Molecular Targeted Agents Combined With Chemo-Radiation in the Treatment of Locally Advanced Cervix Cancer

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Abstract: Despite improvements in survival after the introduction of chemo-radiotherapy (CRT) in the treatment of patients with cervical cancer, loco-regional control of this disease continues to be a major problem. The present article reviews current and emerging therapeutic strategies combining CRT with novel molecular agents that specifically target the abnormal tumor microenvironment, with the aim of improving local control and survival in patients with locally advanced cervix cancer.

The evidence supporting the biological rational to combine novel non-cytotoxic agents with CRT is strong, and drugs targeting different molecular pathways are currently under clinical development (EGFR inhibitors, COX-2 inhibitors, hypoxia targeted agents, etc). Early pre-clinical and clinical strategies also favor the use of vascular-targeted agents with the aim to normalize the abnormal tumor vasculature, increase tumor oxygenation, and reduce interstitial fluid pressure (IFP). The integration of these novel targeted therapies with CRT in clinical trials is discussed, as well as new and promising biomarkers to test drug activity.

Key Words: Molecular targets, chemo-radiation, cervix cancer, hypoxia, IFP.

INTRODUCTION

Cancer of the cervix is the second highest cause of cancer death among women worldwide, with an incidence of 500,000 cases diagnosed per year and more than 288,000 associated deaths in 2006 [1].

In the vast majority of established cervical carcinomas, persistent infection with oncogenic types of genital human papilloma virus (HPV) have been detected, and this is considered the major stimulus for cervical cancer development [2, 3]. New vaccines have become available to prevent HPV infection, however in women already infected with the virus, vaccination has no effect on viral clearance, and the risk of progression to cervical intraepithelial neoplasm (CIN) and invasive cancer is higher among this subset of patients [4]. Thus, the therapeutic impact of these vaccines on cervical cancer incidence will take several generations to determine [5, 6].

Since the publication in 1999 of 5 randomized trials of platinum based-chemotherapy (CT) in patients with cervix cancer and the clinical recommendations announced by the National Cancer Institute (NCI), concurrent radiotherapy (RT) and cisplatin-based CT are considered standard management with a significant improvement in survival rates compared with RT alone [7-11]. Despite this improvement there are still a significant number of patients who do not achieve pelvic control and eventually die of disease. Thus, the development of therapeutic strategies that can target chemo-radiation resistant disease is essential. There is strong evidence that a variety of different targets involved in signal transduction, oncogene transcription factors, cell death, angiogenesis, and prostaglandin biosynthesis are implicated in cervix tumor growth, invasion, metastasis, and resistance to standard treatments. For these reasons, current areas of interest are the combination of standard CRT with novel non-cytotoxic agents that target the tumor microenvironment (e.g., hypoxia, angiogenesis and IFP, growth factors, etc) and oncogenes associated with HPV early protein 6 and 7 (E6/E7). The integration of these novel targeted therapies in combination with CRT is expected to enhance tumor cell killing and provide better therapeutic responses, without the burden of increased or overlapping toxicities.

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Our discussion will focus on current and upcoming studies investigating biologically targeted treatments in combination with CRT with the aim to improve local control and survival in cervix cancer patients.

HPV and Its Influence on the Tumor Microenvironment

HPVs are sexually transmitted agents that contribute to carcinogenesis and are consistently expressed in cervical cancer. More than 30 types of HPV have been described and are classified into low and high risk on the basis of their oncogenic potential [12]. Functionally, high risk HPV infection (HPVs 16, 18, 31, 33, 35, 45, 51, 52 and 56) contributes to carcinogenesis and tumor progression predominantly through the action of two viral-encoded proteins, E6 and E7. Fig. (**1**) One major function of E6 and E7 is to disrupt the activity of two tumor suppressor genes, p53 and pRB, respectively [13, 14]. This disruption results in dysregulated proliferation, unrestricted cell cycle entry, and amplification of oncogenes, transcriptional factors and growth factors (e.g. c-myc, c-fos, c-jun, H-ras, erb-b2, transforming growth factor β (TGFβ), vascular endothelial growth factor - VEGF) [15-18]. These altered phenotypes contribute to a multi-step process in the development of cervix cancer. One critical feature that must be acquired by transformed cells is the ability to recruit their own blood supply, a process known as angiogenesis [19].

In virally infected cells, a link between loss of tumor suppressor genes p53 and pRB, and dysregulation of angiogenesis has been described [20-22]. Analysis of angiogenesis inducers and modulators in different stages of cervix cancer has shown that the presence of HPV-16 in human epithelial cells results in a decrease of the anti-angiogenic factors thrombospondin 1 and 2 (TSP-1, TSP-2) and the expression of proangiogenic molecules, such as basic fibroblast growth factors (bFGF), interleukin 8 (IL-8), TGFβ, tumor necrosis factor α (TNFα) and VEGF [23-25]. More recently, the expression of E6 and E7 proteins in squamous epithelial cells from an HPV16 transgenic mouse model has been associated with early activation of the angiogenic pathway and rapid induction of VEGF in pre-malignant and tumor tissue. [26, 27]. Well documented data indicates the role of VEGF in cervix tumor angiogenesis, [28, 29] and clinical evidence suggests that the over-expression of this pro-angiogenic factor correlates with tumor development and is a prognostic indicator for overall and disease free survival in cervix cancer patients [30].

