Oral Oncology 50 (2014) 19–26

Contents lists available at ScienceDirect

Oral Oncology

journal homepage: www.elsevier.com/locate/oraloncology

Review

Combination antiangiogenic therapy and radiation in head and neck cancers

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ARTICLE INFO

Article history:
Received 8 August 2013
Received in revised form 24 September 2013
Accepted 2 October 2013
Available online 23 October 2013

Keywords:
Antiangiogenesis
VEGFR/PDGFR inhibitor
Radiation
DNA damage
Targeted therapy
Comibination therapy
Head and neck cancer

SUMMARY

Tumor angiogenesis is a hallmark of advanced cancers and promotes invasion and metastasis. Over 90% of head and neck squamous cell carcinomas (HNSCC) express angiogenic factors such as vascular endothelial growth factor (VEGF). Several preclinical studies support the prognostic implications of angiogenic markers for HNSCC and currently this is an attractive treatment target in solid tumors. Since radiotherapy is one of the most commonly used treatments for HNSCC, it is imperative to identify the interactions between antiangiogenic therapy and radiotherapy, and to develop combination therapy to improve clinical outcome. The mechanisms between antiangiogenic agents and ionizing radiation are complicated and involve many interactions between the vasculature, tumor stroma and tumor cells. The proliferation and metastasis of tumor cells rely on angiogenesis/blood vessel formation. Rapid growing tumors will cause hypoxia, which up-regulates tumor cell survival factors, such as hypoxia-inducing factor-1α (HIF-1α) and vascular endothelial growth factor (VEGF), giving rise to more tumor proliferation, angiogenesis and increased radioresistance. Thus, agents that target tumor vasculature and new tumor vessel formation can modulate the tumor microenvironment to improve tumor blood flow and oxygenation, leading to enhanced radiosensitivity. In this review, we discuss the mechanisms of how antiangiogenic therapies improve tumor response to radiation and data that support this combination strategy as a promising method for the treatment of HNSCC in the future.

Introduction

Head and neck squamous cell carcinoma (HNSCC), including cancers of the oral cavity, oropharynx, hypopharynx, pharynx and larynx, is the sixth most common cancer worldwide with approximately 600,000 new cases diagnosed each year [1]. The risk factors are tobacco and alcohol consumption [1], human papillomavirus (HPV) [2,3], Epstein–Barr virus (EBV) [4,5], areca nut [6], and dietary factors, like higher red meat consumption [1]. Two–third of patients are presented with advanced disease, and combined modality treatment with surgery, radiation therapy and chemotherapy is current standard of care [7]. Surgery can be performed if complete tumor resection is possible [8], however the majority of patients with advanced stage HNSCC are inoperable. The most frequent treatment is to combine chemotherapeutic agents with radiation [9]. Although concurrent chemo-radiation protocols are effective in treating HNSCC, treatment outcomes vary

Abbreviations: AA, antiangiogenic agent; AKT, protein kinase B; CA, carbonic anhydrase; CI, confidence interval; CCRT, concurrent chemoradiation; COX, cyclooxygenase; EBV, Epstein-Barr virus; EGF/R, epidermal growth factor/receptor; ERCC, excision-repair cross-complementing protein; FGF, fibroblast growth factor; FHX, 5-fluorouracil, hydroxyurea, radiotherapy; FLK, fetal liver kinase; FLT, fms-like tyrosine kinase; GLUT, glucose transporter; HIF, hypoxia-inducing factor; HNSCC, head and neck squamous cell carcinoma; HPV, human papilloma virus; IFP, interstitial fluid pressure; IGRT, hypofractionated image-guided radiotherapy; IMRT, intensity modulated radiotherapy; KDR, kinase insert domain receptor; Kit, stem cell factor receptor; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; NPC, nasopharyngeal carcinoma; mTOR, mammalian target of rapamycin; OSR, overall survival rate; PDGF/R, platelet-derived growth factor/receptor; PI3K, phosphoinositide 3-kinase; PLGF, placenta growth factor; PFR, progression free rate; RT, radiation; STAT, signal transducer and activator of transcription; TKI, tyrosine kinase inhibitor; VEGF/R, vascular endothelial growth factor/receptor; XRCC, X-ray repair cross-complementing protein.

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http://dx.doi.org/10.1016/j.oraloncology.2013.10.003

considerably and cytotoxicity side effects are significant [10]. In addition, tumor control and survival are still unsatisfactory. Even those who have achieved complete remission have a reported local recurrences incidence of 50–60%, and distant metastases develop in 20–30% of cases, with the 5-year overall survival rate less than 50% [11].

Recent studies have focused on the use of novel molecule-targeting agents as they have non-overlapping side effects and can be incorporated with existing treatment modality of HNSCC to improve outcome. Targeting epidermal growth factor receptor (EGFR) becomes a rational approach for HNSCC treatment since higher expression of EGFR has been associated with resistance to radiation and/or chemotherapy [8,12]. Cetuximab, a monoclonal antibody against epidermal growth factor receptor (EGFR), is an FDA-approved targeted agent for the treatment of advanced HNSCC [3,13]. Combination of cetuximab and radiation improves the overall survival in patients with locally advanced HNSCC, compared to radiation alone (49 months versus 29.3 months, $P = 0.03$) [8,14]. In order to provide personalized medicine and continue to improve outcome, other novel targeting strategies are needed. In the past 5 years, antiangiogenic therapies have seen a rapid ascent into mainstream clinical practice. Since angiogenesis is a hallmark of advanced and metastatic cancers, combining anti-angiogenic agents and radiation seems to be feasible, and warrants further investigation.

