Molecular targets for therapeutic interventions in human papillomavirus-related cancers (Review)

ALAN SAITO RAMALHO[1], ANSELMO DANTAS LOPES[1], ALEYLOVE TALANS[1],
BRUNO YOSHIHIRO PARLATO SAKIYAMA[1], GUILHERME LUIZ STELKO PEREIRA[2],
PAULO MARCELO HOFF[1,2] and GILBERTO DE CASTRO Jr[2]

[1]University of São Paulo, Medical School, Av. Dr. Arnaldo 455, São Paulo, SP 01246903; [2]Clinical Oncology,
Instituto do Câncer do Estado de São Paulo, Av. Dr. Arnaldo 251, São Paulo, SP 01246000, Brazil

Abstract. The infection by the human papillomavirus (HPV) is the origin of several cancers around the world. In some areas of Brazil, cervical carcinoma is still the cancer with the highest incidence among women. After epithelial cell transformation by HPV, several molecular events are observed, resulting in the malignant phenotype. In this review we discuss potential molecular targets for therapeutic interventions in human HPV-related carcinomas, with emphasis on cervical cancer, based on the alterations observed in the signaling transduction pathways caused by HPV infection. With a special attention to tyrosine kinase receptors, and other kinases involved in signal transduction and angiogenesis, these pathological alterations are evaluated as novel targets for anticancer therapies in HPV-related carcinomas.

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1. Introduction

Human papillomaviruses (HPVs) are DNA-viruses capable of infecting basal epithelial cells. The relationship between HPV and cancer is well established and the infection by this virus is required for cervical cancer development, which is

Correspondence to: Dr Gilberto de Castro Jr, Instituto do Câncer do Estado de São Paulo - ICESP, Av. Dr. Arnaldo 251, 5th floor, São Paulo, SP, Brazil
E-mail: gilberto.castro@usp.br

responsible for the death of around 274,000 women per year worldwide (1,2). Besides cervical cancer, HPV is also associated with penile, vulvar, anal and oropharyngeal squamous cell carcinomas (3,4). The infection by high-risk oncogenic HPVs is estimated to lead to around 100% of cervical cancers, 90% of anal cancers, 40% of vulvar and penile cancers and, at least, 12% of oropharyngeal cancers reaching almost 60% of tonsillar cancers (2,5,6). Particularly in Brazil, although screening of precursor lesions and invasive carcinoma by Pap smear is regularly performed, cervical cancer still represents the cancer with highest incidence (22 cases/100,000 inhabitants) in the Northern region of the country (7).

Since the 1970s, when Harald Zur Hausen, awarded with the Nobel Prize in Physiology or Medicine in 2008, focused his research on the relationship between HPVs and cancer, more than one hundred of HPV types have been described and their genetic sequences identified (8,9). This knowledge led to the classification of different viral types and their denomination by cardinal numbers according to the organization of nucleotide sequences of viral genome.

The prevalence of HPV infection is highly variable in different populations, oscillating between 1.4% and 25.6% (10). The highest prevalence is found in women under the age of 25 and there is a progressive decline after this age (11). Currently, HPV infection is the most frequent sexually transmitted disease (12,13). In 2003, mortality-related productivity costs of HPV-associated cancers in the USA reached US$ 3.7 billions, showing the socio-economic challenge of tumors associated with HPV infections (14).

In this review, the authors discuss the molecular targets to be studied with therapeutic purposes in malignant tumors associated with HPV infections, particularly in cervical carcinoma, based on the alterations in signaling transduction pathways caused by this virus in infected cells (Table I), and also taking into account the recent knowledge on HPV carcinogenic mechanisms and the abundance of information on molecular targeted therapies in clinical development.

A data search was performed using Pubmed database for English-language studies from January, 2000, to May, 2010. We searched using the terms 'molecular targeted therapy', 'signaling pathway', 'HER', 'EGFR', 'c-MET', 'PI3K', 'Akt', 'mTOR', 'PTEN', 'MAPK', 'Ras', 'Raf', 'MEK', 'VEGF',

'HIF', 'everolimus', 'temsirolimus', 'cetuximab', linked to 'E6', 'E7', 'tumor', 'cervical cancer', 'anal cancer', 'penile cancer', and/or 'oropharynx cancer'. The list of publications was narrowed to relevant studies of the studied period. References of this list of publications were reviewed, in order to extract other relevant articles. Personal papers of authors were also used in this review.

