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Review Article

A Review of the Molecular Pathways of Oncogenic HPV and Targeted the Rapies in Carcinoma of the Uterine Cervix

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Abstract: Cervical carcinoma is a preventable disease based on its etiopathogenesis. This relies highly on its prompt diagnosis and treatment based on effective screening and treatment methods. Knowledge of its molecular pathways will drive the use of targeted therapies in the treatment of the disease. The objectives of this review are: to explore the biology and immunology of HPV; the various molecular pathways through which oncogenic HPV destroys normal cervical cells; and targeted therapies. Several molecular pathways have been identified but the use of drugs to act on specified molecular targets are at different stages of clinical trials.

Keywords: Cervical Cancer, Molecular Pathways, Targeted Therapies

1. Introduction

Carcinoma of the uterine cervix causes severe morbidity and death in the developing countries where screening services and treatments are deficient [1].

Persistent infection with the high risk Human Papillomavirus (HPV) is a prerequisite for cervical cancer development [2].

HPV is spread by skin-to-skin contact and sexual intercourse [3]. Cervical infections are mostly asymptomatic or unrecognized and a vast majority of the infections clear off with no sequelae within 2 years [3]. The persistent infections progress over a latent period of 10 – 15 years to varying abnormal proliferation of cells and spread of hypervascularized necrotic tissues that characterize cancer of the uterine cervix. The overexpression of E6 and E7 oncoproteins by HPV is the main oncogenic stimulus for the cellular transformation in cervical cancer [4]. The identification of pathways such as PI3K/Akt, Wnt/beta Catenin, EGFR/VEGFR, Apoptotic, etc, provides a window of opportunity for targeted therapies. Most of the treatment modalities; surgery, radiotherapy or chemoradiation are ineffectual when the disease is far advanced [4]. Carcinoma of the uterine cervix is a major cause of cancer related deaths in

women especially in developing countries [5]. Understanding the molecular pathways and the development of safe and effective targeted therapies will improve overall outcomes in this disease.

The objectives of this review are: to explore the biology and immunology of HPV; the various molecular pathways through which oncogenic HPV destroys normal cervical cells; and targeted therapies.

1.1. Biology and Immunology of HPV

Papillomaviruses are small, double-stranded circular DNA viruses with non-enveloped icosahedral capsid [6]. They are epitheliotropic and infect mucosal and cutaneous epithelia in a species-specific manner and induce cellular proliferation [7]. There are five genera; alpha, beta, gamma, mupa and nupapapillomaviruses [8]. Humans are the only known reservoir for HPV which are difficult to grow in-vitro but have been characterized by molecular methods based on the molecular similarities of their genetic materials [7]. Propagation of viruses in organotypic cultures, HPV DNA cloning and molecular hybridization has enabled complete and partial genomic sequencing used in identifying the viruses as well as the mechanisms which regulate viral gene expression and replication [6]. HPV genome contains eight

Open Reading Frames (ORFs) that all transcribe from a single DNA molecule of about 8,000 base pairs. The early region encodes proteins E1, E2, E4, E5, E6 and E7 necessary for viral replication, while the late region encodes the structural proteins L1 and L2 required for virion assembly. There is also the non-coding called long control region (LCR), which contains the *cis* elements that are necessary for the replication and transcription of viral DNA [8]. HPV16 is the most oncogenic of the strains of Human Papilloma Viruses [6].

HPV infects the epidermal cells of cutaneous and mucosal skin, without penetration into the dermal tissues [7]. The body is able to get rid of transient HPV infections while the HPV is still a commensal with no productive viral infection, or the HPV is shed with the dead epithelial cells following desquamation [7]. The virus may persist for months or years because of several mechanisms of immune evasion [9, 10], one of which is regulation of E-Cadherin expression and Langerhans cell density by E6 [8]. Since HPV infection is restricted to the epithelium, with no reliable or effective host immune response, clearance must be mediated by local immune defenses [11]. Cell Mediated Immunity may play a key role at the cellular level for many reason: (a) the squamous epithelium is rich in keratinocytes which can act as non-professional antigen presenting cells (APCs) capable of secreting pro-inflammatory cytokines and chemokines and can induce the activation of CD4 and CD8+ memory T cells into a functional cytokine-secreting and cytotoxic state; [12] (b) there is a preponderance of T lymphocytes, NKT, NK and some B cells at the epidermal-stromal junction beneath the basement membrane of the human cervix; [13] (c) The normal vaginal lavage has also been found to consist predominantly of granulocytes including neutrophils, macrophages, T and B cells [14] which are innate and adaptive effectors; (d) low-grade and high-grade squamous intraepithelial lesions which have persisted or progressed have been shown to have increased numbers of macrophages, neutrophils and lymphocytes in the superficial epithelium compared to the stroma in contrast with uninfected skin or infection with non-carcinogenic types. [11, 15] In the cervix, Langerhans cells are specialized epithelial dendritic cells that function in the uptake of antigens. As they migrate to the draining lymph nodes, these cells change into potent antigen presenting cells (APC) with enhanced expression of Major Histocompatibility Complex (MHC) [6]. The production of Th1 cytokines (interleukin-12, tumor Necrosis Factor (TNFα) and interferon gamma (IFNy) was beneficial while the Th2 response with production of interleukin 4, 5 and B cells) was deleterious [6]. Normal ectocervical epithelium neither expresses detectable levels of class II antigens, nor did expression of HPV gene in culture epithelium result in class II expression. This suggests that the upregulation of class II expression was mediated by the proinflammatory cytokines IFNy and TNFα. HPV E5 oncoprotein blocks IFN-induced surface expression of class II MHC [6].

