Nasopharyngeal carcinoma (NPC) is a malignant epithelial carcinoma of the head and neck region which mainly distributes in southern China and Southeast Asia and has a crucial association with the Epstein–Barr virus. Based on epidemiological data, both incidence and mortality of NPC have significantly declined in recent decades grounded on the improvement of living standard and medical level in an endemic region, in particular, with the clinical use of individualized chemotherapy and intensity-modulated radiotherapy (IMRT) which profoundly contributes to the cure rate of NPC patients. To tackle the challenges including local recurrence and distant metastasis in the current NPC treatment, we discussed the implication of using targeted therapy against critical molecules in various signal pathways, and how they synergize with chemoradiotherapy in the NPC treatment. Combination treatment including targeted therapy and IMRT or concurrent chemoradiotherapy is presumably to be future options, which may reduce radiation or chemotherapy toxicities and open new avenues for the improvement of the expected functional outcome for patients with advanced NPC.

The genetic and epigenetic alterations of NPC have been unveiled by the constant genome-wide studies, which involve cytogenic, allelotyping, CGH, and array-based CGH analysis. Not only copy numbers losses of chromosome 1p, 3p, 9p, 9q, 11q, 13q, 14q, and 16q but also amplification of chromosome 1q, 3q, 8q, 12p, and 12q were detected in primary NPC by CGH. Promoter hypermethylation of RASSF1A at 3p21.3, homozygous deletions of CDKN2A at 9p21.3 and TGFB2 at 3p24 have been regarded as triggers of tumorigenesis. The alterations of the somatic genome were reported when applying NGS technology on normal and tumor specimens, most of which are either EBV-positive or non-keratinizing NPC. As an important cause of NPC, EBV infection leads to the expression of various latent viral proteins, such as latent membrane proteins (LMP1, LMP2), BamH1-A fragment rightward reading frame 1 (BARF1) and nuclear antigen (EBNA1). At the same time, EBV infection also leads to the accumulation of a set of non-coding RNAs. Recently, Hong et al. found that circCRIM, a kind of circRNAs, promote NPC progression by inhibiting FOXQ1. These viral and host’s genomic products affect the signal transductions and cellular mechanisms of normal nasopharyngeal epithelial cells and have major roles in the pathogenesis of NPC. Mutations and repair disorders caused by viral infection are important causes of malignant changes in the nasopharyngeal epithelium. As a main substitution type at NpCpG trinucleotides, the C>T transitions results of spontaneous deamination of 5-methylcytosine, followed by a corrupted DNA mismatch repair signature, while C>G and C>T mutations at TpCpN trinucleotides are linked to catalytic polypeptide-like-mediated signature and the apolipoprotein B mRNA-editing enzyme. What's more, there are mismatch repair (MMR) gene mutations and a primary NPC subgroup with a hypermutation phenotype. C666-1 which is EBV-positive has
its own characteristics of inactivating PMS2 mutation and hypermutation phenotype.

The epigenetic machinery of NPC cells could be altered to reprogram the epigenomes of virus and host cells through EBV-encoded proteins. LMP1 contributes to the expression of DNA methyltransferase and the interaction of EBNA3A and EBNA3C with co-repressor of transcription CtBP could modulate polycomb group protein, which could form higher-order chromatin structures to silence target genes.27,28 The promoter hypermethylation of RASSF1A, BLU, CDKN2A, and DLEC1 could be detected in NPC, which have roles in DNA damage response, stress response, cell proliferation during G1 and STAT3 signal pathway.29-31 What’s more, a variety of tumor-related genes are epigenetically modified, involving transcription factors, enzymes, mitotic checkpoint regulators, cadherins, non-coding RNAs. As the most significantly hypermethylated gene, HOPX is highly associated with early distant metastasis in NPC.32 The antiangiogenic effect and anti-cancer activity of metalloprotease (MMP)-19 are inhibited in NPC by allelic deletion and promoter hypermethylation.33 As a mitotic checkpoint regulator, promoter hypermethylation of CHFR could cancel the impediment to chromatid condensation.34 Sun et al.35 found aberrant methylation of CDH13 could be detected in 89.7% primary NPC tumors with methylation-specific PCR. Long non-coding RNA MEG3 is also silenced epigenetically, which can inhibit proliferation, colony formation, induce cell cycle arrest, and has tumor-suppressive properties in vivo and in vitro.36 Leong also found abnormal histone bivalent switch is linked to suppressing DNA damage repair gene, which indicates another kind of epigenetic modification.

