Review Article

Genetic Footprints of Human Papilloma Virus in Ocular Surface Squamous Neoplasia

Parisima Ghaffarian Zavarzadeh [1], MS; Yalda Yaghooti [2], Pharm.D; Seyed-Hashem Daryabari [3,*], MD

1. Basir Eye Health Research Center, Iran University of Medical Sciences, Tehran, Iran.
2. Faculty of pharmacy, Tehran University of Medical Sciences. Tehran, Iran.
3. Chemical Injures Research Center, Systems Biology and Poisonings Institutes, Baghiyatallah University of Medical Sciences. Tehran, Iran.

***Corresponding Author:** Seyed-Hashem Daryabari
E-mail: shdarya50@yahoo.com

Abstract

Ocular surface squamous neoplasia (OSSN) after melanoma and lymphoma is the third most common tumor. Including a wide range of ocular malignancy from mild form of epithelial abnormality to invasive squamous cell carcinoma (SCC). Overall, the lesions are different in size but mostly occur in specific tissue called limbus due to the structure of the eye ball, that is the most common area for sunlight exposing. Beside exposure to the sunlight and the UV radiation there are other risk factors and genetic co factors related to the OSSN including immune suppression under the infection to the Human immunodeficiency virus (HIV) or Human papilloma virus (HPV) and also mutation in specific genes regulating cell cycles. However, the exact relation between many of these factors and OSSN has not been revealed and need further researches in this area.

In the epidemiology area the prevalence of the disorder is different among geographical context, whereas Africa is with the highest frequency because of major rate of HIV infectants and high level of exposure to the sunlight.

Although surgical procedure is still the gold standard for OSSN treatment, despite that interest in finding the precise relation between risk factors and also efficient medical approaches in case of prevention, early detection and treatment is steadily growing.

In this review we aimed to study OSSN from different views specially risk factors and a glimpse of genetic insights specially related to human viral genome. Later, categorizing the role of them to pave the way in answering different questions related to the OSSN among types of that. On the other hand, although there is no evidence for straight relation between p53 mutations in OSSN, here we tried to mention the separated pathway's role in p53 malfunction or disabled in different process leading to cancers namely OSSN.

Keywords: Human Immunodeficiency Virus; Human Papilloma Virus; Ocular Surface Squamous Neoplasia; p53 Gene; Squamous Carcinoma.

Article Notes: Received: Jan. 10, 2020; Received in revised form: Mar. 02, 2020; Accepted: May. 9, 2020; Available Online: Jun. 23, 2020.

How to cite this article: Ghaffarian Zavarzadeh P, Yaghooti Y, Daryabari SH. Genetic Footprints of Human Papilloma Virus in Ocular Surface Squamous Neoplasia. Journal of Ophthalmic and Optometric Sciences . 2020;4(3): 54-69.

Introduction

Ocular surface squamous neoplasia (OSSN) includes a spectrum of corneal and conjunctival lesions with different severity from epithelial dysplasia to conjunctival intraepithelial neoplasia and invasive squamous cell carcinoma (SCC) [1]. It initially arises from basal members of cells in epithelium and invades the basement membrane while spreading towards the ocular surface. It usually accompanied a major blood vessel called sentinel or feeder vessel which could lead to epithelial basement cells disruption [2].

Individuals with OSSN can be asymptomatic or rarely present as bilateral or multifocal mass, but the tumors are often identified randomly on a routine eye examination [2]. Other symptoms are persistent tearing, redness and pain [2, 3].

In addition, OSSN lesions occur more often at the limbus (within the exposed part of the eyeball between the lids) in which the anatomy of the eyeball makes this a large collecting zone of peripheral sunlight, which, depending on the incident angle and radius of curvature of the cornea [4]. Hence, the limbus, lens and lid margin are the main foci of eye diseases occurred due to sun exposure, such as pterygium, OSSN and cataract [4].

Comprehensively, OSSN would classify into 3 forms benign, pre-invasive and invasive. The term OSSN usually excludes the benign forms, while the invasive type indicates degradation through the basement membrane of the conjunctival epithelium [4]. However, the various morphological patterns of OSSN show considerable overlap, and it usually becomes difficult to distinguish between various masses. Also, it is noteworthy that the large tumor masses invade deeper structures and are usually suggested to of malignant [3].

Conjunctival intraepithelial neoplasia (CIN) type, is non- invasive form, and the basement membrane remains intact [5]. The growth rate of a tumor is slow that could arise from a single mutated cell of the ocular surface [5]. (https://www.reviewofoptometry.com/). CIN could become invasive by breaking through the basement membrane, and reclassified to invasive form of OSSN called squamous cell carcinoma (SCC) which is described as a malignant lesion in which the dysplastic epithelial cells have penetrated the corneal or conjunctival basement membrane with metastatic potentiality [5].

Lastly, corneal epithelial dysmaturation, corneal epithelial dysplasia, and corneal intraepithelial neoplasia refer to neoplastic types of lesions in cornea which are other subtypes of OSSN lesions. [5]. On the other hand, the mucoepidermoid carcinoma count isa rare and aggressive form of SCC(5). However, it must be differentiated from SCC by histopathologic sampling as the invasive type has a worse prognosis with reported local recurrence rate is 5 %, and lymph node metastasis is less than 2 % [3].

