The Interaction of Human Papilloma Virus Oncoproteins (E6, E7) and Retinoblastoma Protein (pRb) in Angiogenesis Regulation

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KEYWORDS	ABSTRACT
	Cervical cancer is a type of cancer that occurs in the squamous cells of the uterus. Different types of Human Papilloma Virus (HPV), a sexual infection, play a key role in endometrial cancer. It has double-stranded DNA with a genome composed of 8000 base pairs and it is classified into two types of low risk and high risk, and it seems that two 16 and 18 high-risk strains are responsible for more than 70% of endometrial cancer cases and they only host those human cells which may not be obvious clinically or they may cause benign and malignant lesions. This type of cancer depends on two E6, and E7 oncoproteins that play role in formation of tumor structurally and angiogenesis compared to other important factors that have a significant role in the progression of this type of cancer. The present study aims to investigate the performance of two E6 and E7 oncogenes and their inhibitory activity in line with suppressing P53 and PRB and also investigating the role of angiogenesis in the function of these oncogenes.

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Introduction

10 years after the publication of Globocan (2008), the results of studies published on December 4, 2019, in Lancet Global Health, have represented new estimations of the cervical cancer rate (Arbyn et al., 2011). This main general health problem considered as the fourth common reason for death among women after breast cancer (2.1 million cases), colorectal cancer (0.8 million), and lung cancer (0.7 million) in 2018 around the world. According to the statistics in last decade, this cancer has become the most common cancer in more than 42 low-source countries, and also it has been considered as the third common cancer among women in the world. A research group in 2018 found a positive and significant relationship between Age Standardized Incidence Rates (ASIR) and estimations related to the incidence of

Human Papilloma Virus (HPV) and almost 570 thousand cases of endometrial cancer and 311 thousand deaths occurred due to this disease (Peng et al., 2015). The main reason for death from cancer in the countries of Central America, South East Asia and South Africa Sahara Desert, especially Eswatini is due to this type of cancer that almost 6.5% of women had endometrial cancer before the age of 75. China and India altogether contain more than one-third of endometrial cancer with 106 thousand cases in China and 907 thousand cases in India and in 146 cases (79%) of 185 countries of the world (Arbyn et al., 2020), endometrial cancer has been considered among three superior influential cancers on women younger than 45 years. The average age of diagnosis in this cancer is between 53-59 years old, although it is an active disease with the higher incidence rate among the youths (12-18) (Bruni et al., 2010). Inappropriate diet, using contra-conceptive drugs, sexual disorders, and sexual diseases are introduced as the factors which endanger the cervix and reinforce this disease. HPV and smoking are two important factors in the spread of this type of cancer (Kumar et al., 2015, Khazaei Z., 2018). The studies show that there is a close relationship between cervical cancer and HPV (the most common sexual infection). However, so many of the patients suffering from cervical cancer are not afflicted by HPV, this matter is demonstrative of other factors' effect such as genetic, cellular changes on the progression of this disease (Woodman et al., 2007). Also, women suffering from HIV are at risk of endometrial cancer and the rapid progression of this disease (Hawes et al., 2006). However, due to the early diagnosis of this disease in developed nations, the death from this disease has been decreased significantly (Vaccarella et al., 2017). HPV in human as its only host, mainly targets skin and mucus epithelial cells which are classified in five evolutionary branches of papillomavirus $\alpha, \beta, \gamma, \mu$ and ν. The biggest group of α-HPV is HPV 64 which is divided into two groups, HPV with low risk, which mainly causes safe lesions, and another type with HR-HPV high risk like HPV16 and HPV18, which may lead to malignant tumors. The most cases of endometrial cancer happen as a result of infecting with HPV16, HPV18 and HPV16 (Bruni et al., 2010). HPV is a virus with double-stranded DNA with a genome composed of 8000 base pairs. HPV genome codes six primary genes (E1, E2, E4, E5, E6, and E7), two late genes (L1 and L2) coded by non-coding region (Shanmugasundaram and You, 2017). HPV usually generates infection in the base epithelial layer. Most of these infections are short-lived and are cleaned by the body's immune system in less than two years. However in 10%-20% of the cases, infectious continues and lead to the progression of the disease. A lesion that is generated is also known as a Central In-uterine Neoplasia (CIN), which is classified according to its intensity.