Targeted therapy involves the design of specific drugs that bind specifically to oncogenic targets within tumor cells to inhibit the development of tumors. MicroRNAs inhibit transcription and translation by binding to the 3′-UTR of target mRNA and affect the expression of target proteins, which has an important role in the genesis and development of tumors.37,38 In addition, abnormal activation and silencing of signal pathways in tumor cells also have a crucial role in tumor activities.39-42 Imbalance of the phosphatidylinositol 3-kinase (PI3K)/Akt/mTOR signal pathway is associated with malignant transformation and apoptosis of tumor cells, and metastasis and radioresistance of tumor tissues,43 while abnormal activation of the VEGF pathway is associated with angiogenesis in tumor tissue.44 Upregulation of the Wnt/β-catenin pathway in tumors is closely related to radioresistance,45 activation of the Notch pathway is widely present in human tumors by regulating self-renewal of cells with inhibition of differentiation.46,47 Abnormal activation of the Mitogen-activated protein kinase (MAPK) pathway is associated with proliferation, migration, invasion, and angiogenesis of tumor cells.48,49

In recent years, NPC patients receiving chemoradiotherapy have a poor quality of life, along with severe side effects such as bone suppression.49 However, targeted therapy can accurately identify and treat NPC cells with low toxic and side effects, suggesting a broad prospect of targeted therapy in the clinical treatment of NPC.51 In this article, we reviewed crucial molecules in signal pathways and miRNAs/lncRNAs in NPC cells studied in recent five years, regarding their roles in the promotion or suppression of NPC and functions as potential therapeutic targets of this disease. In addition, we present future perspectives of biomarker-based treatments and clinical diagnoses in NPC.

CRUCIAL SIGNAL PATHWAYS RELATED TO TARGETED THERAPY OF NPC

Aberrant activation of signal pathways brings about a variety of human diseases. Abnormal transmembrane signal pathways, including prosurvival pathways (PI3K/Akt, NF-κB, MAPK, STAT3, Wnt/β-catenin) and proapoptosis pathways (p53, endoplasmic reticulum stress) in NPC cells, have been proved to be associated with the development, progression, and prognosis of NPC by influencing biological processes such as cell cycle, apoptosis, and DNA repair. They are of potential clinical significance for personalized treatment strategies for NPC. The order of descriptions below corresponds to the depth of the last five years of research.

PI3K/Akt pathway

PI3K phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP2) and converts it to phosphatidylinositol 3,4,5-trisphosphate (PIP3), which is critical for activation of Akt.52 Phosphatase and tensin homolog (PTEN) is a phosphatase that dephosphorylates PIP3, and dephosphorylation of PIP3 can block PI3K/Akt signal pathway.33 mTOR is a serine/threonine kinase that one of the upstream regulators of mTOR is the PI3K/Akt signal pathway.53 In the PI3K/Akt pathway, frequent mutations of activators or regulators (PIK3CA, PTEN, PIK3R1, AKT2, and mTOR) were observed.25 The 110a catalytic subunit of PI3K is encoded by the PIK3CA gene and PIK3CA amplification likely results in the activation of the PI3K/Akt pathway.54,55 The activation of the PI3K/Akt pathway is also related to EBV-encoded latent membrane proteins 1, 2A, 2B.

NPC is highly associated with EBV infection and the major oncogenic proteins of EBV are LMP1 and LMP2A.36,55 LMP1 induces anti-TNF-related apoptosis-inducing ligand (TRAIL) activity in NPC cells by activating the PI3K/Akt pathway, thereby promoting the progression of NPC.60 DNA methyltransferase 1 (DNMT1) can mediate the downregulation of PTEN by LMP1 thereby activating Akt signal.61 Downregulation of DNMT1 can reduce the methylation level of the miR-152 gene and improve the expression of miR-152, leading to lower expression of DNMT1 mRNA and protein which inhibits the migration and invasion of tumor cells.62 On the other hand, Li et al.63 found that DNMT1 can promote EMT and metastasis of NPC by inhibiting the miR-142-3p/Zinc-finger E-box binding homeobox 2 (ZEB2) axis. LMP1 can induce lipid synthesis mediated by sterol regulatory element-binding protein 1 (SREBP1) through the mTOR signal pathway to promote cell proliferation and tumor invasion.64 LMP2A-mediated activation of the PI3K/Akt/mTOR/HIF-1α signal cascade can lead to vasulogenic mimicry (VM).65 Recent studies provide a basis for the selection of potential targets for targeted therapy of NPC.