2. Carcinogenesis in HPV-related cancers

HPV infection can be caused by different types of HPV. They can be classified into high-oncogenic risk, which are mainly found in cervical cancer and in high-grade squamous intra-epithelial lesions, and in low-oncogenic risk, usually found in genital warts. Co-infection by high- and low-risk types is a common event (15).

HPV types 16 and 18 are the most associated with cervical carcinoma, present in more than 70% of the cases, despite some geographic variation (4,8,10). Indeed, the following types have also been classified as carcinogenic: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66 (4).

Usually, cervix infection by HPV is a transitory event, commonly in young woman when sexual life is initialized, and no HPV is detected after 36 months in more than 90% of cases (12). Inside the host cell, HPV DNA can assume two different forms: episomal and integrated. When the viral DNA is in the cell nucleus, but not bound to the host DNA, it is in the episomal form. As soon as it binds to the host DNA, it is considered integrated, which is found in high-grade lesions and invasive carcinoma (15,16).

There are three viral genes related to epithelial proliferation of infected cells. *E5* product seems to be important in the early phases of infection. During the viral integration, *E6* and *E7* command carcinogenesis. *E6* and *E7* products induce increased cell proliferation, causing epithelial atypical features and, ultimately, the malignant phenotype. E6 and E7 proteins are the only viral products conserved and continuously expressed in cervical cancer cells, indicating their importance in cancer progression. Their main function is to enhance the degradation of tumor suppressor proteins p53 and the product of the retinoblastoma gene (pRb), respectively (17,18). pRb degradation by E7 prevents it from binding to the transcription factor E2F that regulates the expression of genes involved in progression to the S-phase of cell cycle, such as cyclin D and *CDK2* genes. As pRb normally acts as a negative regulator of p16^{INK4A} (p16) expression, a tumor suppressor gene located on chromosome 9p21, the inactivation of pRb results in overexpression of p16 protein. The p53 mediated apoptosis is blocked by E6. Thus, E6 and E7 act synergistically to maintain the malignant phenotype (17,19). It is well established that HPV products can affect tumor cell characteristics, including growth regulators independence, resistance to apoptosis, immune escape and angiogenesis, as shown in Fig. 1 (19-23).

3. Molecular targets for therapeutic interventions in HPV-related cancers

Receptor kinases
Human epidermal growth factor receptor family. The human epidermal growth factor receptor (HER) family members include four receptors: EGFR (epidermal growth factor receptor or HER-1), HER-2/neu, HER-3 and HER-4. These receptors are located in the cell surface and have three domains: extracellular, transmembrane and intracellular, and all, except HER3, contain an intracellular tyrosine kinase domain. The activation of these receptors by specific ligands leads to dimerization with auto-phosphorylation and subsequent activation of the tyrosine kinase, which induces a cascade of downstream signaling through several pathways, such as PI3K/Akt/mTOR, Ras/Raf/MAPK and JAK/STAT, resulting in cellular proliferation, diminished apoptosis, increased motility and adhesion properties, and increased DNA repair (Fig. 1).

The amplification of the gene encoding EGFR is strongly associated with overexpression of p16, as shown by Kim *et al* (24). In this study, 71.2% of patients with tonsillar cancer expressed p16 while no expression occurred in chronic follicular tonsillitis.

Since the overexpression of EGFR has been identified as an independent predictor of poor prognosis in cervical carcinoma (25,26), EGFR appears to be an interesting molecular target in these tumors. In the study by Kurtz *et al* (27), 44 patients with advanced cervical carcinoma were treated with cetuximab, a chimeric anti-EGFR monoclonal antibody, in combination with cisplatin and topotecan. Unfortunately, this study was stopped early due to severe bone marrow toxicity and life-threading infections.