Epidemiology

The clinical and pathological characteristics of OSSN are widely variated in different races and geographic areas [6]. OSSN incidence has been reported to range from 0.13 to 1.9 per 100,000 persons, along with the higher incidences in areas with greater exposure to sunlight such as Africa instead of areas with less sunlight (Denmark) [2]. Men in their 6th decade of life are commonly affected, but the disease could occur in youngers either, especially in association with diseases such as xeroderma pigmentosum (XP) or human immunodeficiency virus (HIV) [2, 4]. Studies in Africa report an increased incidence of OSSN in individuals with HIV compared to individuals who are not affected [4].

The highest incidence of OSSN is found in Africa (1.38 and 1.18 cases/year/100,000 in males and females), where both genders are affected equally, unlike other continents where the disease predominates in the male sex [4]. This probably indicates that African woman has increased risk due to their higher prevalence of HIV and HPV infections.

The USA has comparatively lower HIV prevalence, solar irradiance and incidence of OSSN (range from 0.3 to 8.4 per million per year) than Africa [4, 6]. Also, the mean age of diagnosis for African people is around 40 years, it was observed around 60s in Caucasian and Korean people [6].

According to one report from WHO, the prevalence of HIV/AIDS was 36.7 million in worldwide (in 2016). sub-Saharan Africa still remains mostly affected and includes almost two-thirds of the total new HIV infections globally [7]. In studies from sub-Saharan Africa, all OSSN patients went under HIV screening and seropositivity was detected in 49 %–92 % of cases, which indicate a high association between OSSN and HIV status specially in African countries [7]. All these data illustrate the significant relation between HIV and OSSN cases.

Risk factors

Some factors like the UV radiation, the Human immunodeficiency virus (HIV), Human papillomavirus (HPV) and male sex are highly correlated with the occurrence of ocular surface squamous neoplasia (OSSN) [6, 8]. Also, it is referred to immune-associated viruses, as the risk of developing this disease is increased by 20-fold in patients who were under liver transplant, and 10-fold for HIV infected virus types HIV-1 and -2, human papillomavirus (HPV) including serotypes of 16 and 18, and hepatitis B and C virus (which was reported in one study) play a putative role in the pathogenesis of OSSN (Figure 1) [1, 9]. While an Australian population-based study manifests that individuals with fair skin, light color of iris ,susceptibility to sunburn, those who spent more than half of the time under the sunlight during the first 6 years of life, and those living within 30° of the equators are exposed to the most significant risks for developing the tumor [1].

1. Damage in DNA

Damages in DNA are producing pyrimidine dimers in the DNA chain (Figure 2), whereas these CC → TT base pair dimers specially in the p53 tumor-suppressor gene allow cells with damaged DNA past the G1-S cell cycle checkpoint and OSSN occurrence [8]. Damages in DNA of ocular surface cells are probably mainly caused by solar UV radiation, which also could cause photo immunosuppression locally and reactivates viruses such as HPV [8]. The E7 viral proteins of HPV increase the proliferation rate of infected epithelial cells, while E6 viral proteins similarly to the mutation caused by UV radiation prevent the p53 tumor suppressor gene from arresting the cell-cycle of DNA-damaged and infected cells [8].

Overall, HIV, ultra-violet exposure and human papilloma virus (HPV) are the most prominent risk factors for the development of OSSN, though the significant role of HPV with OSSN is not still clear [10]. However other damages in DNA or either change in methylation pattern (specially CpG sites, which decrease the RNA transcription) affects genome stability which leads to cancer [8].

1.1. UV radiation

Different malignancies, including melanoma and SCC, are strongly associated with solar

