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Review

# Overcoming radioresistance in head and neck squamous cell carcinoma

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ABSTRACT

Radiation therapy plays an essential role in the treatment of head and neck squamous cell carcinoma (HNSCC), yet therapeutic efficacy is hindered by treatment-associated toxicity and tumor recurrence. In comparison to other cancers, innovation has proved challenging, with the epidermal growth factor receptor (EGFR) antibody cetuximab being the only new radiosensitizing agent approved by the FDA in over half a century. This review examines the physiological mechanisms that contribute to radioresistance in HNSCC as well as preclinical and clinical data regarding novel radiosensitizing agents, with an emphasis on those with highest translational promise.



## Introduction

Over 320,000 people die each year from head and neck squamous cell carcinoma (HNSCC), making it the eighth deadliest cancer worldwide [1]. Radiation therapy is a crucial treatment modality, but rates of therapeutic success remain unacceptable. The presence of numerous contiguous radiation-sensitive organs in the head and neck area necessitates careful radiation planning to preserve organ function and patient quality-of-life (QOL), while striving to maximize locoregional tumor control and patient survival [2]. A greater understanding of the mechanisms contributing to radioresistance in HNSCC will enable the development of therapeutics that improve tumor remission and reduce the radiation dose necessary to achieve tumor control, thereby mitigating morbidity and improving patient QOL. This review discusses the mechanisms underlying radioresistance in HNSCC as well as the emerging therapeutics that aim to overcome it.

## DNA-damage response

Radiation therapy utilizes ionizing radiation which severs the chemical bonds of DNA with the aim of inducing cell death. However, DNA damage leads to activation of the DNA-damage response (DDR) in order to mitigate this damage and promote cell survival [3]. Derangements of DDR in cancer cells contribute to radioresistance by preventing mutation or cell death that may have otherwise occurred secondary to DNA damage. DNA double-strand breaks (DSBs), which are the most toxic result of ionizing radiation, may be repaired through homologous recombination (HR) or non-homologous end joining (NHEJ). If repair is unsuccessful, cell division may become impossible and cell death can occur through apoptosis, mitotic catastrophe, senescence, necrosis, or autophagy [4,5].

Inability to repair DSBs results in sustained DNA damage that correlates with radiation response in HNSCC [6]. Moeller et al. demonstrated in their biomarker profiling study that increased expression of Ku80, a key mediator of NHEJ DNA repair via protein kinase DNA-PKcs, was associated with markedly worsened locoregional recurrence and overall survival, confirming prior findings that Ku80 is involved in radioresistance [7–10]. The DNA repair enzyme poly (ADP-ribose) polymerase (PARP) has also been implicated in radioresistance due to its role in NHEJ DNA repair [11]. In addition, the enzyme TRIP13 was recently identified as an important contributor to NHEJ-mediated radioresistance [12]. Both mutation and overexpression of the HR factor Rad51 have been associated with worse clinical outcomes after chemoradiation in HNSCC, suggesting that HR also plays an important role in radioresistance [13,14].

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The protein kinases ataxia telangiectasia mutated (ATM) and Rad3-related protein (ATR) are particularly vital in the initiation of DDR and the subsequent promotion of DNA repair through HR and NHEJ [15]. ATM and ATR also regulate cell cycle progression using checkpoint kinases 1/2 (CHEK1/2) to control cyclin-dependent kinase (CDK) activity in order to delay the cell cycle and allow time to repair the cell. Disruption along the ATM-CHEK2 and ATR-CHEK1 pathways appears to effectively elicit radiosensitization in HNSCC [16,17]. These pathways are closely intertwined; deletion of the *ATM*-containing distal arm of chromosome 11q, a primary mutation which occurs in 48% of HNSCC tumors, often produces compensatory ATR-CHEK1 upregulation [18,19]. However, targeting cell cycle control at the level of CDK may be too isolated in its effect to effectively abrogate radioresistance. Knockdown of CDK2 sensitized HNSCC cells to radiation in monolayer culture, but failed to induce significant radiosensitization in a more physiologically representative cell culture model which utilized a three-dimensional extracellular matrix [20].

Though p53 is a central regulator of many cell processes, including angiogenesis and metabolism, it chiefly influences radioresistance in HNSCC by contributions to DDR and cell death. DNA repair proteins, including ATR, ATM, and DNA-PKcs, respond to DNA damage partly by activating p53, which in turn triggers cell cycle arrest through p21 and CDK inhibition to allow for DNA repair [21]. If DNA integrity is too far compromised, p53 initiates pathways leading to cell death, most notably apoptosis [21]. Mutant p53 may circumvent this behavior to help sustain the cancer cell in conditions of stress and is thought to contribute to radiation failure in HNSCC [22–24]. In HPV-positive tumors, TP53 is predominantly wild-type which may contribute to the relative radiosensitivity of HPV-positive HNSCC, although the E6 viral protein attenuates p53 signaling to an unknown extent [25–27].

