Review Article

Reversal of Hypermethylation in Tumor Suppressor Genes using Phytochemicals in Cervical Cancer

Abstract

Cervical cancer is most familiar neoplasm among women ~~of~~ world-wide. Surgery, radiotherapy, and chemotherapy are common treatments, however high stage tumors have frequently poor prognosis. HPV 16 and 18 are major etiological factors for cervix cancer. Likewise, epigenetics is the study of inherited changes and modulated gene expression without alteration in DNA sequences. In mammals epigenetic modifications include DNA methylation, histone modifications and miRNA .

Phytochemicals are mainly contained in fruits, seeds, and vegetables as well as in food~~s~~ supplements. Numerous dietary compounds exhibit potent anti-tumor activities through the reversion of epigenetic alterations associated to oncogenes activation and inactivation of tumor suppressor genes in cervical cancer cell lines SiHa and HeLa, demethylation of the tumour suppressor genes such as RARβ2, MGMT, RASSF1A, and DAPK etc. Reversal of hypermethylated genes as a tumor-suppressor gene is related to inhibition of cell proliferation, development and differentiation. The effect of phytochemicals on genetic and epigenetic modifications and how these modifications help to prevent various types of cancers and improve health outcomes.

1. Introduction

Cervical cancer is a familiar malignancy in females leading cause of death ~~in~~ worldwide (Torre *et al.*, 2015). In 2018, an estimated 570,000 cases of cervix cancers occurred with 290,000 death~~s~~ (Bray et.al; 2018). About~~\~~ 80% death~~s~~ due to cervical cancer occur in developing countries and low middle countries (Yeole *et al.*, 2004). ~~-~~Although recent advances in~~,~~ the prognosis of patients with advanced and metastatic cervi~~x~~cal cancer remains poor~~.~~ ~~R~~remedial options such as chemotherapy, surgery and radiotherapy are recommended ~~for this cancer~~ (Siegel *et.al*; 2016). Human papilloma viruses (HPVs) have been described as the main etiological ~~aspects~~ agents in cervix cancer (Hausen *et al.*, 1987). High risk type is HPV16 that is responsible for the promotion of about 50-60% of all cervix tumor while HPV18 harbour about 10-20% of cervix cancer worldwide (Alshatwi *et al.*, 2013). In India, 122,844 female~~s~~ ~~is~~ are diagnosed with cervi~~x~~cal cancer and 67,477 die~~s~~ from the disease annually. In 2014 high ancient rates in women for cervical cancer recorded in South Asia (22), in Bangladesh(19.2) Sri Lanka(13)and(2.8) in Iran. HPV prevalence among cervix cancer patients in India ~~has~~ varie~~d~~s from 87.8% to 96.67% ~~-~~(Sowjanya *et al.*,2005) Cancer is a set of disorders that are derived by aggregates of genetic and epigenetic alterations (Hanahan *et al.*,2011).Epigenetic changes are equally responsible as genetic changes in the development and progression of cancer. The word epigenetic was ~~composed~~ proposed by Conrad H. Waddington in 1942 (Waddington *et al.*, 1942). Epimutation change can assist determine whether factors are turned

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on or off controlling the production of macromolecules in particular cells (Stalker et al., 2008). These epigenetic processes are necessary which regulate the normal functions of the cell during all stages, including development, differentiation and facilitate adaptation to environmental changes, such as nutritional variation or exposure to cigarette smoke, chemicals, radiation, and hormones (Suter *et al*., 2009).