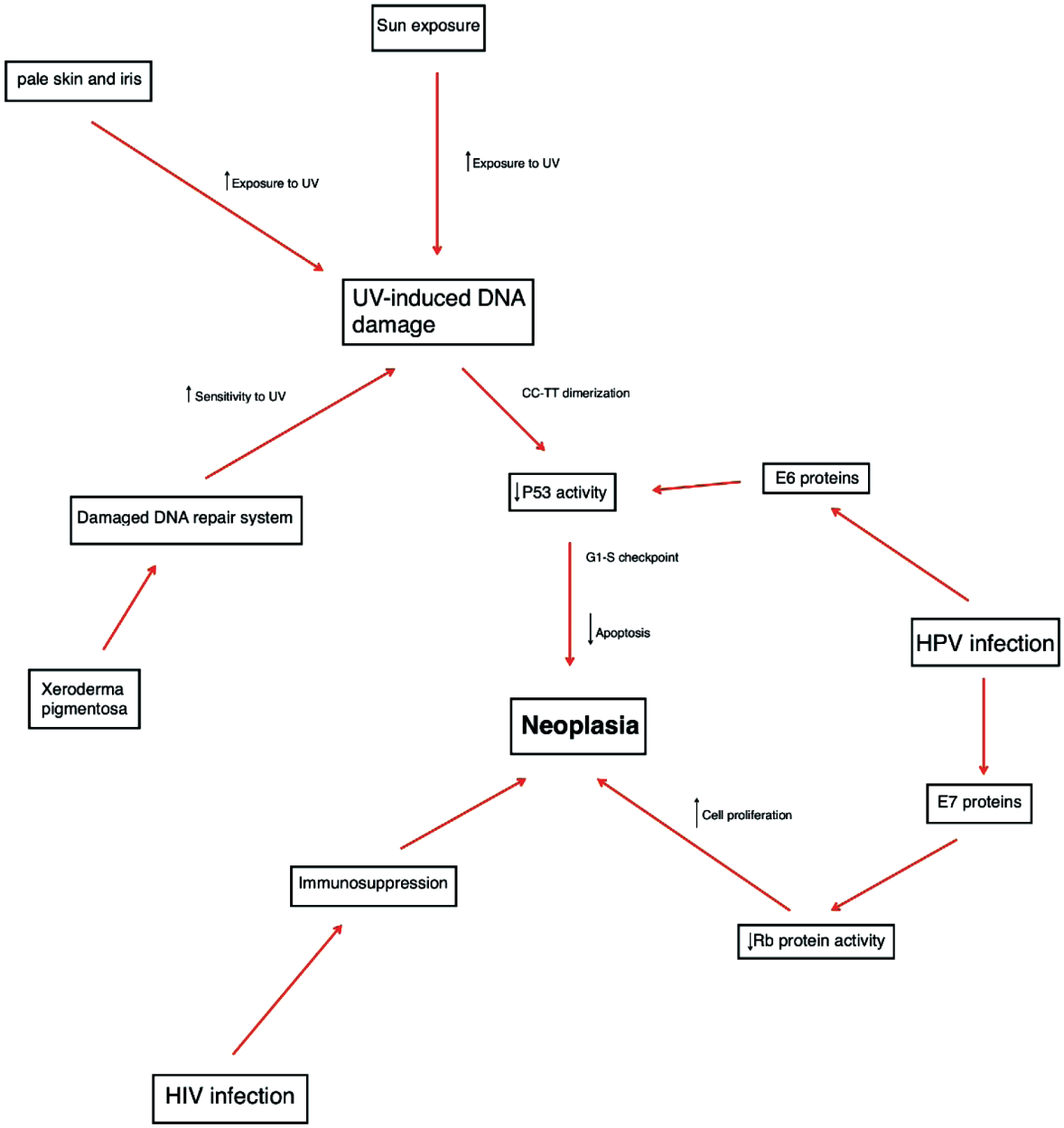

Figure 1: All risk factors included in OSSN

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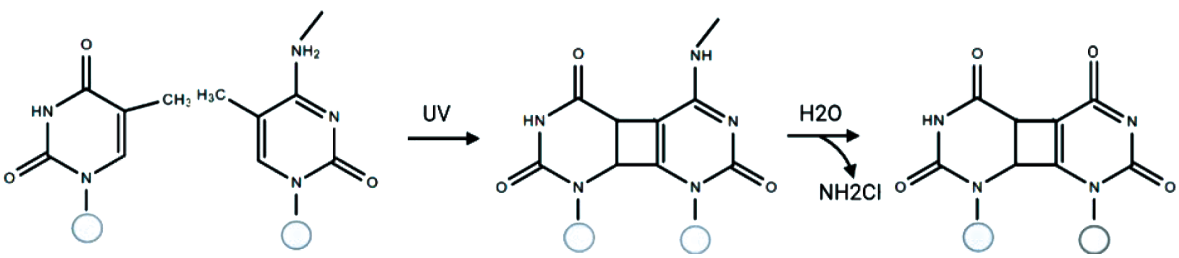

Figure 2: Pyrimidine base dimerization in DNA chain due to the UV radiation

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radiation [4]. Firstly, mentioned in the1960s that SCC was relatively common in East Africa, the most significant risk was related to higher sunlight exposure [4]. More recently, the National Institutes of Health/American Association of Retired Persons (NIH-AARP) Diet and Health Study in the USA found a slightly lower risk of SCC in those who based on the degree of the equator (who lived > 35 ° compared with ≤ 35 °). However, this was not statistically significant [4]. Also, a study in Australia based on case- control reported that the strongest risk factor was a history of skin cancer, although other factors, including amount of time spending under the sun exposure due to the outdoor activity, pale skin and irides and propensity to burn, were also important [4]. Other case–control studies described in Uganda in which 52 % of the cases had mutations in the Tp53 gene, compared with 14 % of controls these mutations were mainly of the pyrimidine dimers (CC TT type), consistent with UV-induced mutagenesis. [4, 11]. To conclude, as the cornea is at the surface and absorbs an approximate rate of about 90 % of the UVB that irradiates the eye's surface,, it is expected that it will sustain DNA damage more than other tissues [11]. Meanwhile, another study suggested that UVR could stimulate the production of the MMPs and growth factors (as downstream effector molecules) [12]. The molecules extensively studied in various human cancers might be related to the invasive and metastatic activities of tumor cells and are involved in the pathogenesis of SCC [12]. Regarding growth factors, an animal model-based study revealed that over-expression of FGF-7 (a member of the fibroblast growth factor (FGF) family) in the cornea could contribute to corneal epithelial hyperplasia or tumorigenesis [13]. Both p53 and PP5 (a serine/ threonine phosphatase) could impact the expression level of this growth factor, which indicates that PP5 overexpression inhibits p53 phosphorylation (and consequently tumor suppressor activity) and increases FGF-7 expression [13]. This study's findings declare that the PP5 plays an essential role in the development of corneal hyperplasia; the mice model of this study with highly expressed PP5 developed corneal hyperplasia and Ocular Surface [13]. Notably, most of the mutated genes driving OSSN pathogenesis are related to the cell cycle and DNA repair systems genes caused by UV sunlight exposure [14]. Hence, there should be actionable drugs targeting these genes and testing novel therapeutic options in OSSN [14].

1.2. cell cycle

The cell-division cycle involves duplicating a cell's intracellular components including the genome and organelles [8]. DNA damage activates checkpoints and related pathways that regulate specific DNA repair systems in the different stages of the cell cycle [8].