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Angiogenesis, Interstitial Fluid Pressure and Hypoxia

The structure and function of the newly formed vasculature in tumors is disorganized, with irregularly shaped vessels with areas of dilation and constriction [31]. Similarly, pericytes lose their association with endothelial cells, contributing to high vascular permeability. These structural abnormalities result in impaired tumor perfusion and high tumoral IFP [32-34]. Interstitial fluid pressure is a new and promising biomarker of vascular complexity and tumor response to therapy in cervix cancer and other solid tumors [32, 35]. Patients with cervix cancer and high IFP are significantly more likely than those with low IFP to recur after RT and die of progressive disease, independent of other prognostic factors [34]. IFP is easily evaluable using a needle probe technique, and correlates with capillary flow resistance, tumor perfusion, and oxygenation [35, 36]. These abnormal micro-environmental features coupled with other factors such as anemia [37] and transient tumor blood flow fluctuations [38] result in profoundly hypoxic regions in tumors. Clinically significant levels of hypoxia have been measured in cervical cancer using needle-electrode-techniques, nitroimidazole drugs that bind in hypoxic regions or endogenous tissue bound or circulating proteins that are up regulated by hypoxia [39-43]. In general, cervical cancer hypoxia has been associated with more malignant phenotypes [44, 45] higher rates of metastatic disease [39, 40, 46] and higher recurrence rates regardless of whether treatment is RT or surgery [44]. It is now well established that hypoxia contributes to radiation resistance by decreasing the availability of reactive oxygen species, up-regulation of angiogenesis and metastasis genes, and altering cell-cycle checkpoints and DNA repair, thereby inducing genetic instability and more aggressive tumor phenotypes[33, 47-49]. Novel therapies designed to target the hypoxic response and defective DNA repair may therefore be effective as chemopreventive agents or as adjuncts to surgery, RT and CT.

There is also pre-clinical data indicating that the overexpression of HPV-16 E6 and E7 oncoproteins contributes to enhanced angiogenesis in cervical cancer cells via stimulation of hypoxia-inducible factor alpha (HIF-α) in a VEGF dependent manner [50]. HIF-1α is a downstream effector of the phosphatidylinositol 3-kinase (PI3K) - alpha serine-threonine-protein kinase (Akt) pathway that modulates apoptosis and regulates the expression of many genes including those involved in blood vessel formation. Figure **2** Pre-clinical data has demonstrated that activation of the PI3K/Akt pathway increases HIF-1α expression in tumor cells through activation of HIF-1α protein translation under hypoxic conditions [51]. The PI3K-Akt pathway can also be activated by members of the growth factor family, such as platelet-derived growth factor (PDGF) and VEGF [52]. The tumor suppressor gene PTEN is a negative regulator of this signaling network. Specifically, the loss of PTEN protein expression and methylation are early events in the development of cervical cancer and are associated with reduced rates of disease free survival [53, 54]. PTEN deficient cells display an exaggerated HIF-1α activation in response to hypoxia, and this finding may partially explain the more aggressive phenotype of these tumors [55].

Increased intra-tumoral phosphorylated Akt has been linked to decreased radiation responsiveness in squamous cell carcinomas, thus inhibition of the PI3K-Akt pathway may provide a direct therapeutic approach and radiosensitization in cervix cancer [56]. Recent studies have focused on the development of inhibitors of the PI3K/AKT/PTEN pathway or its downstream effectors such as HIF-1α, or AKT-phosphorylated targets such as the mammalian target of rapamycin (mTOR). The kinase mTOR is a central regulator of protein synthesis whose activity is modulated by a variety of signals. It was recently shown that mTOR function is down-regulated by hypoxia independently of HIF-1α [57]. Rapamycin is a specific inhibitor of mTOR that can inhibit HIF-1α transcription through suppression of mTOR function in hypoxic cells by increasing the rate of HIF-1α degradation in the proteasome [58, 59]. If rapamycin or other mTOR inhibitors (such as its analog CCI-779), as well as PI3K/Akt/PTEN inhibitors prove to be effective therapeutic targets of hypoxia adaptation in tumors, this could have a tremendous impact on tumor growth, angiogenesis, invasiveness, and metastatic potential in human cancers, and the combination with CRT in cervix cancer patients represents an attractive therapeutic approach.

The extra cellular and extra vascular space is also abnormal in cervix tumors. Integrins are receptors for extracellular matrix proteins (collagens, laminin, fibronectin) that bind the cellular components of the interstitium to the elastic matrix. They play an important role in cellular signaling, and have been shown to promote cell cycle progression in response to mitogens [60, 61]. Integrins, act as mechano- and chemo-receptors to facilitate compaction or expansion of the interstitium in response to acute inflammation and specific cytokines such as PDGF. In this way, integrins are implicated in the regulation of IFP [34, 62, 63].