1.2. Pathogenesis

Infection by oncogenic papillomaviruses requires that the virus gains access to the epithelial basal layer and enter the dividing basal cells [9]. The life cycle is intraepithelial, produces no viraemia, cell lysis, or cell death, and replication is not associated with inflammation [16]. Integrated HPV DNA is found in almost all invasive cancer and in some high grade lesions [17]. p16 INK4A which is a marker of elevated E7 expression can be detected in CIN lesions that show evidence of integration [18]. Integration often results in loss of E2 and leads to deregulation of E6/E7 [9]. HPV E7 protein binds and degrades p105 and p107 which control cell cycle entry in the basal layer as well as p103, which is involved in cell cycle re-entry in the upper epithelial layer [16]. The interaction of E7 with retinoblastoma protein (pRb) results in the degradation of pRb and the release of E2F transcription factors. E2F subsequently transactivates cellular proteins required for viral DNA replication such as Cyclin A and E [9]. The continuous stimulation of S-phase entry and cell proliferation by E7, coupled with the loss of p53-mediated DNA repair pathways as a result of E6 expression, allows the accumulation of secondary point mutations in the cellular genome that eventually lead to cancer.

HPV E5 is a transmembrane protein that resides predominantly in the ER (endoplasmic reticulum), but which can associate with the vacuolar proton ATPase and delay the process of endosomal amplification, leading to an increase in EGF (epidermal growth factor) mediated receptor signaling and the maintenance of a replication competent environment in the upper epithelial layers [6]. E4 accumulates in the cell at the time of viral genome amplification and associates with cyclin B/Cdk2 and relocates the complex to the cytoplasm. This prevents the nuclear accumulation of cyclin B/Cdk2, which is necessary for its progression during mitosis. E4 causes cell-cycle arrest in G2 and antagonizes E7-mediated cell proliferation. E4 can disrupt the keratin network of the cervix and affect the integrity of the cornified envelop. The effect of E4 is to keep the infected cell in a metabolically active state without competing with host DNA synthesis, and so boost viral genome replication [6]. HPV E5 also inhibits gap-junction intercellular communication [19] and withdraws transformed cells from the homeostatic control of neighbouring cells.

HPV E6 and E7 oncogenes also disrupt innate immune response by inhibiting type 1 interferon signal transduction pathways. During natural infection, however, the ability of E7 to drive cell proliferation is inhibited in some cells depending on the levels of p21 and p27 cyclin-dependent kinase inhibitors [9]. High levels of p21 and p27 in differentiating keratinocytes can lead to the formation of inactive complexes of E7 and cyclin E within the cell [9]. High-risk E6 on the other hand mediates p53 ubiquitination and proteosome-mediated degradation, by forming tripartite complex with p53 and cellular ubiquitin ligase E6AP (E6-associated protein), which also associates with Bak and Bax anti-apoptotic proteins [9]. Low-risk E6 proteins bind p53 with a lower affinity than the high-risk types and have no significant ability to bind E6AP and to stimulate p53 degradation. The disruption of the p16/pRb pathway by HPV 16E7 leads to its immortalization [20].

High risk E6 and E7 proteins drive cell proliferation through their association with PDZ domain proteins (PSD-95, a 95 kDa protein involved in signaling; Dlg, Drosophilia discs large protein; and ZO 1, the zonula occludens 1 protein which is involved in maintaining epithelial cell polarity), and antagonize BRCA-mediated inhibition of the hTERT (human telomerase reverse transcriptase) promoter [21]. The increased cellular proliferation induces the expression of hypoxia-inducible factor-1 (HIF-1) which promotes angiogenesis resulting in persistence of HPV and growth of the lesions [22].

