Biological opportunities for personalized radiotherapy of nasopharyngeal carcinoma

Li Lin, Yu-Pei Chen, Ying Sun

Department of Radiation Oncology, Sun Yat-sen University Cancer Center; State Key Laboratory of Oncology in South China; Collaborative Innovation Center for Cancer Medicine; Guangdong Key Laboratory of Nasopharyngeal Carcinoma Diagnosis and Therapy, Guangzhou, China

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Correspondence to: Ying Sun. Department of Radiation Oncology, Sun Yat-sen University Cancer Center; State Key Laboratory of Oncology in South China; Collaborative Innovation Center of Cancer Medicine; Guangdong Key Laboratory of Nasopharyngeal Carcinoma Diagnosis and Therapy, 651 Dongfeng Road East, Guangzhou 510060, China. Email: sunying@sysucc.org.cn.

Abstract: Nasopharyngeal carcinoma (NPC) is highly sensitive to ionizing radiation, and radiotherapy is the mainstay treatment modality for nonmetastatic disease. The advancement of radiation techniques has given radiotherapy the capability to deliver accurate radiation dose based on anatomical and geometrical information, which provides improved tumor target coverage with significantly better sparing of normal tissues, consequently leads to substantial improvement of locoregional tumor control and decreased radiation-induced toxicities. In the era of precision medicine, biology-driven personalized radiotherapy would be a promising approach to further widening of the therapeutic window of radiotherapy. In NPC, personalized radiotherapy is actually an under-researched area; however, the increasing number of basic and translational researches of radioresistance have provided opportunities for the development of novel biology-driven strategies for personalized radiotherapy of NPC. Different from previous reviews that detailed the mechanisms of radioresistance, current work placed emphasis on the clinical implications of these studies. Based on current preclinical experiments and pilot clinical studies, potential personalized strategies will include developing radiosensitizers targeting the mechanisms of radioresistance of NPC, biomarker-driven stratification for use of the radiosensitizers and radiation dose escalation or de-escalation, and personalized hypoxic dose painting intensity modulated radiotherapy (IMRT) guided by hypoxia imaging. In the future, these personalized strategies should be verified in well-designed preclinical studies and prospective clinical trials for parallel investigation of biological mechanisms and patient outcomes.

Keywords: Nasopharyngeal carcinoma (NPC); biology-driven personalized radiotherapy; radioresistance; hypoxia imaging; radiosensitizer

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Introduction

Nasopharyngeal carcinoma (NPC) is highly sensitive to ionizing radiation, and radiotherapy is the mainstay treatment modality for nonmetastatic disease. Modern techniques of radiotherapy, such as intensity modulated radiotherapy (IMRT) and image guided radiotherapy (IGRT), have allowed accurate delivery of radiation dose based on anatomical and geometrical information, which provides improved tumor target coverage with significantly better sparing of normal tissues (1,2). The advancement of radiation techniques has led to substantial improvement of locoregional tumor control (3,4), as well as decreased radiation-induced toxicities for nonmetastatic NPC (5).
Despite this, radiotherapy of NPC has not yet entered the era of precision medicine, in which radiotherapy should be tailored to individual patients based on the biological features.

In oncology, genomics has been gradually integrated into the routine clinical practice to guide personalized chemotherapy and targeted therapy in many cancers (6,7). However, personalized radiotherapy remains largely uninvestigated. A good example comes from the pilot attempts to reduce radiation dose in oropharyngeal cancer positive for human papillomavirus (HPV) since the higher radiosensitivity of HPV-positive oropharyngeal cancer due to lower DNA damage repair capacity (8,9). Furthermore, an important concept relating personalized radiotherapy was introduced as genomic-adjusted radiation dose by Scott et al. (10). They developed a genome-based model to derive an optimum genomic-adjusted radiation dose, which allow the individualization of radiotherapy dose to tumor radiosensitivity for different cancers including head and neck squamous cell carcinoma (HNSCC). For example, a high genomic-adjusted radiation dose suggests of high therapeutic effect of radiotherapy, which may indicate de-escalation of the radiation dose. This study is the first to develop a strategy to integrate biological differences of radiosensitivity into radiotherapy dose and it suggests that biology-driven personalize strategies would be a promising approach to further widening of the therapeutic window of radiotherapy.

In terms of NPC, personalized radiotherapy is actually an under-researched scope. In this review, with the purpose of promoting the development of novel biology-driven strategies for personalized radiotherapy of NPC, we discussed the so far evidence on current clinical practice relating personalized radiotherapy, and biological mechanisms of radioresistance of NPC and targeting the mechanisms to enhance radiosensitivity.