There are three important cell-cycle checkpoints, which will shortly explained in this section. Overall, the G1-S checkpoint prevents cells from damaged DNA to enter the S phase, while the intra-S-phase checkpoint contracts with DNA damage that may occur during S-phase or damage that escaped the G1-S checkpoint. Finally, the G2-M checkpoint simply inhibits cells with DNA damage from undergoing mitosis division [8].

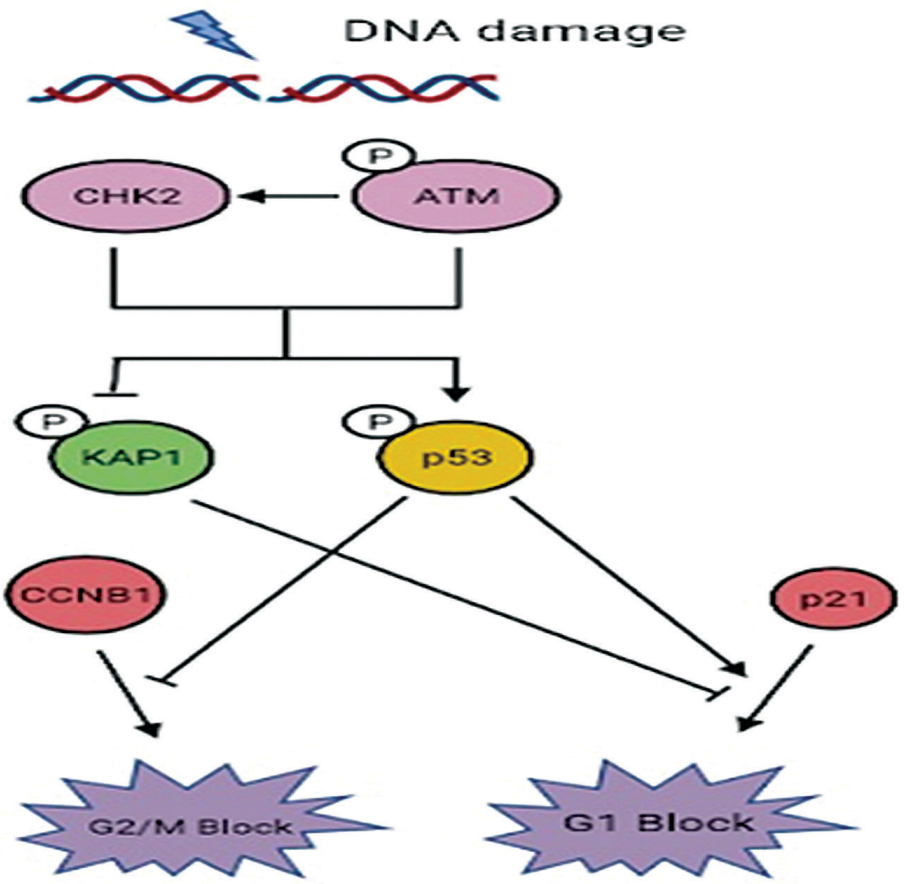
Surprisingly, years of research have declared that p53 (guardian of genome) as a tumor suppressor gene hinders transformation to neoplastic and tumor progression by its vigorous ability in cell cycle and detecting DNA damages [15]. This phosphoprotein is located in the nucleus to regulates cell cycles and protect cells from increasing the mutated

DNA components [8]. The responses are namely, protection at the G1-S checkpoint; functions related to programmed cell death (apoptosis) if the damages are not reparable [8]. Therefore, with all of these responsibilities, the mutated p53 could solely lead to further genomic instability (further information available in figure 3) [8].

1.3. Damage in DNA repair system

1.3.1. Xeroderma pigmentosum

Xeroderma pigmentosum (XP), is a rare genetic disorder related to the skin with a prevalence of 1:250 000 (a high prevalence of 40 % of the Tp53 mutations) worldwide [16]. Characterized by high sensitivity to UV damage owing to deficient DNA repair system associated with various cutaneous and ocular malignancies such as squamous cell carcinoma and basal cell carcinoma [16, 17]. Damages in the DNA repair system routinely could happen through various ways although with the help of DNA repair system it could be reduced. While any mutations in genes controlling this process, could prevent DNA repair system working properly and eventually lead to disease such as Xeroderma pigmentosum (XP). As mentioned above numerous mutated genes are moving to XP which are majorly involved in NER repair systems specially [8]. Therefore, if the system can't prove the existent mutation, XP



Squamous Neoplasia.

Figure 3: Role of damaged p53 in cell cycle arrest after breakage in DNA double strand

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could be attributed with other malignancies, additionally, in corporation with p53 mutations which play a fundamental role in DNA damage repair process [8].

Accordingly, as people with XP have defected DNA repair system, they have a high chance of recurrence or persistence of tumors, hence it requires rigorous follow-up and screening for early detection and treatment of a wide range of oculocutaneous malignancies specially OSSN [16].

Along with details above, the UV radiation, or other spontaneous mutations could also damage in DNA in daily lives, however DNA repair system or other related mechanisms would tackle this issue. Though the damages in DNA repair system or genes controlling of that, could not barrier the consequences from any types of mutations the occurrence of the disorders such as OSSN and association of that with XP or similar syndromes is inevitable.

2. Immunity deficiency

A meta-analysis study revealed the incidence of cancers among both HIV/AIDS patients and immunosuppressed recipients from transplant surgeries is increasing [8]. However, beside a direct association there was no significant clues related to the action of HIV, therefore it was suggested that the primarily immunosuppression in patients is the cause of happening other malignancies [8].