HPV16 E6 direct activation of human Telomerase Reverse Transcriptase promoter [23], is also associated with Myc complexes (Myc/Max) [6], NFXI (Nuclear transcription Factor X-box binding 1 gene) [6], and mTORCI signaling [24]. The specific Myc antagonist Mad, represses E6 transactivation of hTERT [6]. The mTORC1 kinase complex functions as a cellular rheostat integrating environmental cues of energy, growth factors, amino acids and nutrients, and directly inhibiting autophagy [24]. HPV16 E6 triggers mTORC1 through the activation of receptor protein tyrosine kinase (RPTK) signaling and the downstream PI3K/AKT and RAS/MEK signaling pathways [24].

2. Method

A review of current research articles using search engines such as google, professional journals both print and electronic, and conference presentations was done and available evidence on the molecular pathways of oncogenic HPV in carcinoma of the uterine cervix was adduced. Information on targeted therapies was also gathered.

3. Result

3.1. Molecular Pathways

a. The Apoptotic signaling pathways are clearly involved in HPV carcinogenesis. The E6 protein binds to p53 and with the aid of E6AP, prevents p53 from inducing apoptosis by targeting it for degradation via ubiquitin-proteasome pathway [25]. Mitochondrial apoptosis induced channel involving cytochrome c, apoptotic protease activating factor 1 and pro-caspase 9 form apoptosome which releases caspase 9 that activates the effector caspase 3 which carries out the cell death program [26]. HPV E5 protein impairs Tumour necrosis factor Related Apoptosis Inducing Ligand (TRAIL) and Fas Ligand -mediated apoptosis by downregulating the Fas receptor [27].

b. The Wnt/beta-catenin pathway activation has been shown to be necessary to induce the transformation of HPV immortalization [28]. The activation of beta-catenin by the inactivation of negative regulators such as APC through methylation, or by the overexpression of activators such as Wnt ligands, frizzled receptors (Dvl), inhibits GSK3beta and causes the accumulation of beta-catenin in the cytoplasm and then the nucleus where it forms an active transcriptional complex with T-cell factor, which then activates the transcription of target genes including c-myc and cyclin D [4].

An analysis of genome expression conducted in HPV-16 associated cervical cancer cells, showed significant increase in Wnt-4,-8a, Fzd2, GDK3b and beta-catenin when compared with normal epithelia [4]. In chemically derived mouse skin tumors, ablation of the beta-catenin gene results in the loss of cancer stem cells and complete tumor regression [4]. Secreted frizzled-related protein 1 and 2 (SRFP1/2), which function as wnt antagonists decrease wnt signaling and suppress tumorigenicity [29]. Wnt inhibitory factor 1 (WIF1) which induces apoptosis, inhibits cervical cancer growth invasion and angiogenesis, is downregulated by epigenetic gene silencing in cervical cancer [30]. The Wnt-beta catenin pathway may be targeted as a therapeutic option in cervical cancer.

c. The PI3K/Akt signaling pathway is initiated by the activation of PI3K, an ubiquitous intracellular lipid kinase involved in receptor signal transduction and is a downregulator of the Ras signaling pathway [4]. Activated PI3K converts the membrane lipid phosphatidylinositol-4,5-biphosphate to phosphatidylinositol-3,4,5-triphosphate facilitating the phosphorylation of Akt by PDK1 [4]. This phosphorylation stimulates the catalytic activity of Akt, resulting in the phosphorylation of other proteins that affect cell cycle entry, cell proliferation, and anti-apoptosis [4]. PTEN (phosphatase and tensin homologue) a tumor suppressor gene dephosphorylates phosphatidylinositol-3,4,5-triphosphate back to phosphatidylinositol-4,5-biphosphate, and its expression is a biological marker for tumour progression which offer an opportunity for early diagnosis and therapeutic intervention [31]. The phytochemical indole-3-carbinol found abundantly in cruciferous vegetables such as broccoli has been found to inactivate Akt in tumor cells by upregulating PTEN [4]. It inhibits the growth of benign tumors of laryngeal tissue caused by HPV-16 in a mouse model and was shown to be effective in treating and preventing laryngeal papillomas caused by HPVs [4].