**Current clinical practice relating personalized radiotherapy of NPC**

**Risk stratification of tumor recurrence in NPC**

At present, risk-stratified therapy is an effective way to tailor personalized and precise treatment to NPC, especially in combined chemotherapy. For Radiotherapy, a retrospective study demonstrated that a moderately reduced of radiation dose by about 10% delivered with IMRT resulted in comparable prognosis to those with prescription dose of 70 Gy in patients stratified by tumor stage; thus, indicating the effectiveness of de-escalated radiation dose in patients of T1–T3 NPC (11). However, this needs to be validated in a prospective study with a larger sample size and more precise risk stratification is in urgent need.

Other than tumor-node-metastasis (TNM) staging system, lots of studies have proposed different clinical or radiomics models and biomarkers for more precise risk stratification of tumor recurrence in NPC (12-14). In patients with non-metastatic NPC who received radical IMRT, Chen et al. developed pretreatment nomograms, by incorporating clinical and pathological features of the tumors, to predict the risk of local and regional recurrence (12). These nomograms shown more accurate risk stratification performance than TNM staging system. Focused on patients with the most locally advanced NPC (T4 classification), radiomics signature from multiparametric magnetic resonance (MR) imaging was introduced to further improved the performance of clinical nomogram (13). Although these validated models could achieve better risk stratification of tumor recurrence in NPC, the biology-based stratification of radiosensitivity of NPC is urgently needed to guide escalation or de-escalation of radiation dose in tumors with distinct radiosensitivity.

**Dose escalation guided by functional imaging**

In terms of radiation dose escalation, a pilot prospective study has evaluated the effect and toxicities of fluorine-18 fluorodeoxyglucose (18F-FDG) positron emission tomography/computed tomography (PET/CT)-guidance dose painting IMRT (DP-IMRT), which could identify an appropriate tumor volume to be prescribed a higher radiation dose in locoregionally advanced NPC (15). In DP-IMRT group, a higher dose (75.2 Gy in 32 and 77.55 Gy in 33 fractions for patients with T1–2 and T3–4 disease, respectively) was prescribed to the subvolumes defined by the isocontour of maximum 50% standard uptake value (SUV); while the prescribed dose in CT-based IMRT group was 70.4–72.6 Gy in 32–33 fractions. Results showed that DP-IMRT significantly improved 3-year local failure-free survival (98.8% vs. 91.3%; P=0.032), locoregional failure-free survival (97.2% vs. 91.2%; P=0.049), and overall survival (91.8% vs. 82.6%; P=0.049). No statistically significant differences in acute and late toxic effects were observed. The results indicated that 18F-FDG PET/CT or other functional imaging might phenotype tumor heterogeneity of NPC, hence DP-IMRT guided by...
functional imaging might be a way to achieve personalized radiotherapy.

Additionally, emerging radiation technologies like proton therapy and carbon ion therapy have become available, offering new opportunities for escalation of radiation dose, improving tumor control and reducing toxicities (16,17), due to the physical characteristics that allow delivery of a high radiation dose to the tumor and maximal sparing of surrounding normal tissues. At present, these techniques are mainly studied in recurrent NPC. In the future, with the purpose of personalized radiotherapy, the right patients should be identified by biomarkers or functional imaging for dose escalation using proton therapy and carbon ion therapy at their primary treatment.

**Targeting mechanisms of radioresistance in NPC**

Radioresistance remains a major cause of local and regional recurrence in NPC patients; thus, predicting and targeting radioresistance could provide more personalized and effective strategies for radiotherapy and further improve the outcomes of NPC patients. To date, many studies have investigated the mechanisms of radioresistance, such as DNA damage repair, tumor hypoxia and autophagy. Figure 1 demonstrates the example mechanisms involving radioresistance of NPC and their clinical implications. Identifying and targeting the biomarkers could help to enhance the radiosensitivity of NPC and guide biology-driven personalized radiotherapy.

**DNA damage repair**

The most devastating damage of ionizing radiation is DNA double-strand breaks (DSBs), which induce cell-cycle arrest or cell death (18). Many studies have attempted to identify the molecules involved in DNA damage repair. For example, several DNA damage repair-associated genes were found to be significantly differed between radioresistant and radiosensitive NPC biopsy samples, of which replication protein A3 (RPA3) has been identified as a candidate radioresistance biomarker that associated with poor prognosis (19). Given that RPA3 was found to correlate only with radiosensitivity but not metastasis, it may have an advantage and potential application in guiding personalized radiotherapy of NPC.