It is clear that the abnormal microenvironment in cervix cancer contributes to the failure of standard treatments; therefore pharmacological agents that either target hypoxia directly, the upstream regulators of angiogenesis or the abnormal tumor vasculature, and molecular targets associated with HPV, have the potential to improve patient outcome when used in combination with CRT. In addition, the mechanistic link between elevated IFP and abnormal tumor vasculature, and in particular, the strong prognostic effect of IFP may facilitate the evaluation of agents targeting angiogenesis, and could help to identify which patients may benefit most from these therapies.

Incorporating Molecular Targeted Agents with CRT in Cervix Cancer

We are now in the era of rationally designed molecularly targeted therapies against cancer. Targeted agents are increasingly used, either as single agents or in combination with CRT. The choice of agents and combinations is dependent on understanding the biology of cancer and availability of anticancer agents and their toxicities. There is also increasing understanding of the biological effect of radiation on molecular pathways and mechanisms of radiation resistance that will lead to the development of logical synergistic combinations with targeted agents [64, 65]. Current developments in cervix cancer treatment have been to identify biologically active agents, and to combine them with CRT. The burden of identifying improved activity, balanced with acceptable toxicity, is critical. Radiation, much more than CT, has a narrower cumulative lifetime normal tissue tolerance that, if exceeded, may result in significant increase in side effects. In addition, dose-limiting late radiation toxicity particularly involving bowel or bladder is a significant concern yet is usually seen 6 months or longer after treatment. This pattern poses significant challenges for the design of phase I-II clinical trials combining CRT with molecular targeted agents, because toxicity following standard treatment is already at the upper limit of what is considered clinically acceptable. Investigators have adopted novel designs for clinical trials in cervix cancer patients, including delayed assessment of interim end-points to allow the development of late effects, and dose and drug duration escalation schemas, particularly for oral agents given on a daily basis.

Another important issue under debate is the assessment of tumor response. Objective tumor shrinkage has been widely adopted as a standard end-point to estimate treatment efficacy [66]. However, new molecular targets may work by mechanisms unlikely to cause tumor regression, and there remains an important need to develop biomarkers to provide early evidence of drug activity not only in the tumor but also its vasculature. Moreover, while tumor is known to regress substantially in some cervix cancer patients dur-

ing standard CRT, regression has not been shown to correlate with long-term patient outcome {Lim 2008 #283}. Finally, the inhibition of a single-cellular pathway may be not enough to result in therapeutic benefit. Multiple factors such as tumor cell heterogeneity and genetic instability may limit single-target efficacy. More likely, broad spectrum target drugs which hit multiple targets present in the tumor and/or its microenvironment may overcome tumor resistance to treatment and achieve better therapeutic results. If these novel molecular- targeted agents have the potential to kill surviving tumor cell populations after standard treatment, or if they interfere in the mechanisms of resistance, then combination with CRT may lead to an important improvement in local tumor control and survival.

EGFR Inhibitors

The epidermal growth factor receptor (EGFR) is a transmembrane receptor tyrosine kinase (TK) of erb-b (also known as HER) family that is abnormally activated in many epithelial tumors. Several mechanisms lead to aberrant receptor activation, including receptor overexpression, gene amplification, activating mutations, overexpression of receptor ligands, and/or loss of their negative regulatory mechanisms. Receptor activation leads to recruitment and phosphorylation of several intracellular substrates, which, in turn, engage mitogenic signaling and other tumor promoting activities [68, 69].

It was not until the late 1990s that the interaction of EGFR inhibitors with RT was first explored. Several investigators identified the capacity of radiation exposure to induce EGFR phosphorylation and tumor cell proliferation that could be effectively blocked by the addition of an EGFR signaling inhibitor [70, 71]. These findings, as well as work which identified an inverse relationship between the expression level of EGFR and response to RT in tumor model systems [72, 73] and preclinical investigations that identified the capacity of EGFR inhibition to enhance RT response in cell culture and in animal xenografts, [74-78] opened the door for investigations to examine the combination of EGFR inhibitors with radiation.

The common end-point of these preclinical investigations involved augmentation of radiation response after the inhibition of EGFR signaling. Examples of specific mechanisms for enhanced radiation response include the capacity of EGFR inhibitors to abrogate radiation-induced phosphorylation of EGFR (EGFR phosphorylation represents a proposed mechanism underlying accelerated repopulation during RT) [71], enhanced radiation-induced apoptosis, and attenuated radiation-induced expression of DNA repair proteins such as RAD 51. EGFR inhibition either with monoclonal antibodies (m-Ab) such as cetuximab or small molecule agents (gefitinib, erlotinib) has shown improvement in progression-free survival and overall survival in colorectal, lung and pancreatic cancer. In head and neck cancer cetuximab has recently been shown to significantly improve overall survival in combination with radiation alone [79].

Carcinoma of the uterine cervix seems to be an appropriate disease setting where there is a good biological rationale to improve outcome using EGFR inhibitors. EGFR over expression is seen in up to 70% of cervical cancers. Mathur *et al.* [80] have demonstrated increasing EGFR expression with the transition from CIN to malignancy, and this over expression was correlated with poor outcome by other authors [81].