In a phase II study presented by Ferreira *et al* (28), 23 patients with squamous cell cervical cancer were treated with chemoradiation (45 Gy, followed by 4 applications of brachytherapy 600 cGy concurrent to cisplatin 40 mg/m^2/week, 5 weeks), associated with erlotinib (150 mg/d orally), a small molecule EGFR tyrosine kinase inhibitor. There was an impressive 91% rate of complete response (28). However, another recent phase II trial in patients with advanced disease showed that monotherapy with erlotinib was ineffective, with no objective response in 25 studied patients (29).

Hepatocyte growth factor and c-Met axis. c-Met is a membrane-spanning receptor involved in several biological activities including motility, proliferation, survival, invasion and morphogenesis (30). Hepatocyte growth factor (HGF) is the only known ligand of c-Met (31). The HGF/c-Met axis is implicated in a wide variety of epithelial, mesenchymal, and hematological malignancies (Fig. 1).

Walker *et al* (32) reported that HGF and c-Met levels were significantly correlated with the severity of intraepithelial lesions infected with oncogenic HPVs. The correlation between oncogenic HPV infection and overexpression of the complex HGF/c-Met in cervical lesions of squamous epithelia is a strong indication that HGF/c-Met axis may be a potential cancer target for future therapeutic interventions in these cancers. There are currently several HGF/c-Met inhibitors under clinical evaluation, including the monoclonal antibody AMG102, and some small-molecule inhibitors (XL880, PF02341066).

Insulin-like growth factor receptor. Similar to the EGFR pathway, the insulin-like growth factor receptor (IGFR) signaling system comprises multiple circulating ligands, such

Signal transduction pathways, molecular targets and drugs in clinical development for therapeutic interventions in ted cancers.

Receptors and signaling transduction pathways	Molecular targets	Drugs in clinical development
Receptor kinases		
EGFR pathway	EGFR	Cetuximab, panitumumab, nimotuzumab, necitumumab
HGF pathway	c-Met	AMG102, XL880, PF02341066
IGFR pathway	IGF-1R	CP721871, BIIB022, XL228, OSI-906
Intracellular signaling kinases		
PI3K/Akt/mTOR	PI3K	PI103, BGT226
	Akt	Perifosine, GSK690693
	mTOR	Temsirolimus, everolimus, ridaforolimus
Ras/Raf/MAPK	Ras	Tipifamib, lonafamib
	Raf	Sorafenib, PLX4032
	MAPK	AZD6244, XL518
Angiogenesis	VEGF, VEGFR	Bevacizumab, sunitinib, AZD2171
	HIF-1α	Adaphostin

EGFR, epidermal growth factor receptor; HGF, hepatocyte growth factor; HIF-1α, subunit α hypoxia-inducible factor; IGFR, insulin-like growth factor receptor; IGF-1R, type I insulin-like growth factor receptor; PI3K, phosphoinositide 3' kinase; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

as IGF-1, IGF-2 and insulin, interacting with membrane-bound receptors, such as type 1 IGF receptor (IGF-1R) and insulin receptor (IR). The IGF-1R undergoes conformational changes and phosphorylation upon ligand binding and recruits insulin-receptor substrates and/or Scr homology 2 domain-containing proteins. The mitogenic, proliferative and/or anti-apoptotic signals are then transmitted downstream through MAPK and PI3K/Akt/mTOR pathways (Fig. 1) (33).

In the physiological state, IGF-1R plays an important role in fetal development and linear growth of many organs, whereas insulin/IR interaction regulates carbohydrates and lipid metabolism, among other functions. However IGF-1R was implicated in the development and maintenance of malignant phenotype, and interruption of IGF-1R signaling inhibited cancer cell growth and motility both *in vitro* and *in vivo* (34).

Aberrant activation of the IGF-1/IGF-1R pathway was also associated with worse prognosis in many malignant neoplasms, including multiple myeloma, prostate cancer, non-small cell lung cancer and renal cell cancer. Aside from the IGF-1R, abnormally activated IR by insulin or IGF-2 stimulation enhances proliferation in cancer cells, thus highlighting their therapeutic value as molecular targets in cancer. However, IR inhibition may lead to type 2 diabetes mellitus and IGF-1-deficient states have been associated with osteoporotic fractures and ischemic heart disease (35).