Meta-analysis studies and reviews illustrate that the main risk factors for ocular malignancies, such as UV radiation and as mentioned before are HIV and human papilloma virus (HPV); while vitamin A deficiency is a capable risk factor but still needs further investigation [8]. However, the clear risk of ocular surface epithelial dysplasia among HIV infected people (and among immunosuppressed patients due to the renal transplantation) suggests a role for infection to viral genome in the etiology of the disease [18].

A general process called tumor immuno-editing could probably have a role in this area [8]. It is a twofold process of action The immune system may either suppress tumor growth by destroying or inhibiting growth of cancer cells or mistakenly promote tumorigenesis by selecting tumor cells that survive in 3 phases: elimination, equilibrium and escape [8]. Whether a tumor cell pass the equilibrium (between normal-tumor cells) to escape phase, eventually the tumor will growth and occurrence of the disease is no more peculiar.

However, infecting to viruses from the Herpesviridae family including HSV, HCMV, EBV, and KSHV may contribute to the oncogenic process in OSSN, same with the results obtained from their association with other human cancers [19].

2.1. HIV

The human immune deficiency virus (HIV) is a retrovirus that majorly infects and replicates in CD4 T lymphocytes by establishing latency in the resting memory of these cells [8]. In fact, immunosuppression due to HIV-infection has been strongly associated with OSSN, as various studies have demonstrated a 10-fold increase for OSSN in these patients [20].

Uganda, was the first country to report a dramatic increase in the annual incidence of SCC (squamous cell conjunctival) shortly after the outbreak of HIV/AIDS (sixfold increase) [4]. And, in Tanzania, 60-77 % of OSSN patients seen in Africa were infected by HIV [4]. Similarly, remarkable raising in the USA patient observed with the onset of the HIV pandemic (lower results thanto the Africa) [4].

However, the level of contribution of HIV to the development of human malignancies

remains unclear and is likely to be through several mechanisms, but further likely to be related to the immune suppression process [21]. As a result, the disease might manifest more aggressively in HIV patients due to their immune dysfunction, abnormal cytokine and chemokine expression, growth factor production and exposure to ultraviolet rays [21]. Although numerous literature and scientific researchers report that HIV might be in association or in relation to the disease, it is peculiar to conclude whether two diseases disappear with each other based on their same time manifestation and without any other evidence. Hence, there should be further investigations to obtain the relation and molecular basis between HIV and OSSN.

2.2.HPV

The accurate association between the OSSN and HPV is still conclusive, however, it appears that the HPV genotype as well as other associated factors play a crucial role in the pathogenesis of the disease [20].

The prevalence of HPV in OSSN (range from 0-100 %) majorly depends on geographical distribution with significant accompaniment of subtypes HPV16 (one of the most carcinogenic agents known for human) and HPV18. While in overall detail 33.8 % of OSSN and 18.6 % of pterygia cases are HPV positive, further studies have suggested that just the cutaneous subtype of HPV is correlated with the OSSN [4 , 9 , 20].

Notably, as viral DNA sequences can be detected in tumor tissue regarding to continuously expression level of the viral E6 and E7 proteins in tumor cell line, suggest that these proteins are necessary for growing [22]. Overall, detection of E6/E7 mRNA transcripts through the quantitative reverse transcriptase–PCR has been the gold standard method for HPV testing, but it should be considered that RNA as an unstable component would limit the results [4].

Far more, HPV types 6 and 11 have been announced to cause conjunctival papilloma and other dysplastic malignancies of the ocular tissue, while there is a close link between HPV types of 16 and 18 with CIN [3]. As mentioned above, only the cutaneous subtypes seem to be a risk factor associated with OSSN, whereas the mucosal subtype evidencewas weaker [23]. Also, proteins of HPV such as E6 and E7 are oncogenic and have been reported with cell immortalization and antiapoptotic functions [21]. The HPV types such as 6, 11, 16, 18 and 33 have been either observed in benign and malignant conjunctival lesions [21]. By these facts, Investigating the DNA and RNA sequences of HPV cases could help obtain which type of HPV among 170 genotypes of HPV are more related to OSSN and manifest the disease.

In another study, HPV16 was solely detected in 11 % of OSSN cases with specificity in 9 % in SCC patients and 15 % of dysplastic ocular cases [24]. While in minor study of 10 CIN-patients from United States, the DNA and mRNA region of HPV 16 or 18 which codes to the E6 protein was detected in all 10 specimens, and importantly in none of the control specimens [25]. Hence this is consistent with previous studies that showed cervical carcinoma in which the E6 protein was encoded in the cancerous cells by HPV 16 and 18 [25]. The E6 protein has been shown to interact with p53 tumor suppressor genes to promote tumorigenesis, which may be involved in HPV 16 and 18 in the development of OSSN [25]. Although strong evidences have been recorded between prevalence of HIV and OSSN, still further studies needed to confirm this possible association.