A recent phase I study of erlotinib combined with CRT in locally advanced cervix cancer has been reported in abstract form, establishing a maximum tolerated dose for erlotinib of 150 mg with no evidence of increased acute toxicity [82]. According to the NCI Clinical Trial Database there are two studies assessing cetuximab in cervix cancer: one looking at cetuximab with cisplatin in patients with recurrent disease and another investigating cetuximab/cisplatin and RT as a phase-I trial in patients with stage Ib to stage IVa disease. These trials will assess the tolerability and feasibility of adding cetuximab to CRT or CT alone in cervix cancer.

COX-2 Inhibitors

Cyclooxygenase-2 (COX-2) is an enzyme required in the conversion of prostaglandins (PGs) from arachidonic acid. Its tumor promoting activities are mediated via several mechanisms including conversion of procarcinogens to carcinogens, stimulation of tumor cell growth, prevention of apoptosis, promotion of angiogenesis and immunosuppression [83]. COX-2 over-expression has been reported in cervix cancers in association with locally advanced stage, distant metastasis and poor survival [84-86].

Cervical cancer cell lines treated with celecoxib, a non-steroidal anti-inflammatory drug (NSAID) that directly inhibit the enzyme COX-2, are more sensitive to radiation induced apoptosis, and this appears to be due to an increase in G2M cell cycle arrest and inhibition of sub-lethal radiation damage repair [87-89]. In pre-clinical models celecoxib suppresses the growth of corneal capillaries in rats exposed to basic fibroblast growth factors and this potent angiogenesis inhibition seems to be derived from its capacity to inhibit PG production via COX-2 [90].

While there is a biological rationale for combining CRT with COX-2 inhibitors to treat patients with cervix cancer, two recent phase I-II trials have reported increased rates of acute and late toxic effects. A Phase I/II RTOG study accrued 81 patients with advanced stage cervix cancer, treated with celecoxib 400 mg twice daily for one year in combination with RT, cisplatin and 5-fluorouracil (5-FU). Thirty-five of 75 patients (47%) experienced grade 3-4 acute toxicity, which was mainly hematological and gastrointestinal (GI). This high rate, perhaps due to the addition of 5-FU to cisplatin, exceeded the toxicity threshold of 35% that was established in advance and the regimen was therefore considered unacceptable for further clinical development [91]. A recent analysis of efficacy and patterns of failure from this study showed that locoregional control continues to be problematic, with local failures representing 23% of the first recurrences [92].

Our group has recently published the results of a phase I/II trial of celecoxib combined with cisplatin-based CRT. The effectiveness of the drug was assessed at the level of the tumor microenvironment using IFP and oxygen measurements. The acute toxicity, GI and hematological, was acceptable and mainly attributed to CT. However, there were higher than expected late complications, with an actuarial rate of grade 3-4 late toxicity at 2 years of 13.7%, mainly due to recto-vaginal fistulas. Celecoxib was associated with a modest reduction in the angiogenic biomarker IFP but this did not correlate with tumor response and no systematic change in tumor oxygenation was observed [93]. These results again highlight the importance of toxicity assessment as an end-point for trials of biologically targeted agents in addition to CRT.

Anti-Angiogenic Agents

Targeting the angiogenic pathway is an increasingly important therapeutic strategy for cervix cancer. Many of the microenvironmental abnormalities that have been associated with a poor prognosis in these patients, for example hypoxia and high IFP, are a reflection of the chaotic and dysfunctional tumor vasculature. Therefore, therapeutic strategies that combine CRT with vascular-targeted agents that either target hypoxia directly, the upstream regulators of angiogenesis or the abnormal tumor vasculature may lead to improved patient outcome.

Antiangiogenic therapy has been shown in laboratory and clinical studies to increase tumor oxygenation [94], reduce IFP [95, 96], and reduce capillary permeability [97]. More recently, three distinct mechanisms that may help to explain the chemo-sensitizing

activity of these drugs has been described: normalizing tumor vasculature, preventing rapid tumor cell repopulation, and augmenting the antivascular effects of chemotherapy [98].

Jain [32] has hypothesized that antiangiogenic treatment causes time-dependent normalization of the tumor vasculature and that an optimal window exists when vascular efficiency is maximal. If so, the timing of how antiangiogenic treatment is combined with conventional CRT in patients with cervix cancer may be critical to assuring maximal improvement in outcome. This implies the need to incorporate biological monitoring of the tumor microenvironment into future studies of these agents. IFP may be useful in this regard given our clinical results, as may dynamic contrast-enhanced magnetic resonance and computed tomography imaging measurements of vascular permeability [97, 99-102].

The agent that is most advanced in clinical development is bevacizumab, a humanized mAb against VEGF. Bevacizumab has been shown to improve outcome in colorectal and lung cancers when used in combination with CT, and is currently being evaluated in many other malignancies. These studies have identified some of the toxicities that may arise with bevacizumab therapy, particularly a higher than expected rate of hemorrhage and bowel perforation. Severe late radiation GI toxicity occurs in 5-7% of cervix cancer patients treated with conventional CRT [103], which raises concern about the safety of adding bevacizumab. At present, there is one trial underway evaluating bevacizumab in women with recurrent or metastatic cervical cancer, and one in combination with CRT (RTOG 0417).