Figure 1 Example mechanisms involving in radioresistance of nasopharyngeal carcinoma and their clinical implications. RPA3, replication protein A3; RAD51, recombination protein A; EBV-LMP1, Epstein-Barr virus encoded latent membrane protein 1; CHAF1B, chromatin assembly factor 1 subunit B; DNA-PK, DNA-dependent protein kinase; AMPK, adenosine monophosphate-activated protein kinase; CHK, checkpoint kinase; Atg5, autophagy related 5; ANXA6, Annexin A6; HIF, hypoxia inducible factor; CA9, carbonic anhydrase-9; VEGF, vascular endothelial growth factor; MR-PWI, magnetic resonance perfusion weighted imaging; PET, positron emission tomography.

Clinical implications:
1. Rad51 targeted therapy may be investigated as a potential novel agent for the adjuvant treatment of traditional radiation of NPC;
2. The combination of radiotherapy with PI3K/mTOR inhibitor, might be a promising therapeutic strategy for personalized radiotherapy of NPC.

DNA damage repair-associated genes

Clinical implications:
(1) AMPK activators could be used to enhance NPC radiosensitivity;
(2) DNA-PK inhibitor could serve as a radiosensitizer for patients with NPC and high CHAF1B expression.

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<th>DNA damage repair</th>
<th>Cell apoptosis</th>
<th>Tumor Hypoxia</th>
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<td>RAD51↑</td>
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| | MR-PWI | Hypoxic cell radiosensitizer: Nimorazole, Evofosfamide (TH-302), Triapazamine (TP2) |}

Clinical implications:
(1) Reversal of radioresistance through inhibition of the c-MYC-CHK1/CHK2 pathway.
Importantly, some proteins were found to enhance the ability of DNA damage repair and induce radiosensitivity of tumor cells. Lu and colleagues revealed that Epstein-Barr virus (EBV) encoded latent membrane protein 1 (EBV-LMP1) could suppress the DNA damage response through DNA-dependent protein kinase (DNA-PK)/adenosine 5’-monophosphate (AMP)-activated protein kinase (AMPK) signaling to promote radiosensitivity in NPC (23), which provided a mechanistic rationale in supporting the use of AMPK activators for NPC radiosensitization. Another study showed that chromatin assembly factor 1 subunit B (CHAF1B) enhances radiosensitivity by promoting DNA damage repair and inhibiting cell apoptosis, also in a DNA-PK pathway-dependent way, and indicated that DNA-PK inhibitor could serve as a radiosensitizer for patients with NPC and high CHAF1B expression (24).

Additionally, studies have suggested that cancer stem cells (CSCs) are more resistant to chemotherapy and radiotherapy than non-stem cells, mainly due to their differential response to DNA damage (25). In NPC, a recent study showed that overexpression of proto-oncogene c-MYC in CSCs contribute to radiosensitivity through preferential activation of the checkpoint kinase-1 (CHK1) and checkpoint kinase-2 (CHK2) checkpoint response and an increase in DNA damage repair capacity (26). Furthermore, loss of CHK1 and CHK2 expression would enhance radiosensitivity in vitro and in vivo. This study elucidates the role of the c-MYC-CHK1/CHK2 axis in regulating DNA damage checkpoint responses and reveals a potential therapeutic application in reversal of radiosensitivity through inhibition of the c-MYC-CHK1/CHK2 pathway.

Other than coding genes and proteins, non-coding ribonucleic acids (RNAs), such as micro RNAs (miRNA) and long non-coding RNAs (lncRNAs) also play a sizable role in radiosensitivity of NPC (27). Specifically, some miRNAs promote NPC radiosensitivity by targeting cell signaling pathways to promote DNA damage repair (28,29). Conversely, some other miRNAs could reduce NPC radiosensitivity (30). lncRNAs are also proved to have effects on radiosensitivity of NPC by involving in DNA damage repair, cell proliferation and apoptosis (31,32). Targeting radiosensitivity mechanisms of these non-coding RNAs might also be a potential strategy for personalized radiotherapy of NPC.

Despite uncovering mechanisms and biomarkers of radiosensitivity in NPC, which could be used to identify patients who could benefit from a biology-driven personalized treatment, as well as serve as new therapeutic targets for radiosensitization, the clinical use of these biomarkers remains substantially limited and warrant further investigation.