2. Mechanism of Epigenetic modifications

The reversible nature of epigenetic changes targetsing therapeutics processes responsible for epigenetic regulation are DNA methylation, modifications in chromatin , and non-coding RNA (mi RNA) (Lim and Song 2012)

2.1 DNA methylation

DNA methyltransferases (DNMTs) determined DNA methylation in the CpG dinucleotide by activating the addition of CH3 groups from S-adenosyl- L-methionine to the 5' position of cytosine (Hotchkiss *et al*., 1948).The methyl group regulateds the turning off of gene transcription and silencing of genes (Gruenbaum *et al*.,1981). CGs system that is closely positioned among DNA sequences additionally called CpG islands are mostly unmethylated (Jones *et al*.,2006).70–85% of CpG switch in the genome are methylated (Ehrlich *et al*., 1982).DNA methylation is mediated by DNMTs as wellsuch as DNMT1, DNMT2 and DNMT3s. DNMT1 is accountable for the maintenance of –methylation during replication and DNMT3s family methylase CpG dinucelotides de novo pathways and DNMT2 has been shown to specifically methylate cytosine 38 of transfer RNA (Goll *et al*., 2006). Methylation of tRNAs has an impact on the folding and stability of the structure (Jurkowska *et al*., 2010) DNA Hypermethylation are tumor inhibitor genes during initiation and progression in tumor whereas hypomethylated genes lead to chromosome instability and inactivation of oncogenes (Diaz *et al*.,2014)

2.2 Histone modification

The N-terminal tails of histones are widely altered containing acetylation, methylation, phosphorylation, biotinylation and ubiquitination are essential during growth (Kouzarides *et al*., 2011). DNA is wrapped around histones proteins called chromatin.The basic building block of chromatin referred to as nucleosome, which is formed of an octamer of histone proteins (Cedar and Bergman, 2009).There are five basic forms of histones named as H1,H2A, H2B, H3 and H4 containing amino acid residues with an amino group (Fullgrabe *et al*., 2011) Histone alterations are assembled by enzymes like histone methyltransferases (HMTs), histone demethylases (HDMs) histone acetyltransferases (HATs), and histone deacetylases (HDACs). HMTs act to add methyl groups to lysine and/or arginine residues in histones, while HDMs remove the methyl components. In turn, HATs assemble the addition of acetyl groups to the lysine silts of histones, whereas HDACs are accountable for the removal of these groups (Doi *et.al*; 2009).

2.3 mi RNAs

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~~m~~Micro RNAs, tiny non -coding governing RNAs, range in size from 17 to 25 nucleotides (Croce 2009) which are accountable for a lower translation rate and elevated degradation of mRNAs. miRNAs show the vital part in cell cycling, programmed cell death, cell differentiation, tumor development and progression (Negrini *et al.*,2007).Non-coding RNAs, containing miRNAs~~,~~ ~~were~~ aggression were showed to perform catalytic actions in promoting RNA splicing and it was analyzed that they participate within the post transcriptional factor modulation (Mager *et al.*,2008) . Recent phenomenon indicates that non-coding RNA (ncRNA) transcripts play an elementary role in epigenetic modulation of gene intimation and have been involved invaried epigenetic mechanisms likes transposon silencing, X-chromosome inactivation, DNA imprinting, and paramutation. Therefore miRNAs possibly can be used as biomarkers for cancer diagnosis and prognosis (Suzuki *et al.*, 2006).

3. Reversal of hypermethylation

The methylation of CpG islands by DNA methyltransferases is reversible and has been shown to modify the transcriptional activity of key proliferation genes or transcription factors involved in suppression or promotion of cell growth (Palakurthy *et al.*, 2009).The process reversal of methylation or demethylation remove methyl groups and restores normal functions of the gene that has been turned off (Bhattacharya *et al.*,1999). By shutting off genes within a given cell, DNA methylation can disrupt the function of cell so as to cause cancer (Mani *et al.*, 2010) .

3.1 Reversal of hypermethylation in tumor suppressor genes in cervical cancer

3.1.~~,~~1 RASSF1A

The Ras association domain family 1 isoform (RASSF1A) neoplastic genes were regulated like cell cycle suppression, programmed cell death, and heredity instability that shows a vital role in suppressing Ras-mediated oncogenesis (Donninger~~,~~ *et al.*,2015). RASSF1A states that moderation of each allele of a neoplasm inhibitor gene is necessary for carcinoma. Hypermethylation of RASSF1A has been described in a diversity of initial malignancy together with the cervix cancer.RASSF1A is most commonly hypermethylated neoplasm suppressor genes in human tumor and may assist as the biomarker for diseases detection (Garzon *et al.*,2009). Numerous natural compounds such as resveratrol and curcumin ~~all~~ act as antioxidants that inhibits the activity of DNA methyltransferases and reactive RASSF1A in carcinoma, Thus natural compounds could perform vital agents in malignancy prevention or medical care (Burdge *et al.*, 2010)