d. ERK/Mitogen Activated Protein Kinase (MAPK) pathway also known as RAF/MEK pathway lies downstream of several growth factors such as Epidermal Growth Factor (EGF) which when activated triggers a cascade of specific phosphorylation inducing gene expression, transcription factors and cell cycle-related kinases [4]. Once MAPK pathway is activated, the RAF kinase takes action. The Ras/Raf/Mek/Erk pathway has been shown to prevent apoptosis and increase cell proliferation [32]. Anti-EGFR strategies exert their anti-tumour activities by interfering with MAPK and Akt pathways. Epidermal growth factor receptor (EGFR) overexpression is frequently observed in cervical cancer and is a potential target for treatment of cancer [4].

e. Epithelial – Mesenchymal Transition (EMT) is characterized by loss of epithelial markers such as E-cadherin and expression of mesenchymal-related proteins such as fibronectin and N-cadherin as well as changes in the cell morphology [33]. Transforming Growth Factor beta1 is the most well-known growth factor involved in the regulation of EMT [33]. Notch 1 receptor and its ligand jagged 1 have also

been noted to regulate the EMT response through PI3K-dependent signaling [33]. The overexpression of the potassium chloride KCl cotransporter-3 and an increase in this ion transport system weaken the E-cadherin/ß-catenin complex while it accelerates the proteasome-dependent degradation of beta-catenin [33]. Ion transport system such as potassium chloride co-transporter KCC3 triggers EMT through inhibiting the gene expression of E-cadherin CDH1 and promoting the proteasome-dependent degradation of beta-catenin [33]. Enforced expression of SFRP1/2 enhances the expression of SLUG, SNAIL and TWIST transcription factors which promote EMT. Snail transcription factors (snail 1), slug (snail2), smuc (snail3) inhibit E-cadherin gene transcription and initiate EMT [33].

f. Angiogenesis: this is directly related to HPV inhibition of p53 and stabilization of Hypoxia Inducible Factor-1alpha (HIF-1a) both of which increase Vascular Endothelial Growth Factor (VEGF) and promote neovascularization.

3.2. Targeted Therapies

There are several inhibitors of signaling pathways under several phases of clinical trial [34]. Inhibitors of Raf, Rac,

PDK1, HDAC Mek, Erk, JAK, mTOR, Akt, PI3K, PARP, DNA-PK, GSK3, c-Myc, c-Met, wnt/beta-catenin, CDK, S6 kinase and many others are being patented for clinical trials [34].

The degradation of p53 by the ubiquitin-proteasome system could be prevented by increasing p53 levels using MG132 and bortezomib, which induce apoptosis, reduce VEGF and increase radiosensitization of cervical cancer cells [35].

Anti-EGFR activity could be enhanced using drugs such as: Sorafenib, an oral Raf kinase inhibitor; cetuximab, a human/murine chimeric immunoglobulin G_2; Matuxumab, a humanized immunoglobulin G monoclonal antibody; as well as Bevacixumab and paxopanib which target VEGFR [4]