Intraepithelial Lesions (LSIL) move towards High-grade Squamous Intraepithelial Lesions (HSIL) and at last lead to endometrial cancer. Despite tumor regression (return) in response to the primary treatment, most cases of latent infections prevent the virus infection perfect discharge and finally lead to the return of lesion (Shanmugasundaram and You, 2017). This infection mainly transferred through sexual intercourse and causes lesions in in-uterine squamous cells. Most of the lesions disappear because of immunological interferences after 6 to 12 month, although few percent of these lesions will remain which cause cancer (zur Hausen, 2002). After escaping from the primary immune response, the viruses have to protect their nucleus genome in host nucleus to achieve persistent infection. Although insertion in the host genome is an option which is supported by many of chronic viruses, viruses like HPV protect their genome by extrachromosomal episomes which are connected to the DNA host (Bastien and McBride, 2000). Papillomavirus can maintain its content in the host through persistent reproduction in low level in a distinguishable tissue such as base epithelium and also it helps to be hidden from immune system (Frazer, 2009). During this stage of the infection cycle, viral genomes will be able to divide themselves into newborn daughter cells by coordinating their reproduction with the host cell. In the following, the virus enters a stage of vegetative reproduction where high levels of genome products are supposed to come together in viral particles and be repeated. This final stage usually occurs in indistinguishable cellular tissues, like upper epidermal layers which are going to be removed and as a result, they are not protected accurately by host immune system; also a higher level of viral reproduction and montage is observed in such layers because they are not inclined to generate immune response (Groves and Coleman, 2015). Constant HPV is created in 10% to 15% of the cases, and around 1% of the HR-HPV infections lead to cervical cancer. The results of other studies show that HPV mostly occurs at the age of 25 (Burd, 2003). Various studies have explored that genetic factors and lifestyle may increase the probability of suffering from persistent infection significantly (Haukioja et al., 2014).

The Performance of E6 and E7 Oncoproteins in the Generation of Cervical Cancer
At the first stage, virus DNA is combined with the host genome by E6 and E7 virus oncoproteins which are stopped, and it is essential for guiding the replication process resultant from HPV (Georgescu et al., 2018, Oyervides-Munoz et al., 2018). The virus conflicts with cellular DNA methylation system to transform itself or a part of its viral cycle. Therefore, DNA changes epigenetically derives from E6 and E7 oncoproteins during HPV19 infection (Au Yeung et al., 2010, Leonard et al., 2012). This DNA methylation may be a defensive mechanism in the host cell to extinct viral DNA (Whiteside et al., 2008). On the other hand, E7