The TK VEGF inhibitors do not appear to present the same high risk of GI toxicity as bevacizumab, making them more attractive candidates to use with conventional cytotoxic agents. They also have the theoretical advantage of inhibiting other TK domains including PDGF, which plays a central role in vascular and lymphatic development [104, 105]. PDGF has been shown to modulate angiogenesis by recruiting VEGF-producing fibroblasts, enhancing endothelial cell survival and promoting pericyte coverage and stability of newly formed vessels [106]. Inhibition of PDGF has been associated with reduction in IFP, tumor specific increases in drug uptake and greater CT cytotoxicity. [34, 104, 107].

We have implemented a clinical trial with sorafenib in women with high risk cervix cancer being treated with CRT. Sorafenib is an oral multitargeted TK inhibitor that has potent anti-angiogenic properties, targeting both tumor cells and the tumor vasculature. It was originally developed as an inhibitor of Raf-1, a member of the Raf/MEK/ERK signaling pathway [108]. Sorafenib was subsequently found to have activity against B-Raf, VEGF receptor–2, PDGF receptor, Fms-like TK-3 (Flt-3), and the stem-cell growth factor c-KIT [109]. In phase-I studies investigating various oral dosing schedules, sorafenib was generally well tolerated. Dose-limiting toxicities at continuous doses higher than 400 mg twice daily were diarrhea, fatigue, and skin rash [110, 111]. In patients with renal cell carcinoma, and more recently hepatocellular carcinoma, phase III trials have shown improvement in survival and quality of life with sorafenib, and it is now approved by the Food and Drug Administration for the treatment of metastatic renal cancer [112-114].

However, there is no in-vivo data assessing the risk of GI toxicity with this anti-angiogenic agent combined with RT, and this is currently being tested in animal models in our lab. Our clinical trial will assess the safety and tolerability of the combination with cisplatin and RT in a phase I setting, and also the pharmacodynamic changes on tissue oxygenation and IFP.

Hypoxia - Targeted Agents

Several pharmacological strategies have been proposed for overcoming the adverse effects of tumor hypoxia in patients with cervical cancer, including the connection of anemia with erythropoietin, radiation sensitization of hypoxic cells using nitroimidazole compounds, and direct killing of hypoxic cells with mitomycin C or tirapazamine.

There is substantial clinical evidence to indicate that anemia prior to and especially during RT for cervical cancer is associated with poorer patient outcome [37, 115]. The underlying mechanism is not known, although it is possible that the lower oxygen carrying capacity of blood in anemic patients contributes to the development of tumor hypoxia, radiation resistance and dysregulated angiogenesis. An important question is whether or not the normalization of hemoglobin levels either by transfusion or with recombinant human erythropoietin overcomes the adverse consequences of anemia and improves patient survival. The only randomized trial to date that has addressed this issue demonstrated that transfusion to maintain hemoglobin levels above 120 g/l during RT was associated with better local tumor control than transfusion for hemoglobin levels < 100 g/l; [116] however, this result was based on a subgroup analysis, as not all patients were transfused, making the results difficult to interpret. Erythropoietin stimulates red cell production, and has been shown to increase hemoglobin levels and improve the quality of life of cancer patients when administered weekly before and during RT or CT. A large intergroup randomized study was designed to test the benefit of maintaining hemoglobin levels >120 g/l throughout treatment in patients with advanced cervical cancer treated with CRT, but was closed prematurely because of an excess risk of thrombosis, and new information linking erythropoietin to inferior patient outcome in other randomized studies [117, 118]. This latter effect may have been due to direct stimulation of erythropoietin receptors on cancer cells, activation of anti-apoptotic pathways and angiogenesis promotion [119-122].

The nitroimidazole family of drugs has been shown to sensitize hypoxic cells to RT [123]. There are at least seven phase III studies in which patients with advanced cervical cancer were randomized to receive standard RT with or without a nitroimidazole [124-130]. Misonidazole has been the most common drug used in these studies; all pre-dated the modern era of CRT. Only two of these studies showed improved patient outcome [125, 130]. In addition, two meta-analyses, which pooled the results from these studies, found no difference in local tumor control or survival rate [131, 132]. Neurotoxicity was significantly higher in nitroimidazole-treated patients, which may limit their use with other neurotoxic agents such as cisplatin. These disappointing results have been attributed to drug levels that were inadequate to achieve sensitization, and the fact that some of the patients in these studies probably had relatively well oxygenated tumors and could not have benefited from the treatment. In general, the nitroimidazoles have fallen out of favor in cervical cancer, being displaced by enthusiasm for drugs that are directly cytotoxic under hypoxic conditions.

Mitomycin-C and tirapazamine are examples of hypoxia-activated drugs that have been studied in patient with gynecological cancer; both undergo reduction in the absence of oxygen to form reactive compounds that cause DNA damage and inhibit DNA repair. There have been numerous phase I-II studies of RT and concurrent mitomycin-C (with or without other drugs such as 5-FU) in cervix cancer. In addition there have been at least two phase-III studies of RT plus mitomycin-C vs. RT alone in locally advanced cervical cancer. These studies demonstrated improved disease-free survival and a reduction in the risk of distant recurrence, with no apparent increase in late treatment complications [133, 134]. However, other studies have identified unacceptably higher rates of late GI toxicity when mitomycin C was combined with RT to treat cervical cancer [135].