Sodium butyrate (NaBu) may inhibit Akt/mTOR axis activity by promoting the degradation of EGFR in histone deacetylase 6 dependent way in NPC cells.66 Mitogen-activated protein kinase-activated protein kinase 2 (MK2) is a serine/threonine kinase.67 MiR-296-3p can block MK2-induced PI3K/Akt/c-Myc signal pathway by directly targeting and downregulating the expression of MK2 thereby inhibiting cell cycle and EMT as well as cisplatin resistance.68 Studies have demonstrated that miR-374a inhibits PI3K/Akt signal pathway, cell cycle, and EMT signal by directly targeting and downregulating Cyclin D1 (CCND1), while CCND1 can activate the PI3K/Akt pathway to increase the expression of c-Jun to downregulate miR-374a.69 As one of the most upregulated IncRNAs, FAM225A could enhance the expression of integrin β3 (ITGB3) by functioning as a miR-590-3p and miR-1275 sponge, thus activating FAK/PI3K/Akt signal pathway related to proliferation and invasion in NPC.70 FOXO1 can inhibit the expression of myosin heavy chain 9 (MYH9) by inhibiting the PI3K/Akt/c-Myc pathway and activating p53/miR-133a-3p axis thereby inhibiting the stem cell characteristics, metastasizing and enhancing the sensitivity to cisplatin of NPC cells.71 Mir-9 inhibits the activation of the PI3K/Akt pathway by targeting and downregulating miR-122 (MDK).72,73 MiR-92a promotes NPC cell migration and invasion by targeting and downregulating PTEN causing the activation of the PI3K/Akt pathway.74 EBV-miR-BART7-3p, an EBV-encoded BART-microRNA highly expressed in NPC, activates the PI3K/Akt signal pathway, induces the expression of c-Myc and c-Jun, and promotes the growth, proliferation, and tumorigenesis of NPC cells.75 For the
downregulation of fibroblast growth factor receptor 2 (FGFR2) can enhance the activation of the caspase pathway caused by cisplatin. Fibroblast growth factor 2 (FGF2) is the upstream molecule of the PI3K/Akt signal pathway so that FGF2/FGFR2 has become a crucial target in the targeted therapy of NPC as well. 

MI-R-16 can directly target and inhibit FGF2, resulting in the inhibition of both PI3K/Akt and MAPK signal pathways, causing the inhibition of proliferation, migration, and invasion of NPC cells.

Collagen type 1 alpha 1 (COL1A1) can regulate the radiosensitivity of tumor cells through the PI3K/Akt pathway.

MI-R-29a can induce radiosensitivity in NPC cells by directly targeting and downregulating COL1A1.

RNA-binding motif protein 3 (RBM3) inhibits cancer cells apoptosis by activating the PI3K/Akt/Bcl-2 signal pathway thereby enhancing radiation tolerance.

Ionizing radiation (IR) can increase the expression of phosphatase 1 nuclear-targeting subunit (PNUTS) in NPC cells, which induces EMT by activating the PI3K/Akt signal pathway.

Annexin A1 inhibits the autophagy pathway of NPC cells by increasing Akt phosphorylation level and membrane transport to activate the PI3K/Akt pathway thereby upregulating Sequestosome-1 (SQSTM1) and Snail, inducing EMT in NPC cells and promoting NPC cell migration, invasion, and metastasis.

Besides, IL-8 can regulate NPC metastasis by means of activating Akt signaling and inducing EMT of NPC cells. Downregulation of cyclin-dependent kinase inhibitor 3 (CDKN3) decreases the phosphorylation of Akt and inhibits the increase in the size and weight of transplanted tumors.

C-Src in NPC cells promotes the EMT process to improve the metastatic ability of cancer cells by activating the PI3K/Akt pathway. The cell membrane alteration also makes an important impact in metastasis. Flotillin-2 (Flot-2), a key component of lipid rafts, is found to be a high sensitivity biomarker for lymph node metastasis in NPC.

Silencing Flot-2 expression in 5-8F cells suppressed metastasis and proliferation as a result of inhibiting NF-κB and PI3K/Akt signal pathways. Besides, the important role of Flot-2 in the progression of NPC might be partially linked to its interaction with PLC-63 (PLCD3).