oncoprotein may influence DNA methylation which is another form of epigenetic changes. E7 suppresses gene by increasing DNMT1 level which methylates E-Cadherin promoter and decreases the level of E-Cadherin in the keratinocytes including HPV16 perfect genome (Laurson et al., 2010); also it targets oncoproteins of genes related to apoptosis and disrupts them which finally causes the resistance of cells with cells mutation, and reproduction. The main target of these oncoproteins are inhibiting proteins of P53 tumor and Retino Blastoma (Sagae et al., 2016). P53 tumor suppressor protein is a transcription factor which is mainly known as "the guardian genome" which removes the protective performance of P53 tumor suppressor protein due to TP53 gene mutation in at least 50% of the whole human cancers (Donehower et al., 1992). In this performance, P53 connects itself specifically to its target genes promoter to regulate their expression and as a result it controls cell cycle and cell death (Moon et al., 2019). So the P53 has been activated by E6 and E7 and connects to SP1 proteins (transcription activator), chromatin remodeling and DNMT1 and prevents SP1 transcription from DNMT1 and as a result genes which restore damaged DNA, are stimulated and prevent from the reproduction of cells including damaged DNA and malignant cells and remove them through apoptosis induction (Lin et al., 2010). Otherwise, E6 and E7 by means of virus replication and through employing cell
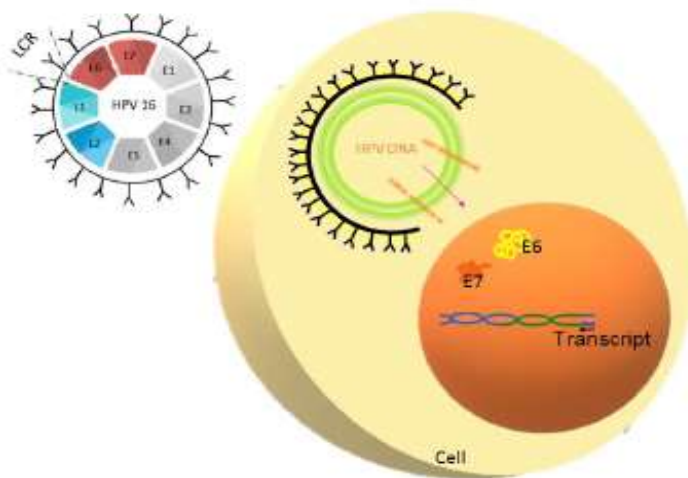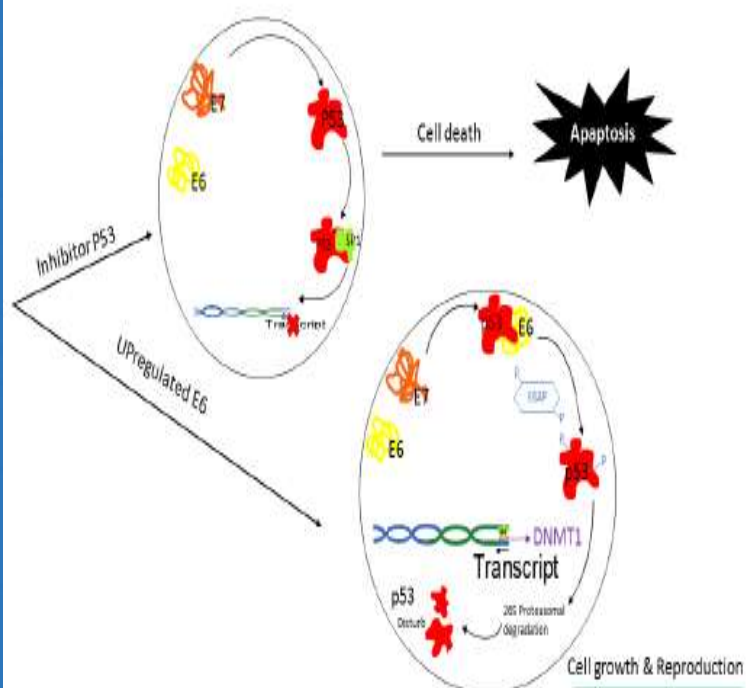

reproduction cycle force it to reproduce uncontrolledly (Langsfeld and Laimins, 2016).