Tirapazamine is the most promising hypoxic-cell cytotoxic drug currently in clinical testing. It selectively kills hypoxic cells in tumors that are resistant to the effects of RT, and also potentiates

cisplatin cytotoxicity [136]. A phase I study has demonstrated the feasibility and safety of using cisplatin CRT and tirapazamine every 2 weeks to treat cervical cancer [137]. A recent phase I study has been reported in abstract form evaluating the weekly dosing of tirapazamine in combination with CRT. The combined treatment was associated with more grade 3-4 acute toxicity than anticipated, predominantly neutropenia and thrombotic events (total of 4 of 11 patients with grade 3/4 complications), and the dose limiting toxicity was established at tirapazamine 260 mg/m^2 and cisplatin 30 mg/m^2 [138]. The role of agents targeting hypoxia still needs to be elucidated. An ongoing Phase III randomized trial is comparing weekly cisplatin and alternate weekly tirapazamine to cisplatin alone.

Pro-Apoptotic Agents

Apoptosis can be induced by many stimuli; growth factors, RT, CT, immunotherapy or activation of death receptors. Tumor resistance to treatment is commonly cause by a loss of the tumor cell's ability to enter apoptosis. Therefore, modulation of specific molecular pathways leading to increased tumor cell death could widen the therapeutic window. Enhancing apoptotic cell death by modulating the survival pathway and combining this with RT induced cell killing may be synergistic. This may be more relevant in cervical cancer as the normal apoptotic signaling pathways may be disrupted by the HPV genome [139].

The extrinsic apoptotic pathway is initiated by activation of the death receptors on the cell membrane, among them the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). TRAIL became attractive as a potential anti-tumor therapeutic agent due to the selective induction of apoptosis in tumor cells while sparing normal cells. This challenging attribute stands in contrast to TNF and FasL, which have been shown to induce cell death in tumor cells, but to also cause lethal systemic toxicity. TRAIL binds to five different receptors, but only the death receptor 4 (TRAIL R1) and death receptor 5 (TRAIL R2) elicit an apoptotic response [140, 141]. TRAIL-R1 and R2 are expressed in a broad range of cancer cells including carcinoma of the cervix [142-145].

Recently, Mapatumumab (HGS-ETR1), a mAb that binds with high affinity to TRAIL-R1 and activates the extrinsic apoptotic pathway has been tested as a single agent in a phase I trial. Two out of 49 patients developed hyperbilirubinemia and respiratory distress as grade 3-4 acute toxicity. Fatigue, pyrexia and myalgia were the most common grade 1-2 acute effects. No evidence of objective tumor responses were observed, however the study included a heterogeneous population of solid malignancies with no specific selection of patients whose tumors over-expressed TRAIL-R1 [146].