**Tumor hypoxia**

Intratumor hypoxia is a characteristic feature of solid tumors and hypoxic cells are proved to be resistant to ionizing radiation (33). Higher radiosensitivity of normoxic compared with hypoxic cells is most likely due to fixation of reactive oxygen species under normoxic conditions (34). Hypoxia has been constantly shown to be negatively associated with radiotherapy treatment response in head and neck cancers (35,36). Pathological hypoxic biomarkers have been studied in NPC biopsy samples; in particular, the transcription factor hypoxia inducible factor (HIF) 1-α and the genes up-regulated by HIF-1 such as carbonic anhydrase 9 (CA9) were studied as endogenous markers (37,38). Such intrinsic marker of hypoxia would have the advantage of being assessable on routine clinical biopsies. Besides, parameters of magnetic resonance perfusion-weighted imaging (MR-PWI) were found to be correlated with the expression of hypoxia-labelled markers, such as HIF-1α, vascular endothelial growth factor (VEGF), and microvessel density (MVD) in NPC, which is a promising new approach to predicting tumor hypoxia (39).

In addition to molecular profiles, hypoxia PET imaging has been gradually put into clinical use to detect tumor hypoxia, so as to selecting patients for radiation dose escalation or hypoxia-targeting therapy (40). In HNSCC, studies have suggested that boosting hypoxic tumor volume using baseline hypoxia imaging is feasible in principle, since 70–80% of the hypoxic volume is reproducible in test-retest experiments, while a small transient component was also seen in some patients (41-43). However, this test-retest experiment should be performed in NPC to confirm the stability of hypoxic volume in NPC tumor before its implementation in clinical practice. Moreover, if multiple images are obtained, it might be possible to increase the stability of hypoxic volume definition.

In NPC, using the hypoxia imaging agent fluorine-18 fluoromisonidazole (18F-FMISO) with positron emission tomography, tumor hypoxia was demonstrated in 100% of primary tumor and 58% of cervical lymph nodes metastases (44). Hypoxia detection using 18F-FMISO PET/CT and fluorine-18 fluoroazomycin arabinoside (18F-FAZA)
PET/CT imaging have been found to have a prognostic and personalized radiotherapy potential in many cancers including NPC (45,46). A preliminary study showed that 18F-FMISO PET/CT-guided imaging hypoxia-targeted dose boost (an escalation of 20% of the prescription dose) was technically feasible by using volumetric-modulated arc therapy (VMAT) in NPC (45).

In addition to dose boost, the hypoxic cell radiosensitizer nimorazole has been successfully introduced to reduce the negative impact of hypoxia on the radiosensitivity of tumor cells in HNSCC (47,48). 18F-FAZA PET has shown some advantage in guiding personalized radiotherapy using nimorazole as radiosensitizer in rhabdomyosarcomas and esophagus adenocarcinoma in preclinical studies (46,49). However, the effectiveness and safety of nimorazole in NPC need to be tested in preclinical and clinical studies. Moreover, several hypoxia-activated prodrugs, such as evofosfamide (TH-302) and tirapazamine (TPZ), have been evaluated in preclinical studies in NPC to show therapeutic potential (50,51). However, there is still a long way to go before putting into clinical use.

**Autophagy**

Autophagy is another crucial mechanism of tumor cell death induced by radiation (52), and inhibition of autophagy enhances radiosensitivity of cancer cells in several cancer cell types (53) as well as in NPC (54). However, the mechanism remains unclear. Mo et al. demonstrated that inhibition of autophagy enhances the susceptibility of NPC cells to radiation by suppress recombination protein A (Rad51) expression (55). Rad51 is a key protein of homologous recombination that has been demonstrated to play a critical role in the repair of DNA DSBs induced by radiation (55). Therefore, Rad51 targeted therapy may be investigated as a potential novel agent for radiosensitization of NPC.

Contrarily, another study revealed that Annexin A6 (ANXA6) could promote autophagy by inhibiting the PI3K/AKT/mTOR pathway and it thus contributes to radioresistance of NPC, while ANXA6 siRNA suppressed cellular autophagy by activating the PI3K/AKT/mTOR pathway, ultimately leading to radiosensitization (56). Consequently, the combination of siANXA6 and CAL101 (an inhibitor of PI3K, p-AKT, and mTOR, concurrently) significantly reversed the above siANAX6-reduced autophagy. Preclinical study has also demonstrated that targeting the PI3K/mTOR pathway by dual PI3K/mTOR inhibitors (GSK2126458 and PKI-587) increased the radiosensitivity of NPC (57). GSK2126458 and PKI-587 are highly selective and potent small-molecule inhibitors which could effectively suppress both multiple class I PI3K isoforms and mTOR kinase activity (58,59). Both studies indicated that the combination of radiotherapy with PI3K/mTOR inhibitor, might be a promising therapeutic strategy for personalized radiotherapy of NPC.