On the other hand, beside UV radiation's role on OSSN, it has been revealed that it could reactive the HPV virus and cause local immunosuppression [26, 27]. Also, it has been reported that only E6 and E7 proteins of the HPV genome enhance human Rb and p53 genes respectively [4, 20]. Where the E7 protein induce proliferation in infected epithelial cells with the help of the Rb gene, and at the same time, E6 proteins stop cell cycle arrest of infected and damaged cells by restriction of the p53 gene in [4, 20]. (Figure) On the other hand, as the p53 could also downregulates the replication of HPV type 16, suggesting that its mutation separately or any inhibition of hat may allow replication of HPV particles [4]. Aims for detection of p53 protein in HPV-transformed cell lines beside high levels of p53 mRNA resulted in nothing observable. [22]. Therefore, the lack of p53 proteins in cancer cell lines, suggested that the translation level of p53 mRNA was in some way repressed, or that the turnover of p53 protein was increased in HPV-transformed cells [22]. Further studies revealed that the E6 proteins from HPV-16 and HPV-18 are able to bind to p53, this binding process result in the degradation of p53 with the ubiquitin pathway [22]. To expand the pathway related to degradation of p53 protein, first the E1 which is a ubiquitin-activating enzyme activates ubiquitin pathway (Ub), later transferred by the E2 ubiquitin-conjugating enzyme to the E3 enzyme (a type of ubiquitin-protein ligase) [22]. HPV E6 binds to E6-AP, activating the E3 enzyme which specifically binds and ubiquitinates p53, and finally the p53 protein which is polyubiquitinated is then degraded by the proteasome complex (Figure 4) [22].

Here, in contrast with other types of cancers

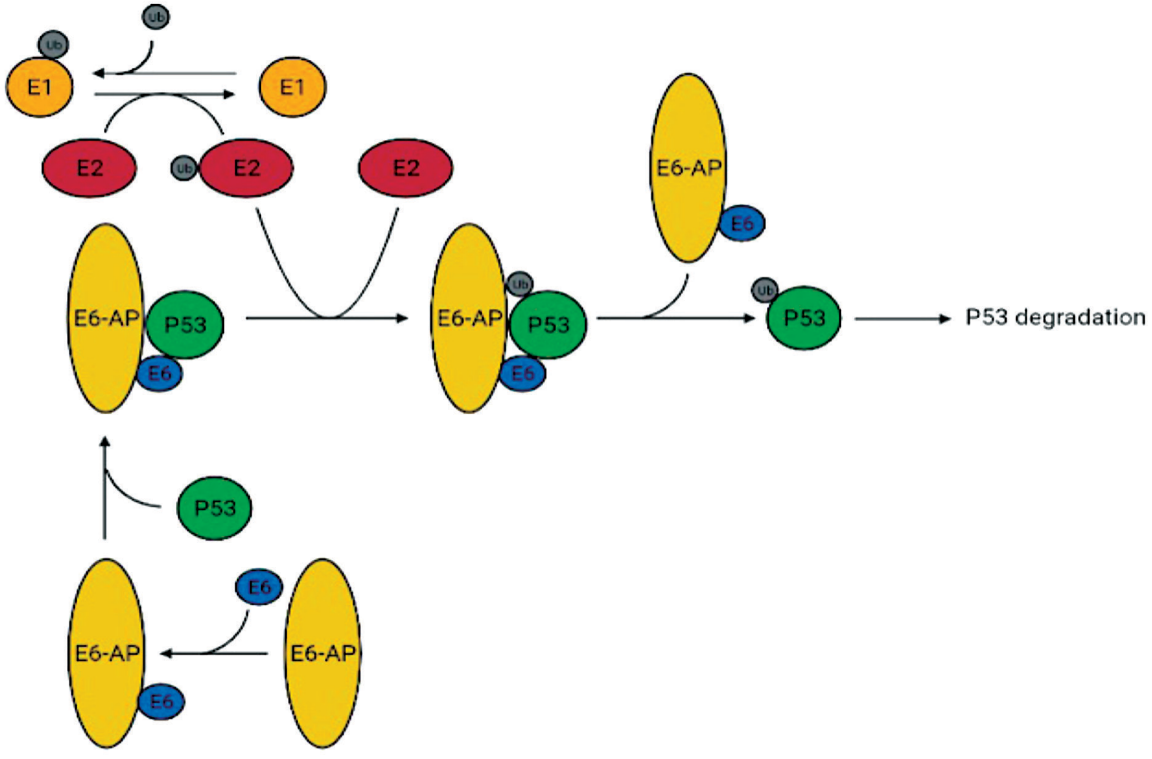

Figure 4: Role of HPV proteins and E6-AP in p53 degradation

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the p53 is almost wildtype (E6 are analogous to an inactivating mutation), however it could affected improperly owing to the E6-p53 complex that prevents the p53-mediated transcriptional repression of promoters that contains TATA-binding sites, result in repressing the function of promoters which are related to the p53-responsive elements, in addition it inhibits the p53's growth arrest and apoptotic processes [22].

Other point is that although the E6 protein from HPV-1, which is clinically classified as low-risk, has been shown to have numerous effects p53 gene, this could be concluded that even low-risk type of HPV could have deleterious consequences [22].

According to the cell cycle, the G1 checkpoint activity in cell might become abandoned due to the degradation of p53 [22, 28]. Also, the G2 checkpoint is not affected by this mutation but there is increasing in the chromosomal instability rate of cells which express E6 is probably because of the weaken G2 checkpoint function, as the p53 is not efficient to control this checkpoint either [22, 28].

From another point of view, the p53 have two polymorphisms including p53 Arg and p53 Pro at residue of 72 (where is reported to be increased risk of neoplasia in one study from Uganda including 107 cases- 115 controls), while they show completely different action [18]. Therefore, it is important to attention to the interaction of each residue with HPV E6 protein.

As we have discussed above, the exact interaction of the E6–p53 could differ according to HPV type, and intra-typic variations in E6 sequence [22]. To conclude, both p53 gene and HPV types could interact differently. However, understanding the specific relation between p53 variants and any type of HPV especially variation in E6 protein needs deeper investigations.