3.1.2 p16

The p16 gene additional called CDKN2A encodes p16INK4A that suppress the CDK45: cyclinand CDK6: cyclin D complexes (Duthie *et al.*, 2011). Hypermethylation of the p16 neoplasm suppressor gene and has effects on transcriptional down-regulation or silencing. ~~,t~~This has been shown as one of the vital structures of p16INK4a gene inactivation in varied kinds of carcinoma such as colon cancer, thyroid cancer and cervical cancer (Hayashi *et al.*, 2001). The tea leaf polyphenol EGCG suppress DNMTs and reactivates repress neoplastic suppressor genes and DNA regulate genes through their demethylation in cancer cell lines (Yang *et al.*, 2002).The promoter methylation of the p16INK4a sequence is a common event in

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cervix cancer and may have promising objective used as a marker for the promotion and diagnosis of diseases(Jeong *et al.*, 2006).

3.1.3 RARβ2

Retinoic Acid Receptor (RARβ2) is known as a cancer inhibitor gene by correlating with retinoic acid. Expression of retinoic acid receptor β (RARβ) is defined to be absent or disease controlled in cancer (Jha *et al.*, 2010) Epigenetic silencing by promoter hypermethylation ends up in retinoic acid therapy failure in breast carcinoma (Mittag *et al.*,2006)In cervix cancer cell lines SiHa and HeLa, demethylation of the RARβ2 promoter by the natural compounds curcumin and genistein led to reexpression of the genes. Thus, RARβ2 could be associated epigenetic therapeutic purpose of natural compounds. Enumerate to resveratrol improved the activity of adenosine analogues to suppress methylation of the promoter of RARβ2 gene that corelating with rising expression but resveratrol alone was ineffective (Yamanaka *et al.*,2010).

3.1.4 DAPK

DAPK is, a positive mediator of the programmed cell death possessed by gamma-interferon (Deiss *et al.*,19~~9~~95). Loss of DAPK expression has been shown to occur in a ~~varied~~ variety of human malignancies, primarily by promoter hypermethylation, reported in cervical cancer (Shohat *et al.*,2002) DAPK associates with actin microfilament and include a distinctive multidomain structure and methylated at CpG islands in numerous tumors that can be used as a biomarker for diseases identification and management(Inbal *et al.*, 1997) DAPK mRNA and macromolecules expression levels were absent or remarkably decreased in SiHa and HeLa during which the DAPK promoter was hypermethylated. The exposing levels of DAPK might be renovated after demethylation operate with 5-aza-2'-deoxycytidine (Dong *et al.*,2001).To date, 5-AZA-CdR, the methyltransferase substances, ~~was~~ has been stated to reverse gefitinib resistance caused by DAPk promoter methylation (Jacobsen *et al.*,2017). Curcumin (turmeric extract), the best natural antitumor agent usually sold out as associate herbal supplement , performs its cytotoxic effects via stimulation of DAPK1 macromolecules and messenger RNA over ~~er~~expression in U251 cells(Yokoyama *et al.*,2015)

3.1.5 CDM1

The cell adhesion molecule 1 (CADM1) gene additional called TSLC1 (Tumor suppressor of lung cancer) has been normally examined as a neoplasm inhibitor gene in numerous tumors as varied cervical cancers etc (Kuramochi *et al.*, 2001). CADM1, as a TSGs, is related to suppression of cell proliferation, moreover as induction of programmed cell death in cancer and vital role of the hypermethylation in cervix cancer (Steenbergen *et al.*, 2004). ~~In r~~Recent studie~~d~~s analyzed the macromolecules and mRNA expression of CADM1 in numerous cervical neoplastic cell lines, containing C33A (HPV-negative), HeLa (HPV18 positive), and SiHa and CaSki (HPV16-positive) cells,and ~~situated~~ stated that CADM1 was considerablelly downregulated in three HPV-induced cervical cancers (Fukuhara *et al.*,2002). In addition intimation of CADM1 is modulated by genetic or epigenetic mechanisms (Lung *et al.*,2006) Resveratrol is a polyphenol extracted from plants such as blueberries, cranberries, and grape