Vascular endothelial growth factor (VEGF) and its receptors: the role in HNSCC

VEGF plays a central role in the formation of new blood vessels and its importance in HNSCC has been well established [15]. The VEGF family of proteins consists of seven ligands, including VEGF A–E and placenta growth factor (PLGF) 1 and 2 [16]. PLGF, VEGF-A, VEGF-B are known to bind VEGFR-1. VEGF-A, VEGF-C and VEGF-D are known to bind VEGFR-2 [17,18]. VEGF-C and VEGF-D also bind to VEGFR-3, which is expressed by lymphatic endothelial cells and hematopoietic progenitor cells [19,20]. VEGFR-1/FLT-1 (fms-like tyrosine kinase) and VEGFR-2/KDR/FLK-1 (fetal liver kinase) are primarily involved in angiogenesis. Previous reports show that among VEGF family proteins, VEGF-A is the most common and can bind to two receptor tyrosine kinases, VEGFR-1 and VEGFR-2, promoting endothelial cell differentiation, migration, survival and induction of matrix metalloproteinase (MMPs) [21,22]. VEGFR-1 is more involved in the development of the vascular system during angiogenesis. VEGFR-2 is the predominant mediator of the angiogenic functions attributed to VEGF that exerts its mitogenic, chemotactic, and vascular permeabilizing effects on endothelial cells [23]. It also activates signaling pathways such as PI3K/AKT and Ras/MAPK pathways to help with endothelial cell proliferation and survival [16]. Meanwhile, tumor cells and stromal cells, like endothelial cells and fibroblasts, can produce VEGF. Through a paracrine loop, tumor cell VEGF can increase endothelial cell survival [24]. Since VEGF and PDGF receptors, as well as their ligands, are highly expressed in HNSCC, over-expression of PDGF enhances tumor formation by stimulating VEGF expression in neovessels and by attracting vessel-associated pericytes [25]. Dual inhibition of VEGF and PDGF can markedly decrease angiogenesis and inhibit tumor growth *in vitro* and *in vivo* [26,27]. Therefore, these could be good targets to inhibit angiogenesis for the treatment of HNSCC.

Radiation and hypoxia

Radiation-induced DNA double strand breaks trigger cell cycle arrest and cell death by apoptosis and/or necrosis. Oxygen is known to be a potent radiosensitizer that can promote reactive oxygen species (ROS)/free radicals production, essential for the induction of radiation-induced DNA damage [28]. As tumors grow, the microenvironment lacks an adequate blood supply, leading to regions that are underperfused and poorly oxygen ated or hypoxic [29]. This can lead to radiation resistance as a tumor microenvironment in oxygen deficit cannot facilitate radiation-induced DNA damage. Hypoxic tumor cells are particularly known to up-regulate hypoxia-inducing factor 1α (HIF-1α), a key transcription factor which increases the expression of VEGF [30]. After radiation exposure, the induction of a variety of transcription factors can activate transcription of growth factors, cytokines and cell cycle-related genes involved in multiple pathways and affect tumor cell survival or alter tumor cell proliferation. As for angiogenesis, radiation exposure can result in activation of EGFR which can activate PI3K/AKT and STAT3 pathways, and upregulate VEGF production [31]. The release of angiogenic growth factors like VEGF and FGF have been recognized as part of the radiogenic response of epithelial tumors [32]. Protection of tumor vessels by high VEGF levels could thereby contribute to the radio-resistance of tumors [33]. It has also been shown that Hsp90, EGFR, VEGF and AKT are known to play a role in radiation resistance [34,35]. Radiation therapy itself contributes to radioresistance by upregulating angiogenic and pro-survival factors, like Bcl-2, Bcl-xL and Survivin [36,37]. The increased tumor cell proliferation that is often seen after radiation may be the result of up-regulated angiogenic pathways [38,39]. This may lead to factors contributing to radiation resistance such as increased interstitial fluid pressure and vascular permeability, decreased tumor perfusion, increased oxygen consumption, increased hypoxic microenvironment, and up-regulated survival pathways, which makes radiation less effective [22].

Antiangiogenic interactions and radiation

Antiangiogenic agents with radiation have been tested in experimental conditions with various tumor models, tumor host strains, starting tumor size, final tumor volume measured, and dosing and scheduling [40]. Tumor size can affect oxygen tension, nutrient supply, and pH, which are all factors in determining radiation response [38]. As tumor size increases, oxygen tension and pH decrease because of a greater demand for oxygen and nutrients, and glycolysis dominates, leading to acidosis [41]. A previous study also showed that radiation dose required to achieve the same biologic effect is around 3 times higher in the absence of oxygen than in its presence, the so-called "oxygen enhancement effect" [42]. Antiangiogenic therapy produces a specific "vascular normalization window", a break when function, structure of tumor blood vessels and microenvironment temporarily become normalized [22]. Since tumor growth and angiogenesis are part of a codependent cycle and antivascular treatments can break this cycle and prevent revascularization after radiation [43], the potential function behind this, is to decrease interstitial fluid pressure (IFP) in tumor tissues and increase blood perfusion, so that antitumor drugs can easily penetrate into the tumors. Additionally, it will temporarily overcome hypoxia, improve oxygenation to produce more free radicals, result in more DNA damage, apoptotic cell death and increase the sensitivity to radiotherapy [44]. Therefore, the alternation of radiotherapy and short term antiangiogenic therapy is what produces this seemingly paradoxical effect of antiangiogenic therapy via vascular normalization. The concurrent administration of radiotherapy of contiguous antiangiogenic therapy will not produce a decrease in IFP and increased blood perfusion.

Postulated mechanisms

The precise mechanism by which angiogenesis inhibition improves clinical outcome is not fully understood yet. On one hand,