Figure 1: HPV including small double-stranded DNA, six primary genes (E1, E2, E4, E5, E6, and E7), and two genes (L1 and L2) codes by a non-coding region. Virus DNA combines with the human genome by E6 and E7 oncoproteins.
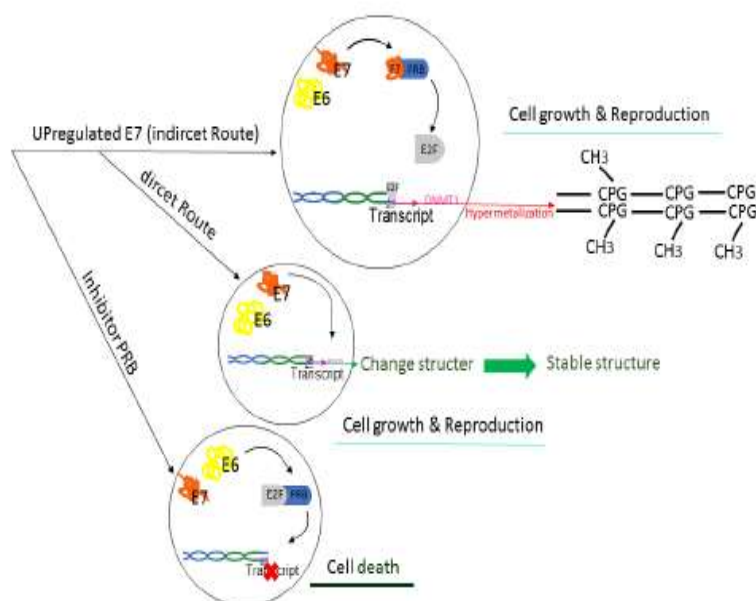
The performance of P53 tumor suppressor protein is suppressed by the performance of E6 through ubiquitin route and by means of a protein cell known as the protein related to E6 (E6AP) which is a member of ubiquitin ligase (HECT) E3 family (Kruiswijk et al., 2015). So that the E3 cell of E6Ap ubiquitin ligase is targeted by E6 and E7 and transfers ubiquitin peptides from E6AP to P53 and transfers P53 for destruction by 26S proteasome route, as a result SP1 connects to DNMT1 promoter and regulates gene expression, and since P53 has been destroyed, the expression and the activity of DNMT1 increased (Martinez-Zapien et al., 2016, Paek et al., 2016). It has been reported that the removal of E6 is accompanied by the increase of P53 and the decrease of DNMT1 expression in SIHa and caSKI cervix cancer cellular range (Leonard et al., 2012)(figure 2).

The RB tumor suppressor has a significant role in the progression of the cell cycle and acts as a potent tumor suppressor, which is inactive in most human cancers (Dick and Rubin, 2013). PRB protein prevents cell reproduction by inactivating E2F transcription family members and suppressing S phase genes' expression. PRB family, including PBL1 and PBL2, are targeted by E7 oncoprotein. Then, PRB protein has destroyed, removed, and connected to PRB and disrupts its performance by preventing the connection of PRB to E2F (Zhang et al., 2006). Since E7 inactivates the PRB, E2F is reregulated and released to connect to DNMT1, which causes hyper-methylation of the CpG structure. In the case of cervical neoplasia, the CpG structure is inactivated in the promoter region of tumor suppressor genes mostly through hyper-methylation. As the degree and the stage of neoplasia progress, hyper-methylation increases by mixing with the methyl-3CH group, and cell reproduction genes are activated (Schiffman et al., 2016). Moreover, it has been shown that E6 and E7 form complexes with hundreds of other protein in the host cell. E7 adjusts DNMT1 expression in two separate methods, and E7 connects to PRB through an indirect mechanism and releases the E2F transcription factor (Yin et al., 2016). As E2F exists in the transcription starting site of the DNMT1, it activates the release of DNMT1 promoter E2F, and E7 connects to DNMT1 through direct mechanism (Leonard et al., 2012). This connection changes the structure of DNMT1 and exposes the active area.

A sustainable DNA/DNMT1 set is formed after E7 separation from this set. This leads to genome methylation due to tumor suppressor gene extinction, so this provides the necessary route for uncontrolled reproduction and cancer (Batlle et al., 2019).

Figure 3. E7 oncoproteins prevents the inhibitory effect of PRB by connecting to it and releases E2F, which E2F places on the DNMT1 promoter and causes methylation in CPG structure which removes the effect of suppressor genes. Otherwise, E2F connection to PRB prevents it from connecting to promoter, and cancer cells are removed. In another state, E6 and E7 have an indirect effect on the structure of the DNMT1 promoter and make DNMT1 sustainable structure with DNA by E7 connection to promoter and after causing the uncontrolled reproduction and cancer after E7 separation and the extinction of suppressor genes by E6 and E7.