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which has shown anti-inflammatory effects via the regulation of pathways like~~s~~ cell cycle, programmed cell death and tumor metastatis (Roy *et al.*,2011)

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3.1.6 MGMT

The O6 methylguanine DNA methyltransferase (MGMT) is a polymer repair macromolecules that protects the human regulating from agents and tumor conduct of endogenous cancer (Fang *et al.*, 2007). MGMT additional induces protection to alkylating agents and it removes alkyl adducts from the O 6 position of guanine in DNA, (Chen *et al.*, 2003).MGMT is then degraded by the proteasome that being in roughly 17% loss of intimation of cervical cancer, Several studies have mediated the effects of natural products on the methylation standing and release of repair genes (Yang *et al.*, 2003). In cervical cancer cells line HeLa cell lines reactivation of MGMT by Epigallocatechin-3 gallate (driven from green tea) and genistein (soybeans extract) have been shown by natural compound antioxidants potential to impact diseases prevention (Fang *et al.*, 2005)

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4. Involvement of Phytochemicals in cancer treatment

Phytochemicals are non-nutritive plant chemicals that have defensive or disease preventive properties (Doughari *et al.*,2009).Greek word 'phyto' means plant and chemicals indicating to a vast variety of compounds that take place naturally in plants. It is foretold that quite 5000 particular phytochemicals are recognized in grains ,fruits and vegetables but an outsized proportion area unit still unknown and should be identified before understanding their health benefits in whole foods (Liu, ;2004). A number of studies suggest dietary plant products as actively affecting the development and promotion of tumor, "Nutri-epigenetics" focuses on the impact of dietary agents on epigenetic mechanisms. This approach has gained significant attention; since in contrast to genetic alterations, epigenetic modifications are reversible affect early carcinogenesis (Uramova *et al.*,2018).These phytochemicals are as follows:

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4.1 Phenolics : The secondary metabolites phenolics are extensively found in fruits (Levin *et al.*,1971). These compounds have on~~e~~e more aromatic rings ~~with~~ than other hydroxyl groups (Watson *et al.*, 2013). The main phenolic compounds ~~that area unit~~ is found in food may well be classified into three groups: simple phenols and phenolic acids, hydroxycinnamic acid derivatives and flavonoids. They play a~~n~~ fundamental role in the proliferation and production of the plants besides performing as protecting system mechanisms against pathogens, parasites ,and predators(Acharya *et al.*,2010). Simple phenols and phenolic acids are precursor to the synthesis of other complex compounds such as Flavonoids and tannins (Islam *et al.*, 2013). They act in the natural defense effective of plants to prevent them from infectious diseases and reduce the growth of pathogenic bacteria, viruses, and fungi. Hydroxycinnamic acids and their esterified derivatives in fruits and vegetables are almost completely originated from p-coumaric acid (PCA), caffeic acid (CA), and ferulic acid (FA) (Mateos *et al.*, 2006). Flavonoids as a major class of phenolic compounds exhibit~~ed~~ high~~ly~~ antioxidant activity. These compounds have been connected to reducing the risk of main chronic diseases and have been recognized largely

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in fruits, vegetables, and other plant foods (Forster *et al.*, 2013).