### *Metformin*

Metformin, a diabetes drug, exhibits anti-neoplastic activity in HNSCC through a mechanism that is thought to be primarily driven by adenosine monophosphate-activated protein kinase (AMPK) blockade [28]. TP53 mutational status may impact the radiosensitizing effects of metformin in HNSCC. Skinner et al. showed that metformin potentiated the effects of radiation in cells with disruptive TP53 mutations using in vitro (2–6 Gy of radiation) and in vivo models (a single dose of 5 Gy radiation and 8 daily metformin treatments at 250 mg/kg with orthotopic mouse model), but had no influence on cells with wild-type TP53. Cells with mutated TP53 have been shown to favor the glycolytic metabolic pathway for energy production, which may explain their sensitivity to metformin [29]. Among patients receiving post-operative radiation therapy, those concurrently treated with metformin had less locoregional recurrence and improved survival compared with matched controls (85% vs. 41%) [28]. A Phase I dose-finding study examining the addition of metformin to chemoradiation in HNSCC patients is underway (NCT02325401).

### *ATR*

In vitro studies have shown that ATR inhibition via siRNA can induce DNA damage and reverse radioresistance in head and neck cancer (single fraction of 5 Gy) [30]. Additionally, the clinical grade ATR inhibitor, VX-970, significantly enhanced the efficacy of cisplatin both in vitro and in a patient-derived xenograft model [31]. A phase I clinical trial for locally advanced HPV-negative head and neck cancer patients using VX-970 with or without radiation was recently initiated (NCT02567422).

### *Cell cycle modulation*

SAR-020106, a CHEK1 inhibitor, promotes mitotic entry in cells with radiation-induced G2/M arrest, producing increased apoptosis in p53-deficient HNSCC cells [32]. In the study, SAR-020106 reduced tumor growth when administered alongside 5 fractions of 2 Gy in a xenograft mouse model, with no evidence of metastasis or toxicity [32]. There are currently no clinical trials for SAR-020106, but a Phase I trial for the CHEK1 inhibitor CCT245737 recently started recruiting patients with advanced cancer. Another compound of interest is P276-00, a plant-derived flavone which inhibits the cyclin-D/CDK4/P16/pRB/E2F axis and can induce apoptosis by triggering G1/S arrest [33]. Results from a Phase II clinical trial of P276-00 for radiosensitization of recurrent or locally advanced HNSCC are forthcoming (NCT00824343). Olaparib, a PARP inhibitor, had an additive effect with PF-0477736, a CHEK1 inhibitor, on radiosensitization of HPV-positive HNSCC cell lines at a single fraction of 3 and 6 Gy [34]. A Phase I trial combining olaparib with RT in advanced HNSCC is currently recruiting (NCT01758731).siRNA knockdown of the Polo-box domain of Polo-like kinase 1 (PLK-1), an oncogene necessary for mitotic entry and activity, enhanced radiosensitization in a manner associated with increased apoptosis, G2/M arrest, and DNA damage (in vitro and in vivo models, two doses of 4 Gy) [35]. Inhibition of PLK-1 may not lead to direct toxicity as loss of function mutations in PLK-1 appear to have minimal effect in normal cells but can cause apoptosis in transformed cells [36]. Currently, several Phase I clinical trials to assess monotherapy in patients with advanced solid tumors are ongoing, but no trials are investigating its combination with radiotherapy (NCT01145885, NCT01348347, NCT01014429).

## Tumor microenvironment

The tumor microenvironment (TME) is composed of stromal cells, immune cells, vasculature, and extracellular matrix. TME impacts radioresistance through the interactions of hypoxia with cell proliferation, angiogenesis, and metabolism. Numerous clinical studies have associated tumor hypoxia with worsened prognosis after radiotherapy in HNSCC patients [37–40]. In addition to modulating nutrient metabolism and angiogenesis to mitigate the effects of radiation, hypoxia can prevent the oxidative fixation of DNA damage caused by ionizing radiation [41]. Hypoxic, underperfused areas of tumor exhibit reduced accumulation of DSBs in response to radiation when compared to adequately perfused areas [42]. However, the timing and context of hypoxia influence its effect on radiation response. While acute hypoxia reliably induces radioresistance, chronic hypoxia can actually sensitize cancer cells to radiation by decreasing synthesis of proteins involved in HR, including Rad51 and Xrcc3 [43].

Anemia in head and neck cancer patients correlates with reduced efficacy of radiation and chemoradiation therapy, which is hypothesized to occur due to its exacerbation of tumor hypoxia [44–47]. Unfortunately, efforts to circumvent the hypoxic effects of anemia using blood transfusion or erythropoietin have not demonstrated a benefit in terms of radiosensitization or patient survival [48–50]. In addition, smoking during radiation treatment promotes hypoxic radioresistance in part due to higher levels of circulating carboxyhemoglobin [51–54]. For this reason, smoking cessation is strongly advised during radiation treatment.

### *Vascular endothelial growth factor*

The state of hypoxia chiefly stimulates hypoxia-inducible factor-1 (HIF-1) stabilization, leading to promotion of numerous downstream target genes, including vascular endothelial growth