Figure 2. After the entrance of oncoproteins into the cell two mechanisms happen. In the first state, after the entrance of E6 and E7, the P53 suppressor protein is secreted, which prevents transcription by connecting to SP1 protein and does not allow that transcription activator to be located on promoter (DNMT1) to do transcription; as a result cancer cell removes through apoptosis. In the second case, E6 inhibits its function and binds to it, releasing P53 into the ubiquitin pathway and also increases E6AP, in which case the E6AP peptides are transferred to P53 and insert it into the S26 proteasome pathway, and P53 is eventually destroyed, and SP1 attaches on the promoter and transcribes, and the cancer cell grows and multiplies. Retinoblastoma tumor suppressor (RB) also plays an important role in cell cycle progression[52-50-51] and acts as a potent tumor suppressor that is inactive in a wide range of human cancers[53]. The pRB protein inhibits cell proliferation by inactivating members of the E2F transcription factor family and suppressing the expression of S-phase genes[54]

The Role of Angiogenesis in Cervical Cancer and the Performance of E6 and E7 oncoproteins

Angiogenesis in Cervical Cancer (CC)

Angiogenesis plays an essential role in the growth and progression of tumors by forming new blood vessels. Angiogenesis stages include protease, endothelial cells migration, the formation of vascular tubes, the anastomosis of formed tubes, synthesis of the new base membrane, and the combination of priests and smooth muscular cells (Rajabi and Mousa, 2017). After the activation of endothelial cells by angiogenetic stimuli, proteolytic enzymes are produced, which destroy Extra Cellular Matrix (ECM) and base membrane, which causes the reproduction and the migration of endothelial cells around vessels. Finally, it forms the "primary nucleation."(Koch and Distler, 2007). The next reconstruction of the primary nucleation leads to the formation of a capillary ring which consequently cause

the synthesis of new membrane and the maturity of blood vessels to complete tube-like structures (Thanapprapasr et al., 2012). Signals may disrupt the formation of blood vessels or support the removal of vessels systematically. The inhibitors act by controlling several proteins which have been known as angiogenesis activator, such as Vascular Endothelial Growth Factor (VEGF), Fibroblast Growth Factor (FGF2), angiotensin, Transforming Growth Factor (TGF), Tumor Necrosis Factor (TNF)-α, Platelet-derived Endothelial Growth Factor, Granulocyte Colony-Stimulating Factor (G-CSF), Placental Growth Factor (PGF) Interleukin 8 (IL-8), Liver Growth Factor (LGF) and Epidermal Growth Factor (Ward, 2008). Oxidative stress is vital in regulating angiogenesis. Endothelial cells (ECs) and Smooth Muscular Cells (SMCs) have different mechanisms of measuring oxygen including NADPH oxidases sensitive to oxygen such as Endothelial Nitric Oxide Synthase (ENOS) and heme-oxygenases (Mousa, 2000). When cancers are growing, endothelial cells are free due to the release of proteins like EGF, estrogen, basic and acidic FGF, IL8, E1, and E2 prostaglandin, TNF-α and VEGF which are very free and can activate and be occurred when the growth of endothelial cells and when anti-angiogenesis factors have been decreased (Finetti et al., 2008). E2 prostaglandin (PGE2) is a mitogen in epithelial tumor cells, which is another example of one angiogenesis non-protein stimulus in vascular endothelium. Also, it has been raveled that the increased expression of cyclooxygenase2 (an enzyme to transform arachidonic acid to H2 prostaglandin) is accompanied by expression and production of factors such as VEGF, HIF-1, FGF-2, Matrix Meta Proteinases (MMPs) and integrin family adhesion receivers. Therefore, it was evident that high amount of PGE2 through cyclooxygenase-2 expression leads to the development of angiogenesis (Kajdaniuk et al., 2011). VEGF acts as the main factor of tumor angiogenesis and stimulates the growth of new blood vessels (Bhattacharya et al., 2016). Moreover, VEGF has direct effects on cancer cells and may reinforce the reproduction of cancer cells through VEGFR1 signaling activation (LaGory and Giaccia, 2016). Hypoxia in cancer microenvironment is an essential factor in stimulating this phenomenon (Bodily et al., 2011). Angiogenesis is generated by hypoxia or through reducing the level of oxygen in the tissue which resulted from the accumulation and reproduction of tumor cells. Cellular response to hypoxia is first regulated through the activity of hypoxia induction-factor transcription factor-1. In the reasonable condition of oxygen (normoxia), the HIF-1α subunit has a short half-life because of hydroxylation dependence on oxygen and its consequent destruction Von Hippel-Lindau (VHL) route/proteasome (Nakamura et al.,2009). Cells with HPV genome increase HIF-1α level and also increase the expression of HIF-1 target genes under hypoxic conditions so, under hypoxic conditions, the reduction of oxygen level leads to the HIF-1α accumulation that activates the expression of HIF-1 target genes (Yoo et al., 2006).