Serrano *et al* (36) showed that the expression of IGF receptors are different between HPV positive (Siha) and negative (C33a) human cervical cancer cell lines. Siha cells express the three receptors (IGF-1R, IR-A, IR-B, and hybrids) whereas C33a mainly express IR-A. They found that both IGF-1 and IGF-2 can stimulate IR and IGF-1R autophosphory-lation while insulin only stimulates IR autophosphorylation at physiological concentrations. In the C33a cell line the three ligands stimulate the phosphorylation of IR, but lower concentrations of IGF-2 are needed in comparison to IGF-1. This result is consistent with the predominance of IR-A in C33a and the higher affinity of IR-A for IGF-2 than IGF-1. In agreement with the presence of receptors in C33a and SiHa cells, it was observed that activation of PI3K and MAPK pathways occurs in response to stimuli with all three ligands. It was also described a higher basal Akt phosphorylation in C33a cells that mainly express IR-A and have PTEN (phosphatase and tensin homolog) mutations, which was increased by treatment with IGF-I and IGF-II, but not with insulin, in comparison to SiHa cell line. Thus, studies of therapeutic agents that interact with IGFR in the treatment of cervical cancer should consider the different patterns of receptor distribution of the IGFR pathway. HPV-positive tumors express significantly more IGF-1R, and IGF-1 has an important role in the survival of these cells, which makes the complex IGF-1/IGF-1R an interesting target in the treatment of cervical cancer.

There are several IGF-1R-targeting agents in clinical development, including monoclonal antibodies, such as CP-721871 and BIIB022, and small molecule inhibitors, such as XL-228 and OSI-906, but none are approved for current clinical use in oncology.

