich islands and the function of DNA methylation. *Nature*, 1986; 321: 209-213.
32. Antequera F, Bird A: Number of CpG islands and genes in human and mouse. *Proc Natl Acad Sci U SA*, 1993; 90: 11995-11999.
33. Robert MF, Morin S, Beaulieu N, Gauthier F, Chute IC, Barsalou A,MacLeod AR: DNMT1 is required to maintain CpG methylationand aberrant gene silencing in human cancer cells. *Nat. Genet*, 2003; 33: 61-65.
34. Wang KY, James-Shen CK: DNA methyltransferase Dnmt1 and mismatch repair. *Oncogene*, 2004; 23: 7898-7902.
35. Laird PW, Jaenisch R: DNA methylation and cancer. Hum Molec Genet, 1994; 3: 1487-1495.
36. Greger V, Passarge E, Hopping W, Messmer E, Horsthemke B: Epigenetic changes may contribute to the formation and spontaneous regression of retinoblastoma. Hum Genet, 1989; 83: 155-158.
37. Bachman KE: Histone modifications and silencing prior to DNA methylation of a tumor suppressor gene. Cancer Cell, 2003; 3: 89-95.
38. Ehrlich M: DNA hypomethylation, cancer, the immunodeficiency, centromeric region instability, facial anomalies syndrome and chromosomal rearrangements. J Nutr, 2002; 132: 2424-2429.
39. Bochman ML, Schwacha A. The Mcm complex: unwinding the mechanism of a replicative helicase. Microbiol Mol Biol Rev, 2009; 73: 652–683.
40. Sanchez B J, Mesa P, Ibarra A, Martinez J MI, Blanco L, Mendez J, Boskovic J, Montoya G. Molecular architecture of a multifunctional MCM complex. Nucleic Acids Res, 2012; 40: 1366–1380.
41. Honeycutt KA, Chen Z, Koster MI, Miers M, Nuchtern J, Hicks J, Roop DR, Shohet JM. Deregulated minichromosomal maintenance protein MCM7 contributes to oncogene driven tumorigenesis. Oncogene, 2006; 25: 4027–4032.
42. Ojesina AI, Lichtenstein L, Freeman SS, Pedamallu CS, Imaz-Rosshandler I, Pugh TJ, Cherniack AD, Ambrogio L, Cibulskis K, Bertelsen B, Romero-Cordoba S, Trevino V, Vazquez-Santillan K, et al. Landscape of genomic alterations in cervical carcinomas. Nature, 2014; 506: 371–375.
43. Parkkila S, Pan PW, Ward A, Gibadulinova A, Oveckova I, Pastorekova S, Pastorek J, Martinez AR, Helin HO, Isola J. The calcium-binding protein S100P in normal and malignant human tissues. BMC Clin Pathol, 2008; 8: 2.
44. Yu M, et al. Metabonomics research progress on liver diseases. Can. J. Gastroenterol. Hepatol, 2017; 2017: 8467192.
45. Zielinski DC. Systems biology analysis of drivers underlying hallmarks of cancer cell metabolism. Sci Rep, 2017; 7: 41241.
46. Yin MZ, et al. Identification of phosphatidylcholine and lysophosphatidylcholine as novel biomarkers for cervical cancers in a prospective cohort study. Tumour Biol, 2016; 37: 5485–92.
47. Wu J, Fang J, Yang Z, et al. Wnt inhibitory factor-1 regulates glioblastoma cell cycle and proliferation. J. CLIN NEUROSCI, *2012; 19: 1428-1432.*
48. Borcherding N, Kusner D, Kolb R, et al. Paracrine WNT5A Signaling Inhibits Expansion of Tumor-Initiating Cells. CANCER RES, 2015; 75: 1972-1982.
49. Zhang J, Tian XJ, Xing J. Signal Transduction Pathways of EMT Induced by TGF-beta, SHH, and WNT and Their Crosstalks. J Clin Med, 2016; 5.
50. Gordon MD, Nusse R. Wnt signaling: multiple pathways, multiple receptors, and multiple transcription factors. J BIOL CHEM, 2006; 281: 22429-22433.
51. Wang D, Wise ML, Li F, Dey M. Phytochemicals attenuating aberrant activation of beta-catenin in cancer cells. PLOS ONE, 2012; 7: 50508.
52. Matsuzawa SI, Reed JC. Siah-1, SIP, and Ebi collaborate in a novel pathway for beta-catenin

degradation linked to p53 responses. MOL CELL, 2001; 7: 915-926.
53. Chung MT, Lai HC, Sytwu HK, et al. SFRP1 and SFRP2 suppress the transformation and invasion abilities of cervical cancer cells through Wnt signal pathway. Gynecol. Oncol, 2009; 112: 646-653.
54. Misra JS, Srivastava AN, Sharique A, Srivastava KR Cervical Cytology Associated with Pelvic Inflammatory Diseases.JCytol Histol, 2015; 6: 343.
55.