4.2. Carotenoids: Carotenoids as the most extensive natural pigment have gained considerable attention regarding their pro vitamin and antioxidant properties (Stahl *et al.*, 2005). Thisese pigments are naturally found in plant and animal kingdoms. This group of fat-soluble pigments comprises more than 600 different carotenoids have been recognized in nature responsible for red, orange and yellow colors. They may be of plants, microorganisms, and/or animals origin. They have a skeleton of isoprene units containing 40-carbon (Russo *et al.*, 2007). All color fruits and vegetables area unit smart carotenoids sources. The forms of carotene pro vitamin A are found in dark green and yellow-orange leafy (like orange, tomatoes, carrot and spinach). Darker colors are associated with higher levels of pro vitamin .Carrot, tomato and papaya represent important dietary sources of β-carotene and lycopene ((Ambrósio *et al.*, 2006)).

4.3 Alkaloids: Alkaloids are a group of round structured nitrogen containing organic compounds with a wide range of anticancer activity (Deiters *et al.*, 2004). These compounds take part in tumor suppression via prevention of enzyme topoisomerase activity that is involved in DNA imitation, inducing programmed cell death and expression of p53 gene (Su *et al.*, 2015).

4.4 Organo sulfur compound: Organosulfur compounds are organic compounds that can be identified similar to their sulfur containing functional groups (Giardi *et al.*,2004). Vegetables such as onion, garlic, shallots etc. belonging to allium family possess various water and fat soluble organosulfur compounds (OSCs) that have been widely implicated for numerous health-related benefits including diseases prevention, improved immunity, cardiovascular health and anti–microbial activity (Iciek *et al.*,2009). In colon cancer Caco-2 and HT-29 cells, DADS was suppose to suppress cell proliferation and cause G2 cell cycle arrest by inhibiting HDAC activity, inducing histone hyper acetylation as well as p21 (Waf1/cip1) expression (Druesne *et al.*,2004).

4.5 Quercitin : Quercitin is a flavonoid found in fruits and vegetables such as onions, red wine, green tea, and apples. In tumor cells, quercitin blocked cell cycle and induced pro-apoptotic effects without affecting normal cells (Chirumbolo *et al.*, 2013). Quercitin is consumed daily by millions of people through nuts, tea, vegetables and herbs in the diet. It is also available as a commercial dietary supplement, and it is now being included in functional foods. The bioavailability of this compound differs among totally different food sources, being affected by the kind of glycosides containing it. Onions are considered better sources of bioavailable quercetin than apples and tea that contain rutin and other glycosides (Graefe *et al.*,2001).

4.6 Eugenol ; Eugenol, a methoxyphenol element of clove (clove , dicot family), has been reportable for variety of medical specialty effects, as well as the suppressor , anti inflammatory, analgesic, anesthetic, antipyretic, anti platelet, anti anaphylactic, medicine, medicament, anti hyperglycemic, antibacterial drug, antifungal and antiviral effects. Eugenol and its derivatives in clove square measure potent antioxidants (Avila *et al.*,2014) that can be because of their ability to create complexes with reduced metals. Clove could be a wealthy supply of free eugenol, eugenol acetate, caryophyllene, sesquetrepene organic compound, phenyl propanoid and β-caryophyllene, (Miyazawa *et al.*,2003).

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5. Conclusion

Dietary phytochemicals hold nice promise in cancer hindrance and in medical care by ~~causation~~ causing epigenetic modifications. ~~because~~ Since the importance of epigenetic modifications in cancer is well recognized, precise contribution of epigenetic mechanisms and cellular targets of epigenetic alterations by dietary phytochemicals in human cancer ~~desires~~ needs more investigations. T~~t~~hough recent advances within the field of cancer epigenetics has increased our understanding of epigenetic changes in traditional cellular processes and abnormal events resulting in tumorigenesis, ~~but~~ deeper understanding of the worldwide patterns of epigenetic modifications by phytochemicals in cancer can result in style higher methods to forestall and cure cancer. ~~v~~Variety of studies demonstrate dietary plant product as actively touching the event and progression of tumor. "Nutri-epigenetics" focuses on the influence of dietary agents on epigenetic mechanisms. This approach has gained respectable attention; since in distinction to genetic alterations, epigenetic modifications are reversible and has summary sed the an effect on early carcinogenesis This review shows the importance and role of natural compounds in targeting epigenetic alterations, progression, in addition as its potential chemoprevention within the context of practice of medicine.

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