Intracellular signaling kinases.
PI3K/Akt/mTOR pathway. The phosphatidylinositol 3-kinase (PI3K)/Akt/mTOR (mammalian target of rapamycin) pathway acts as a cell sensor for nutrients and growth factors, integrating signals from multiple receptors to regulate cellular growth and metabolism (37). Although this pathway is often described as linear and vertical, its regulation is complex,
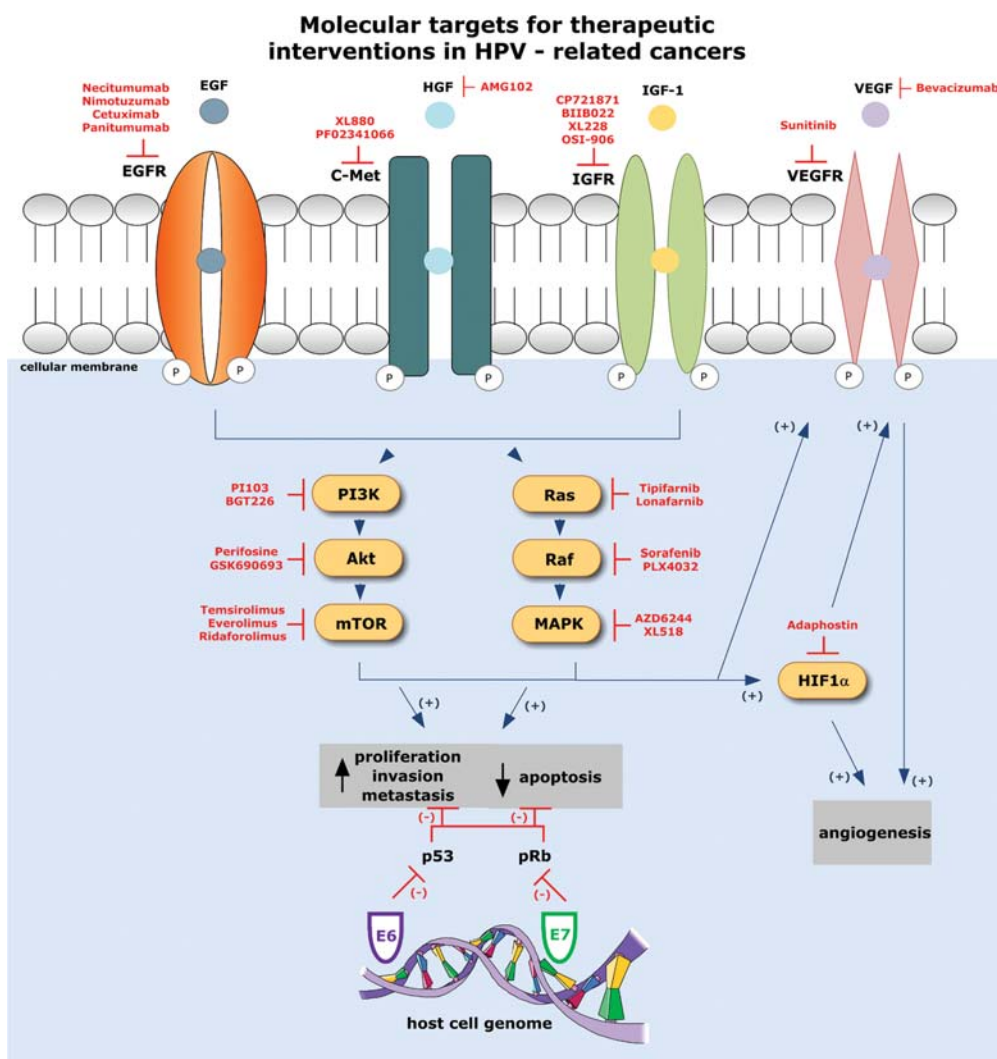
Figure 1. Molecular targets for therapeutic interventions in HPV-related cancers. Targeted therapies are indicated in red. c-MET, HGF receptor; E6, HPV early protein 6; E7, HPV early protein 7; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; HGF, hepatocyte growth factor; HIF-1α, subunit α of the hypoxia-inducible factor 1; IGF-1, type I insulin-like growth factor; IGFR, insulin-like growth factor receptor; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; p53, protein 53; PI3K, phosphoinositide 3'-kinase; pRb, retinoblastoma protein; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

with internal feedbacks and crosstalks with other intracellular signaling pathways, such as MAPK.

PI3K is a lipid kinase that generates the 3'-phosphoinositides (PIP3) in the cell membrane when activated by receptor kinases. This leads to the recruitment of phosphoinositide-dependent kinase (PDK1) and Akt to the cell membrane. The formation of PIP3 is negatively regulated by PTEN. Akt is activated by multiple enzymes, including PDK1, mTORC2 and IRS-1, leading to inhibition of protein 2 tuberous sclerosis complex (TSC2). Inhibition of TSC leads to mTOR-mediated activation of 4EBP1 and p70S6K, which in turn regulate cellular mechanisms of gene transcription and translation (Fig. 1) (37).

The PI3K/Akt/mTOR pathway is a promising target for molecular therapies for several reasons. The kinases of this pathway are overactivated in a variety of solid tumors types, as a result of loss of PTEN function, amplification of Akt, mutations of TSC and/or constitutional activation of upstream kinases (38).

Fausch *et al* (39) showed that the activation of the PI3K in Langerhans cells stimulated by HPV virus-like particles was associated with reduced immune response, due to repression of transcription of important genes related to immune function. When PI3K activation was suppressed, the Langerhans cells were able again to trigger an efficient immune response.

Among the compounds targeting the PI3K/Akt/mTOR pathway, mTOR inhibitors are furthest along in development. The mTOR protein is a serine-threonine kinase discovered in the 1990s when the mechanism of action of rapamycin was investigated. Rapamycin (or sirolimus) is a macrolide first discovered as a product of *Streptomyces hygroscopicus*, a bacterium isolated in a soil sample from Easter Island, and has been widely used as immunosuppressive agent in organ transplantations (37).