A systematic analysis of the interactions of E6 prototypes and the polymorphic forms of p53 related to the E6-AP complex, may obtain different results which may shed a light for treatment and targeted therapies [22]. Although the presence of p53 in viral replication complexes at the same time is not clear that whether it is for attempt to reduce the replication or because the virus has recruited p53 to assist replication [22].

However, it has been imposed that p53 might be involved with much different DNA of tumor cells at the level of viral DNA replication [22]. The E6–p53 association is undoubtedly of fundamental importance in the pathogenesis of HPV and it will be an important target for controlling and therapeutic procedures among HPV related cancer.

However, investigation of viral sequences in OSSN samples, could pave the way to understanding the relationship betweenthese viruses and cancerous cells and the differences between natural immune system and immunosuppressed system for resonance to the disease [21].

With the progress in sequencing methods, more than 100 genotypes of human papillomaviruses have been sequenced, resulting ina significant amino acid variation within the cases to suggest different HPV types, which are continuously being found in the future [4]. Investigation through viral sequencing obtained from HPV and specific variants show that each of them specifically, role as oncogenic and might trigger cell proliferation leading to OSSN and other cancers in patients with immunodeficiencies [4, 20, 21].

In this section, we aimed to obtain details scientific report among cases prepared to illustrate significant relation among HPV and OSSN disorder. In one study, the 38 patients

with who underwent surgical excision of OSSN, went through tissue sampling using IHC and DNA chip test to obtain 24 HPV serotypes [20]. As only 12 cases (31.6 %) illustrated strong positivity, this study conclude that maybe there is a weak association between two diseases [20]. Other studies related to this association in Africa and Australia revealed conflicting results, for instance African study found strong p16 immunoreactivity (IHC) in 67 % of the OSSN cases, whereas in Australia related to corneal and conjunctival squamous lesions reported just a 6.5 % positivity of HPV [20].

Among HPV serotypes, the high-risks such as types 16 and 18 are currently considered as the most strongly associated with OSSN, however p16 IHC had some pitfall on accurate detection of these types, hence DNA chip test and PCR-microarray-based method could be beneficial in viral detection [20]. To conclude, Herpes simplex virus might be considered as possible contributor factors for developing OSSN. Same as immunosuppressive disease namely HIV, which thought to have same effect related to OSSN [20]. In other words, HPV might not manifest as the exact cause of OSSN but still it may be involved in the tumorigenesis process. It is noteworthy to mention that in most of researches in area of measuring the association of HPV and OSSN, the exact HIV status of patients were not recorded therefore, the result might be further reliable if these data were provided [20]. A study combined the interaction of HPV with head and neck carcinoma including oral, oropharyngeal and SCC revealed that among total 93 patients with different malignancies [29]. Among these, 23 patients were HPV positive in which they show that the value of HPV- related cervical metastasis is highly significant or a primary oropharyngeal tumor [29]. A wide range of methods has been utilized for HPV detection, in another study p16 immunohistochemistry was used in OSSN case series and this method showed an excellent correlation between HPV positivity and OSSN [30]. However, other ways failed to detect evidence for HPV in patients with OSSN, hence the conflicting in results about the correlation between HPV infection and occurrence of OSSN would be problematic in case of obtaining the exact influences [30]. Although, through all observations, p16 induction is not directly linked to HPV infection, the results suggest that HPV could contribute to more aggressive type of OSSN [30]. Consistently, further studies are demanded to determine if the higher expression of p16 in SCCs is related to the HPV infection, or from other etiologies such as genetic damages, various radiation or inflammation. [30].

Another scientific review gathered data from twenty-two separate studies, where in four studies performed in Uganda and one in Mozambique reveal the association between both cutaneous and mucosal HPV subtypes and OSSN, although only the cutaneous subtypes is possible to be a risk factor which is in contrast with results from small sample size studies yielded stronger estimates between the mucosal subtype of HPV and OSSN [23]. At this stage, according to the conflicting results on the likelihood of relationship between HPV and OSSN, and as the mucosal subtype got lower frequency, targeted studies ought to be designed to look for declaring the subtypes with the help of viral DNA sequencing.

According to recent information, and genetic factors namely mutations in p53 gene, environmental factors, such as UV radiation, HIV co-infection and, various HPV genotypes appear to function a significant role in lesion pathogenesis [20]. The disagreements in the results might be due to the different

geographical contexts and the genetic methods of detecting HPV serotypes [20]. Although each method has different sensitivity to obtain viral genomes, however a gold standard procedure should be taken that beside efficiency it is cost effective either. In addition, further researches are needed to declare exact relation of HPV and OSSN in the case of the molecular process which would undoubtedly pave the way in prevention or treatment of HPV- related disorders. Along with this issue we could decide if the purpose of using antiviral drugs or vaccination against HPV is true or not.

On the other hand, finally it is noteworthy to consider the role of HIV (at least by declaring the HIV infection status of patients) , as it increases the risk of obtaining HPV and far more HPV- associated carcinomas, due to host immunosuppression [30]. Along with this fact, as the prevalence of OSSN recorded to be higher in HIV patients (3-30-fold), and with the controlling role of HIV on HPV, hence it is not so peculiar to construct a vigorous connection between HIV and HPV [30].

Diagnosis and treatment approaches

In the case of ocular disorders, through the development of high-resolution imaging modalities, histopathological investigation of specimens obtained from the biopsy was traditionally the sole method for diagnosis, especially the grade of OSSN [31]. On the other hand, "optical biopsy" with Anterior Segment High-Resolution Optical Coherence Tomography (HR-OCT) is a novel diagnostic approach for Clinically Ambiguous Ocular Surface Lesions [32]. It is a non-invasive, non-contact method of detecting thickness and differentiating epithelial lesions from another area, primarily used for OSSN diagnosis [31].