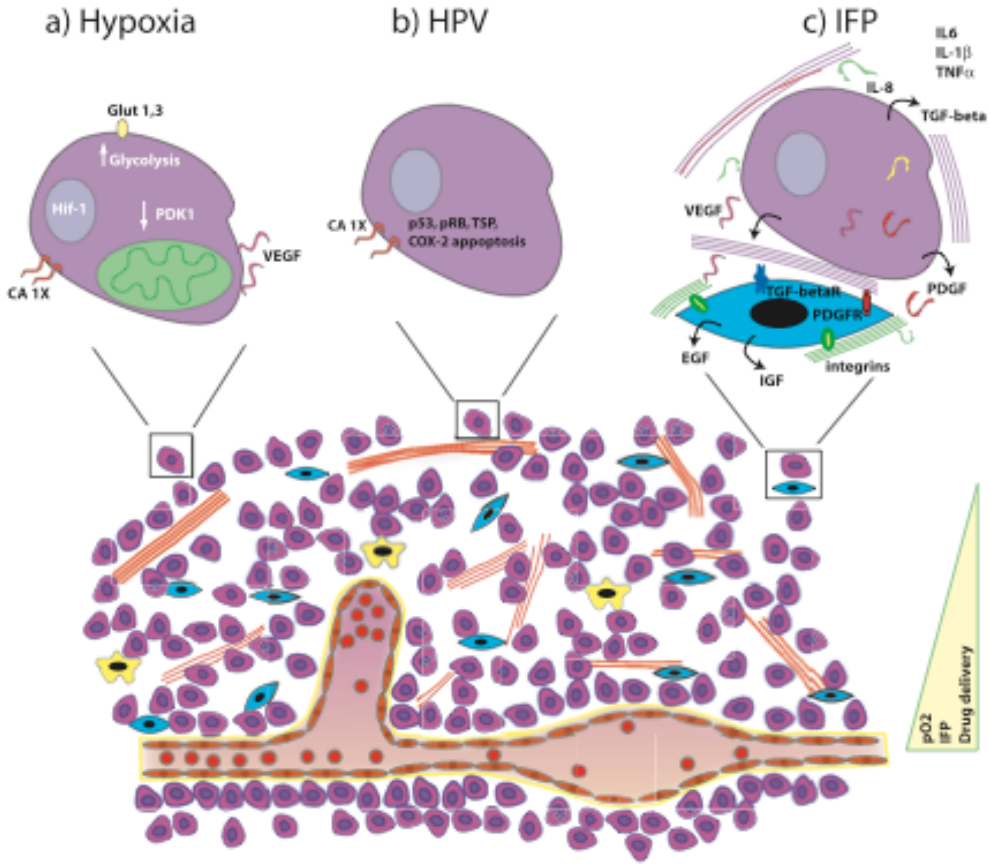

Fig. (1). Microenvironmental Condition of the Tumor Present Barriers to Therapy Adapted from Cairns, R. *et al.* Mol Cancer Res 2006;4:61-70. The genetic, biochemical, and physiologic factors that regulate these barriers to therapy represent potential targets for novel molecular targeted agents. a) Chaotic tumor vasculature contains unstable endothelium, leaky vessels, and instability in RBC flux. Poor oxygen delivery by the defective vasculature and oxygen consumption by the tumor cells result in hypoxic areas. Oxygen deprivation and activation of HIF-1 mediates adaptation of tumor cells to hypoxia. b) Major function of viral proteins E6 and E7 is to disrupt the activity of both tumor suppressor genes, p53 and pRB which results in dysregulated and unrestricted cell proliferation. Early activation of the angiogenic pathway with over-expression of the proangiogenic factor VEGF and decrease of the anti-angiogenic factors thrombospondin 1 and 2 have been associated with HPV infection. c) Induction of VEGF contributes to angiogenesis, increased numbers of activated fibroblasts and macrophages contribute to the formation of highly contractile ECM, rich in collagen fibers, raising the IFP. The release of cytokines and angiogenic factors by cancer and stroma cells creates a complex network of interactions regulating the plasticity of the connective tissue and the perfusion of the tumor.