Activation of PI3K/Akt/mTOR pathway was associated with a worse prognosis and chemoresistance in cervical cancer. Faried *et al* (40) found that the survival of patients with mTOR-negative cervical adenocarcinomas was significantly higher than those of mTOR-positive tumors, suggesting that the expression of mTOR is an independent marker of worse prognosis.

inhibition of mTOR increases the response to cyto-motherapy. Treatment of CaSki cervix cells with rapamycin increased the sensitivity to paclitaxel and inhibited several proteins involved in cell survival, suggesting a synergistic role between rapamycin and cytotoxic agents in this model (41). Brown *et al* (42) showed that in most cases of oropharyngeal carcinoma, there is an increased constitutional activation of the mTOR pathway. It was also observed that rapamycin caused a reduction in the expression of p-mTOR (phosphorylated Ser-2448) in these tumors, which inhibited cell growth in a dose-response pattern.

Akt is a serine-threonine kinase that regulates mTORC1 and it is implicated in carcinogenesis in several malignancies. Akt is equally attractive as mTOR for molecular targeting in therapeutics due to its participation in important cellular functions, including cell cycle progression, gene transcription and translation, apoptosis and cellular metabolism. Bertelsen *et al* (43) observed that in cervical carcinogenesis there was an increased activation of Akt due to *PI3KCA* gene amplification, raising the intracellular levels of PI3K. The mechanism of Akt activation by amplification of *PIK3CA* was also demonstrated in anal squamous cell carcinoma. Furthermore, in squamous cell carcinoma of head and neck, *PIK3CA* was found overexpressed in HPV-positive lesions when compared to those HPV-negative.

It was shown that E7 protein is able to modulate the cytoplasmic localization of p27, a regulator of cell motility, which acts by inhibiting the activation of the cytoskeleton organizer RhoA. Modulation by E7 on p27 occurs through the activation of the PI3K/Akt pathway. It follows, therefore, that mobility and cell migration can also be dependent on activated Akt (44). Moreover, Menges *et al* (45) showed that the expression of E7 protein of HPV-16 in cervical keratinocytes cause inhibition of cell differentiation and proliferation, and increased activity of Akt.

The increased activity of Akt is dependent on the ability of E7 to bind with and to inactivate the Rb protein. The knockout of *Rb* with shRNAs, small hairpin RNAs capable of blocking mRNA by RNA-induced silencing complex, was capable of increasing Akt phosphorylation in many cell cultures. As HPV is strongly associated as a cause of cervical cancer, it was also evaluated whether the expression of Akt was increased in cervical biopsies, which in fact has been confirmed, as there was a higher expression of Akt in neoplastic in comparison to normal tissue, strongly correlated with the expression of *E7* (45).

Another oncoprotein involved in this process is E6, which modulates the activity of PTEN, leading to a decrease of its activity. Since PTEN is a negative regulator of Akt, E6 expression results in the stimulation of Akt pathway. Contreras-Paredes *et al* (46) showed that E6 was also able to activate PI3K and MAPKs, particularly ERK1 and ERK2. Thus, the inhibition of Akt signaling, either directly or by interfering with regulators of PI3K pathway is a potential treatment strategy in cervical cancer.

Given the pivotal role of Akt in the cellular homeostasis, its inhibition leads to more frequent severe adverse effects than the inhibition of mTOR, and it is one of the possible reasons for the disappointing development of Akt inhibitors (47).

Ras/Raf/MAPK pathway. Mitogen-activated protein kinase (MAPK) pathway is referred to be as a major connector between extracellular and intracellular stimuli such as cytokines, growth factors, oncogenes and cellular responses related to adhesion, mobility and malignant transformation (48). Ras is a GTPase protein which transmits activating signals from growth factors, cytokines, and oncogenes to Raf and then to MAPK. Following, MAPK phosphorylates and activates the extracellular signal-regulated kinase (ERK) (Fig. 1).

H-Ras, K-Ras and N-Ras genes, in humans, have been localized to chromosome 11, 12 and 1, respectively, and their activations by point mutations have been suggested to play an important role in the carcinogenesis process (49,50). It is estimated that activating Ras mutations are found in more than 20% of human cancers (51).