**Radiosensitizers**

Radiosensitizers are intended to enhance cancer killing by ionizing radiation while having much less effect on normal tissues. Gemcitabine, a commonly used chemotherapy agent in NPC, can be used as a radiosensitizer and recent investigations have suggested that it markedly decreases oxygen consumption in tumor cells (60). Other than hypoxic cell radiosensitizer nimorazole, radiation sensitizers targeting the cellular mechanisms involved in the cell cycle, DNA repair or apoptosis pathways, have been studied in NPC. Sodium glycididazole (CMNA) is a commonly used radiosensitizer, when combined with radiotherapy in treating patients with locally advanced NPC, it could improve curative effects without increasing adverse reactions, and significantly increase survival rates of the patients (61). Preclinical study revealed that CMNA enhance the radiosensitivity of the NPC cells via enhancing DNA damage and promoting cell apoptosis (62). Besides, a bisbenzylisoquinoline alkaloid which is isolated from the roof of the Chinese herb *Stephania tetrandra*, termed as tetrandrine, is found to be effective in enhancing the radiosensitivity of NPC cancer cells and the underlying mechanism could be associated with abrogation of radiation-induced G2/M arrest via activation of the CDC25C/CDK1/Cyclin B1 pathway (63,64). However, these agents are not routinely used in clinical practice of NPC. In the future, radiosensitizers targeting the mechanisms of radioresistance should be further investigated in preclinical and clinical studies.

**Future perspectives and challenges**

The experimental evidences to date suggest that mechanisms such as DNA damage repair, tumor hypoxia and autophagy, play sizable roles in radioresistance of NPC, targeting these mechanisms is one of the most promising aspects in personalized radiotherapy. In order to guide the most effective way to develop radiosensitizers,
it will be important to investigate in depth: (I) genes, signaling pathways, miRNAs or lncRNAs to regulate radioresistance; (II) blocking approaches for tumor cells to endow CSC phenotype or sensitize CSCs to iron irradiation; (III) how EBV interact with cancer cells or adjacent cells to promote NPC radioresistance. Most importantly, biomarker-driven stratification for use of radiosensitizer and radiation dose escalation or de-escalation are worthy of exploration in the future studies. On the other hand, pilot studies have demonstrated that hypoxia imaging has the potential to guide personalized hypoxic dose painting radiotherapy. In the future, these personalized strategies should be verified in well-designed preclinical studies and prospective clinical trials for parallel investigation of biological mechanisms and patient outcomes.

The arrival of new era of immunotherapy have provided unprecedented opportunities for personalized radiotherapy by optimization of strategies combining radiotherapy with immunotherapy, to improve both local and systemic tumor control. It is now recognized that, in parallel with killing tumor cells, radiotherapy can enhance the efficacy of immunotherapy agents by releasing vaccine in situ, improving antigen presentation, removing the inhibitory immune microenvironment, and increasing programmed death-ligand 1 (PD-L1) expression of tumor cells (65-68). Promising results have been reported with radiotherapy and immunotherapy in pre-clinical and pilot clinical studies (69,70). Widely clinical translation requires deep understanding of the complex interaction between radiotherapy and immune cells, cancer cells and particular immunotherapy agents; as well as investigation of the sequencing of the immunotherapy agent relative to radiotherapy, and the site and route of delivery of the immunotherapy agents.

A major challenge emerging for clinical research on personalized radiotherapy is that patient cohorts when precisely stratified by biomarkers get smaller. Hence, traditional phase III randomized control trials, which require large numbers of patients with the same kind of cancer, is no longer the best trial design. This warrants adaptation and further development of trial designs to assemble solid evidence for personalized treatment options. In the future, the important issue is optimization of the trial design and quality of preclinical studies in order to achieve positive clinical results.

**Conclusions**

In NPC, personalized radiotherapy is actually an under-researched area. Based on current preclinical experiments and pilot clinical studies, potential personalized strategies will include developing radiosensitizers targeting the mechanisms of radioresistance of NPC, biomarker-driven stratification for use of the radiosensitizers and radiation dose escalation or de-escalation, and personalized hypoxic dose painting radiotherapy guided by hypoxia imaging. In the future, these personalized strategies should be verified in well-designed preclinical studies and prospective clinical trials for parallel investigation of biological mechanisms and patient outcomes.

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