Wortmannin, a naturally occurring metabolite of penicillium funiculosum is a potent irreversible inhibitor of PI3K, enhancing apoptosis by inhibiting Akt activation. Quercetin, a naturally occurring flavonoid, as well as indole-3-carbinol (I3C), a phytochemical present in cruciferous vegetables, inhibit a broad range of protein kinases and have been shown to arrest or reverse the progression of cervical neoplasia [4].
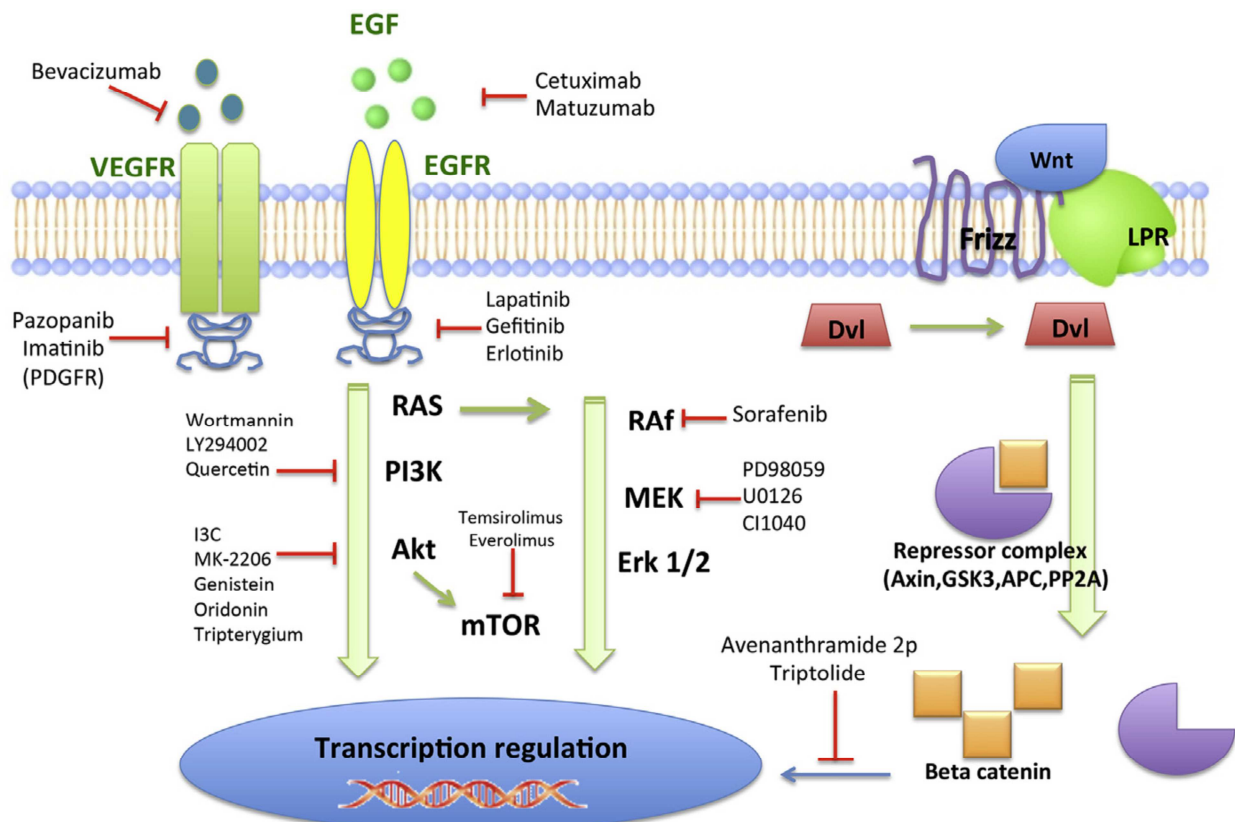

Figure 1. *MAPK, PI3K/AKT and wnt pathways and therapeutic interventions (adapted from Manzo-Merinoet al; Archives of Medical Research, Elsevier).*

Two more natural products shown to inhibit Akt activation are oridonin, and triptolide. Oridonin is isolated from Rabdosia rubescence, while triptolide is the main active component of the traditional Chinese herbal medicine Tripterygium wilfordii Hook [4]

Withaferin-A, the active component of withania somnifera,

a medicinal plant is able to downregulate the expression of HPV E6 & E7 oncoproteins and to induce p53-dependent apoptosis in human cervical cancer cells [36].

Cidofovir, a US Food and Drug Administration approved anti-viral against VMV retinitis reduces E6 & E7 expression in cervical and other HPV associated carcinomas at the

transcriptional level, which lead to an accumulation of active p53 & Rb associated with the induction of cyclin-dependent kinase inhibitor p21 [37]

4. Discussion

There is increasing knowledge on the biology, immunology and molecular pathways of HPV-induced carcinogenesis. It is expected that when the clinical trials of targeted therapies are finally concluded, medical options may supersede surgical options especially following early disease diagnosis. Figure 1 shows some targeted therapies in clinical use, and some in clinical trials. Bevacizumab, cetuximab, pazopanib and Sorafenib have shown remarkable clinical usefulness.

The best strategy for cervical cancer control is primary prevention, screening and treatment of pre-invasive disease [38].

5. Conclusion

Carcinoma of the human cervix is a preventable disease that still remains a cause of morbidity and cancer-related mortality especially in the developing regions of the world.

The molecular pathways by which the oncogenic HPV inflict the damage are still being studied. The apoptotic signaling pathway is inhibited not just by the E6 protein binding with E6AP and preventing p53 from inducing apoptosis, but by increased expressions of the Wnt/β-catenin pathway, PI3K/AKT and activation of MAPK by the Ras/Raf/Mek/Erk pathways. Targeted therapies thus try to enhance apoptosis either by increasing the number of death receptor ligands or antagonizing the anti-apoptotic pathways. While drugs such a bevacizumab, cetuximab, Sorafenib and some others are in clinical use with varying percentages of success depending on the tumour type and a host of other factors, clinical trials are ongoing and targeted therapies may become a popular therapeutic option in the treatment of the disease.

Abbreviations

Mammalian target of rapamycin (mTOR)
CDH1 (Cadherin 1 or Epithelial cadherin)
SFRP1 (secreted frizzled-related protein 1)
GSK (Glycogen synthetase kinase)
HDAC (Histone deacetylase)

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