The exact mechanisms linking overexpression of Ras genes and cervical carcinogenesis remain to be elucidated, however, a cooperative effect between Ras and *E6/E7* genes in cellular transformation is considered (52,53). The activation of ERK1 and ERK2 as a consequence of *E6/E7* transcription has been already observed (46).

Regarding the association between high-risk HPVs and genetic alterations in H-Ras, there is evidence of a more aggressive tumor behavior in patients whose tumors harbor H-Ras mutations, as an early predictor of faster tumor progression or higher risk of invasion (54). Actually, cells with H-Ras mutations were reported to present higher proliferation rate when infected by HPV, leading to reduced duration of G1 phase of cell cycle, dependent on MAPK and/or PI3K/Akt/mTOR activation (55). As a consequence, poorly differentiated tumors were observed in patients with cervical squamous cell carcinoma carrying H-Ras mutations (56,57).

Besides its prognostic value, the expression of the oncogenic Ras-family proteins seems to be predictive of resistance of cancer cells to radiation therapy. It has been reported that inhibition of H-Ras, by treatment of a transformed embryonic rat fibroblast cell line with a farnesyltransferase selective inhibitor, resulted in higher levels of apoptosis after radiation (58,59). Post-translational modifications, such as farnesylation, are required for the membrane localization and activation of Ras. This information has led to an interest in developing farnesyltransferase inhibitors (FTIs) which could prevent the proper functioning of Ras. Some examples are tipifarnib and lonafarnib, which unfortunately did not show relevant clinical activity in phase II and III studies (60,61).

The *RASSF1A* gene, identified at chromosome region 3p21, encodes a novel Ras-binding protein. The precise function of this gene is not well known, but it is suggested that its overexpression induces apoptosis, demonstrating tumor suppressor gene properties (62,63). *RASSF1A* is inactivated by DNA promoter hypermethylation in several human solid tumors, including cervical cancers, being probably the most commonly inactivated gene thus far reported in human cancer (64,65).

The prevalence of *RASSF1A* promoter methylation in cervical adenocarcinoma is around 20%, and there is an inverse correlation between *RASSF1A* inactivation and the presence of HPV-transforming gene products (65,66). It may suggest that *RASSF1A* promoter methylation plays an important

role in the development of cervical adenocarcinoma, independently of HPV infection status. The epigenetic alteration that interferes in Ras pathway may have therapeutic implications, as it could explain some possible differences in therapeutic responses between cervical adeno- and squamous cell carcinomas.

Angiogenesis. Angiogenesis describes the formation of new vessels from existing vasculature. It is vital in some physiological process, such as wound healing, menstrual cycle and embryogenesis, but it is dysfunctional in malignant tumors (67,68). In hypoxic conditions, cells produce a large number of cytokines and growth factors that induce proliferation, migration and formation of new vessels by endothelial cells (69).

Vascular endothelial growth factors and receptors (VEGF, VEGFR) are now well validated targets in cancer therapy (70,71). Small molecules that inhibit the tyrosine kinase domain of VEGFRs, like sunitinib and sorafenib, as well as the monoclonal antibody bevacizumab, directed to circulating VEGF, are now used in the daily clinical practice in oncology.

Hypoxia-inducible factors (HIF)-1 and HIF-2 are recognized as important mediators involved in tumor angiogenesis and metabolism (72). HIF-1α subunit is rapidly degraded by the ubiquitin-proteasome system under normal oxygen conditions. Nonetheless, it is upregulated in a hypoxic tumor microenvironment, resulting in accumulation of HIF-1α (69,73). Besides, aberrant HIF-1α expression can be observed through MAPK or PI3k/Akt/mTOR stimulation, activation of oncogenes, or loss of tumor suppressor genes such as VHL, p53 and PTEN, which can lead to tumor progression, making HIF a rational target for anticancer therapies (Fig. 1) (69).

Angiogenesis is an early event in the progression of HPV-related cervical lesions (74). A strong association between higher VEGF expression in cervical cancer and worse prognosis has been observed (75,76).