HIF-1α is regulated by several factors such as P53/CBP, P53, and HDAC under normal conditions. Although HDAC repetitively inhibits transcription, HDAV activity is necessary for HIF-1, and angiogenesis (Seo et al., 2009). HDAC5, HDAC4 (Qian et al., 2006), HDAC7 (Kong et al., 2006), HDAC1 (Fath et al., 2006), HDAC6 (Kong et al., 2006) and SIRT-6 (Longworth and Laimins, 2004) have been reported to activate or regulate the activity of HIF-1.

E6 & E7 Oncoproteins Activities

The extra limited factors by E7 are Histone Deacetylase (HDAC), which catalyzes histone deacetylation and other transcription supervisor proteins (Denslow and Wade, 2007). HDAC connection by E7 leads to the activation of cellular promoters, which are necessary for the virus life cycle dependent stage (Narisawa-Saito and Kiyono, 2007). E7 ability is related to reinforcing HIF-1 transcription activity through inhibiting HDACs connection to HIF-1α. It has been revealed that HDACs use different mechanisms to adjust HIF-1 activity. E7 translocate several HDAC of HIF-1α, including HDAC1, HDAC4, and HDAC7, which are related to activation of the E6 promoter. E7 prevents the connection of HDAC1, HDAC4, and HDAC7 to HIF-1α protein and leads to an increase in their activity. E6 first blocks the inhibitory effects of P53 in HIF-1 activity. It is concluded that E7 viral protein is responsible for reinforcing HIF-1 transcription activity and only E7 expression is enough for increasing the level of HIF-1 targets in keratinocytes (Nakamura et al., 2009). The performance of pathogenesis is related to E6, and E7 proteins that E6 destroys P53. P53 in healthy cells by arresting the cell cycle guides cells to regeneration and apoptosis, and therefore, the cell expansion will be continued. While E7 inactivates RB protein, the destruction of P53 may activate angiogenesis through producing VEGF and E7 is produced to release E2F transcription factors to promote the expression of genes with the condition like S phase (Toussaint-Smith et al., 2004). Destruction of P130 protein is expected to postpone distinguishable E7 and provides the virus with an opportunity to reproduce genome and inhibits thrombospondin-1, the potent angiogenesis inhibitor factor (Toussaint-Smith et al., 2004). E7 leads to abuse from PRB performance, which causes disorder in P21-RB route regulation, and as a result, the increase of VEGF and E7 is responsible for increasing HIF-1α activity. Studies show that E7 improves HIF-1-dependent transcription by inducing HDAC cleavage from HIF-1α. [92]. HIF-1α is a transcription factor that controls the expression of more than 40 different genes and encodes various cytokines and growth factors involved in angiogenesis, including VEGF. Also, E7 and P53 act independently to change the activity of HIF-1 and the effect of each does not change by the other (Bosch et al., 2002). As a result, these mechanisms contribute to angiogenesis in endometrial cancer HPV.

Conclusion

HPV has been implicated in the development of cervical cancer, which is a sexually transmitted infection, but can be prevented by following good hygiene principles. The mechanism of this cancer begins with the destruction of DNA by the HPV virus. Then, E6 destroys the P53 tumor suppressor gene, and TSP-1 angiogenesis decreases. On the other hand, E7 disrupts P21-RB by destroying PRB and increases HIF-1 through replacing HDAC4, HDAC1, HDAC7, and HDAC7. HIF-1 and other factors increase VEGF. As a result, they lead to the angiogenesis process in cervical cancer. It is hoped to generate methods through the advancements of technology and scientific knowledge to prevent the cancer progression by disrupting angiogenesis in the near future.

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