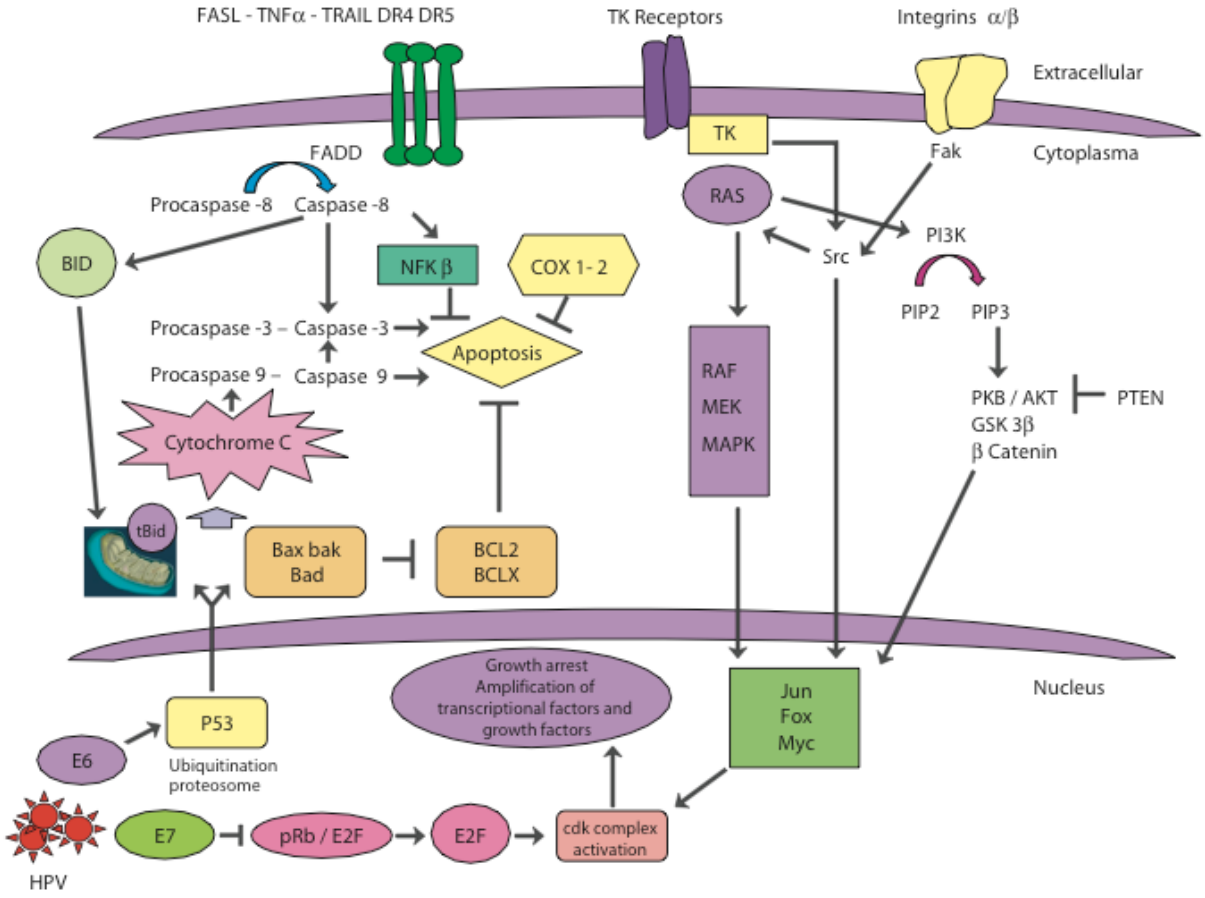

Fig. (2). Biological events in cervix cancer oncogenesis. HPV E6 induces degradation of p53 through ubiquitin dependant proteolysis causing G1 cell cycle arrest. HPV E7 binds pRb, rendering it unable to bind and regulate E2F, which then activates the cell cycle, decreases DNA repair thereby increasing mutations and genetic instability. This disruption also results in amplification of oncogenes, transcriptional factors and growth factors (e.g. cmyc, c-fos, c-jun, H-ras, erb-b2, TGF , VEGF. P53 induces transcription of a number of genes including BAX. BAX translocates into the mitochondria where it triggers cytochrome-C release, activating the caspases cascades and leading to apoptosis. Cells with defective function of p53 have increased resistance to apoptosis. Growth factors such EGF, VEGF, PDGF, and many others act by binding to a transmembrane TK receptor, activating the Ras protein signalling pathway. Ras activates the Ras/Raf/MEK/ERK pathway including MAPK, which ultimately activates the gene transcription factor family AP-1, resulting in increased gene transcription of cyclin proteins that drive the cell cycle. Integrins (surface proteins mediating cell motility, attachment and associated growth signals) can also actívate Ras. Both the p53 and ras promote VEGF secretion -- promoting angiogenesis and metastasis. Over-expression of E6 and E7 oncoproteins contribute to enhance angiogenesis in cervical cancer cells via stimulation of HIF- in a VEGF dependent manner. HIF-1 is a downstream effector of the PI3K/Akt pathway that modulates apoptosis and regulates the expression of many genes including those involved in blood vessel formation, contributing to more aggressive phenotypes and resistance to cancer therapies.

Apo2L/TRAIL, a dual (R1&R2) pro-apoptotic receptor agonist have also been tested as a single agent in phase I studies, and results have been reported in abstract form demonstrating that it can be safely administered in humans solid tumors with modest antitumor activity [147, 148].

In vitro and in vivo preclinical models combining CRT with mAbs that bind TRAIL R1&R2 have demonstrated enhanced anti-tumor efficacy and synergistic effect in a variety of solid tumors including cervix cancer, however no clinical data in cervix cancer patients is available to date [149, 150].

The proteasome also plays an important role in apoptosis by regulating intracellular protein degradation. HPV targets the ubiquitin-proteasome system: the viral oncoprotein E6 and E7 target host-tumor suppressor gene products p53 and pRB for accelerated proteasomal degradation and inactivation, causing cellular immortalization and transformation. The proteasome inhibitor bortezomib induces apoptosis and inhibits radiation-induced activation of nuclear factor κβ (NFκβ), which reduces tumor growth, enhances radiosensitivity, and has the potential to reverse chemo resistance [151, 152]. It has been recently shown that proteasome inhibition alters the response to tumor hypoxia and HIF production by a hypoxia independent mechanism [153]. This observation warrants assessment of bortezomib in combination with CRT in cervix cancer.

Anti-viral Therapies

New strategies are under study to enhance the anti-tumor effect of RT in HPV related cancers. Recently, experimental studies have shown that cidofovir, a DNA incorporated nucleoside analogue which has anti-viral activities through inhibition of the DNA polymerase, can enhance the therapeutic effect of ionizing radiation through down-regulation of E6-E7 and subsequent restoration of the tumor suppressor gene pathways [154, 155]. Specifically, in cell cultures treated with RT and cidofovir, significant inhibition of E6 and restoration of p53 function was observed with a simultaneous decrease in both VEGF expression and endothelial cell migration suggesting that the anti-tumor efficacy is due to inhibition of tumor angiogenesis [156].

Another promising agent under investigation is nelfinavir, which belongs to the protease inhibitor family and is currently used

in the treatment of human immunodeficiency virus (HIV) [157]. Interestingly, in-vivo and in-vitro studies have shown that its anti-tumor activity is mediated via inhibition of the PI3K-Akt pathway, leading to a decreased expression of both VEGF and HIFα. When combined with radiation nelfinavir exerted significant radio-sensitization in pre-clinical studies [158].

CONCLUSIONS AND FUTURE DIRECTIONS

HPV infection and its tumor promoting activity are associated with the micro-environmental changes observed in cervix cancer. Cytology screening programs and vaccination will have a tremendous impact in reducing cervical cancer worldwide, as well as other HPV-associated neoplasms. However, for many women who are already infected with the virus the effect of vaccination will take longer to evaluate as the progression from infection to cancer occurs over decades. In women with locally advanced cervix cancer CRT has become the standard treatment. However despite improved survival, loco-regional control still constitutes a major problem and other targeted treatments are necessary to improve effectiveness. Advances in the understanding of the tumor microenvironment such as hypoxia, angiogenesis, and apoptosis will open the window to implement new therapeutic approaches. Innovative targeted drugs alone and in combination with CRT are under investigation and there is a biological rationale to combine these agents with CRT in cervix cancer patients. Understanding the molecular biology of tumor microenvironment is essential in order to validate not only tumor but also vasculature biomarkers in clinical studies and optimize targeted delivery of these novel therapies.

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