The expression of HPV genes can contribute to production of angiogenic factors. Among the most studied pro-angiogenic factors, VEGF is consistently overexpressed in cervical neoplastic lesions (77). Overexpression of E6 is associated to higher production of VEGF in cervical cancer (22,77). Studies using transgenic mice as well as samples of human cervical tissue suggest that both E6 and E7, besides hypoxia, can stimulate VEGF production (78,79). López-Ocejo *et al* (77) have demonstrated that E6 positive cervical carcinoma cells expressed VEGF mRNA levels two to three times higher than E6 negative cells. Moreover, reduction in VEGF expression was able to produce suppressive effects over angiogenesis and tumor growth *in vivo*. In HPV-16 positive cells, E6 oncoprotein can contribute to amplification of epigenetic alterations that increase the expression of VEGF.

In HPV-16 positive cells, expression of pro-angiogenic molecules, such as bFGF, IL-8, TNFα, TGFß and VEGF was higher as compared to control keratinocytes. In contrast, expression of the anti-angiogenic factors trombospondin (TSP)-1 and 2 were decreased in cells infected by HPV. It demonstrates that cervical cancer expression of HPV-16 integrated genes is able to contribute to a pro-angiogenic phenotype (referred as angiogenic switch) that might support tumor growth and invasion (22,74,80).

Bevacizumab is a monoclonal antibody that neutralizes circulating VEGF, blocking its action. Wrigth *et al* (81) described the first use of bevacizumab in recurrent cervical cancer. In six patients, bevacizumab was combined to cytotoxic chemotherapy (5-fluorouracil or capecitabine) and clinical benefit (any response or disease stabilization) was verified in 67% of patients. Although the number of patients was small, the benefits and safety of this therapy suggested that VEGF is a potential molecular target in recurrent cervical cancer. In the phase II study published by Monk *et al* (76), bevacizumab was evaluated in 46 patients with recurrent cervical cancer. Patients received bevacizumab 15 mg/kg intravenously every 21 days until disease progression or severe toxicity. Of these patients, 23.9% had at least six months of progression-free survival and 10.7% had, at least, partial response. Median progression-free and overall survival were 3.4 and 7.3 months, respectively. Treatment-related toxicity was high: eight events of hematologic grade 3 toxicity were observed, five patients had deep venous thrombosis, one patient presented grade 4 vaginal bleeding, one had a grade 4 urinary fistula and one patient died as a consequence of infection.

Besides VEGF, HIF-1α is commonly overexpressed in cervical cancer, as a consequence of HPV genomic integration, and it is related to a diminished response to radiation therapy and unfavorable prognosis (82). Besides, HIF-1α overexpression occurs not only in tumor cells, but also in pre-malignant lesions (83). Both E6 and E7 act independently by interfering in HIF-1α production.

Therefore, HPV E6 and E7 oncoproteins can contribute to cervical cancer progression not only by deregulating the cell cycle, but also by promoting angiogenesis as well. VEGF and its receptors, besides HIF-1α, are promising targets to be explored as therapeutic interventions in these HPV-related cancers.

4. Conclusion

Advancing the knowledge of the molecular biology of HPV-related tumors allowed to identify molecular targets, such as those here described, as growth factor receptors, signaling transduction pathways and angiogenesis. Molecular targeted drugs that are already in use in the daily practice, or in different stages of clinical development, may inhibit tumor progression and increase apoptosis, resulting in tumor response or stabilization. Due to a more rational mechanism of action, the toxicity profile and effectiveness are different from those observed with the classic cytotoxic chemotherapy. Some of these new drugs have enormous therapeutic potential for clinical applicability in the treatment of these HPV-related cancers, reducing morbidity and mortality and improving patients' quality of life. The development of these drugs through clinical studies with appropriate design and procedures, preferably with exploratory translational aspects, should be encouraged.

One aspect that must be stressed is how to best assess the HPV positivity status. Currently, p16 immunohistochemistry, *in situ* hybridization and E6 messenger RNA RT-PCR are being investigated and p16 immunohistochemical expression appears to be a reliable surrogate marker for relevant HPV infection (84,85).

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