Traditionally various surgical approaches were the only way to tackle OSSN, however, it has proven inadequate for preventing disease recurrence and became less favored to around 51 % after 2012 [31, 33] Currently, among corneal specialists, topical chemotherapy is more favored in that it counts as a "Standard of care" for managing the OSSN [31]. Whether it is utilized for preoperative procedures (chemo reduction) or hindering disease reoccurrence after the surgery (chemopreventive) [31].

The interferon-α2b (IFN-α2b), 5-fluorouracil, and mitomycin C (MMC) are three well-known and Morlu used in this area, though anti-vascular endothelial growth factor (anti-VEGF), retinoic acid and aloe vera have also been studied they are less prominent. The efficacy is not apparent [31, 33]. Hence, this article aims to include the top three chemotherapy approaches previously mentioned. Interferon-alpha is an immunomodulatory cytokine produced by human leukocytes and, along with interferon beta, forms the group of type I interferons [31]. They have various capabilities, including apoptosis induction and inhibition in protein synthesis, whereas their prominent effect is in virus-infected and neoplastic cells [31, 33]. It has been illustrated that they would inhibit viral replication and mitosis in cancerous cells [31, 33].

In the case of managing OSSN with IFN-α2b, two different formulations are used, a topical drop or a subconjunctival perilesional injection [31, 33]. As a primary option, IFN-α2b's overall success rate was 76–100 % 1. It is mainly used for successfully treating recurrences (decline the rate from 13 % to 4 %) [31]. Patients receiving each formula ought to use different doses. Side effects are minor, namely, mild irritation and follicular conjunctivitis [31, 33].

Interferon also has alternative uses. For instance, it is administered for infectious diseases, including chronic hepatitis B and

C, and for treating conjunctival papilloma, lymphoma, and conjunctival Kaposi sarcoma in the case of ophthalmology [33].

On the other hand, 5-FU (analog of uracil) inhibits the thymidylate synthase (TS) and impairs DNA and RNA synthesis. Hence limited the accelerated cancer cell proliferation (also impacts normal cells with rapid growth such as epithelial cells and fibroblasts) [31, 34]. Although long-term effects of 5-FU have not been documented, one study on OSSN cases observed a range from 1.1 % to 43 % rate of tumor reoccurrence with more side effects- less cost than the interferon [31]. In ophthalmology, other alternative uses of 5-FU are for glaucoma, pterygium, and vitreoretinal surgery [33].

Due to the antitumor activity (antineoplastic/ antibiotic features), MMC is used as a chemotherapic agent [31]. It is an antimetabolite agent obtained from Streptococcus caespitosus, inhibiting mitosis [33]. Various dosages of these agents have been used to treat OSSN remarkably; the higher concentrations result in more epithelial toxicity [31]. It could be used for the treatment of recurrences, in which it has successful results of decreasing recurrence from 66.7 % to 5.9 % [31, 33]. Other uses of this chemotherapic agent in the ophthalmology area include the treatment of pterygium, glaucoma, refractive, and oculoplastic [33].

Discussion

OSSN as the third most common malignancy after melanoma and lymphoma, is described with tumors ranging from surface of epitheliums to inner basement cells [4]. UV radiation, HIV and HPV include the major risk factors of OSSN and with higher prone ability to these factors in Africa, this may explain the high incidence rates of the disease in the area. Although the effect of xeroderma pigmentosa is still unclear [4].

Thoroughly, during the early stages, OSSN does not affect the sight health however, after years it could lead to eye disfigurement and finally death in the last stages [4] Hence, regardless of the current available options such as early detections and some accurate treatment, we should aim to observe further non-invasive diagnostic methods for diagnosing and proper treatment.

Various models have been proposed to distinguish the role of risk factors in OSSN cancer specially the social and environmental factors. Besides, attention to the biological is necessary to make a clear knowledge about the disease. Due to the hidden impact of OSSN, it has been widely neglected by both eye and HIV care programs.

As mentioned before, no early non-invasive diagnostic methods have been proposed. Therefore, investigating the DNA and RNA sequences of HPV cases could help us obtain which type of HPV according to 170 genotypes of HPV are more related to OSSN. The progressions in non-invasive diagnostic techniques and treatment procedures could lead to a considerable decline in tumor-related morbidity [1]. Also, diverse sequencing approaches could be beneficial as provide further clues between the HPV and OSSN or to investigate which type of HPV and their proteins are significantly involved in mechanisms of the disease. On the other aspect, prolonging between the time of manifestation of symptoms and diagnosis of tumor is noted in a few studies from Africa, hence, such observations and largeness of the lesion imply that early sufficient medical care has not been approached by most of patients and in addition they received a wrong treatment for other ocular conditions due to misdiagnosis [7].

All in all, numerous years of research and

clues in the area of OSSN come up with questions in the mind of researchers about why the disease is mostly unilateral while both eyes receive equal sunlight exposure? And also, further investigation is needed to obtain unknown driver genes and molecular level that convert intraepithelial neoplasia to squamous cell cancer which could be metastatic and hazardous still demand to be answered.

Authors ORCIDs

Parisima Ghaffarian Zavarzadeh:
https://orcid.org/0000-0002-9549-3260
Seyed-Hashem Daryabari:
https://orcid.org/0000-0002-7264-8637

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Footnotes and Financial Disclosures

Conflict of interest:

The authors have no conflict of interest with the subject matter of the present manuscript.