In addition, as a type I transmembrane glycoprotein, the role of epithelial cell adhesion molecule (EpCAM) in different cancers is distinct which might depend on the cell type and microenvironment. In head and neck squamous cell carcinomas (HNSSCs), the extracellular domain of EpCAM (EpEX) has a role of a ligand of EGFR that induces EGFR-dependent proliferation but counteracts EGFR-induced EMT.

On the contrary, the overexpression of EpCAM could promote EMT and stemness and metastasis through PTEN/AKT/mTOR signal pathway in NPC cells.

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In the view of the key role of inhibitor of NF-κB kinase (IKK) in the activation of the NF-κB pathway, IKK inhibitor is considered as one of the strategies of targeted therapy for cancer. The natural flavonoid glycoside vitexin can inhibit the activity of IKK thereby inhibiting the activation of the NF-κB signal pathway and inducing apoptosis in NPC cells. BST2, also known as CD317 or HM1.24, is found to be highly expressed in cisplatin-resistant NPC cells. It can activate the NF-κB signal pathway and increase the expression of downstream anti-apoptotic proteins. BST2 overexpression is associated with a low survival rate in patients with NPC. Therefore, BST2 could be used as a prognostic marker and therapeutic target for NPC.

Decreased expression of various components that inhibit the NF-κB pathway in NPC cells is one of the reasons for the abnormal activation of the NF-κB pathway. SIRT6, a kind of deacetylase, is downregulated in NPC cells. Lei et al. found that overexpression of SIRT6 might inhibit the expression of NF-κB by means other than acetylation and promoted apoptosis of NPC cells. In addition, knockout of NEAT1, a cancer-related long non-coding RNA, can inhibit the proliferation of NPC cells and promote apoptosis via promoting the activity of the miR-124/NF-κB axis. Deleted in liver cancer-1 (DLC-1) is a member of the GTPase-activating protein (GAP) family that is able to inhibit multiple tumor processes. Huang et al. demonstrated that DLC-1 could induce mitochondrial apoptosis and inhibit EMT and related processes by inhibiting the activation of the EGFR/Akt/NF-κB axis. Tumor necrosis factor-alpha-induced protein 3 (TNFAIP3), which has the function of the ubiquitin-editing enzyme, can inhibit the upstream signal transduction of the NF-κB signal pathway. Relative studies have shown that miR-19b-3p and miR-125b can directly suppress TNFAIP3 expression, thereby activating the NF-κB signal pathway, promote the proliferation of NPC cells, and inhibit the apoptosis of NPC cells. Ras-like estrogen-regulated growth inhibitor (RERG) is regarded as a potential tumor suppressor gene, expressed in a variety of normal tissues. Zhao et al. found that RERG was silenced by DNA hypermethylation in NPC cells. The extracellular-signal-regulated kinases (ERK)/NF-κB pathway is suppressed after RERG demethylation activation, which results in downregulating the expression of MMPs and pro-angiogenic cytokines, reducing the proliferation, migration, invasion, colony formation, and angiogenesis of NPC cells. Being a key active subunit of the NF-κB pathway, p65 is closely involved in the deregulation of the NF-κB pathway. Li et al. found that Epigallocatechin-3-gallate (EGCG) could inhibit NF-κB p65 activity, thus suppressing cancer progression, which is relevant to CSCs and EMT.

The NF-κB pathway can also be combined with immunotherapy. Hu et al. found that treating drug-resistant NPC cells with sunitinib could promote the expression of nature killer group 2 member D ligands (NKG2DLs) on the cell membrane by activating the NF-κB signal pathway, thereby enhancing NK cell-mediated cytotoxicity.

To sum up, the targeted inhibition of p65, LMP1, IKK, CDH6, Pim1, BST2, NEAT1, and the targeted activation of SIRT6, DLC-1,
TNFAIP3, RERG, NKG2DLs in the targeted therapy of NPC could be a potential therapeutic strategy through the NF-κB pathway.

**MAPK pathway**

MAPK is a member of the serine/threonine kinases family. There are three main subfamilies of MAPK: the ERK, the c-Jun N-terminal (JNK) or stress-activated protein kinases (SAPK), and MAPK14 (P38-α). The cascade activation of MAPK is an important pathway for the survival, proliferation, and drug resistance of cancer cells.127 In the MAPK pathway, recurrent mutations of its activators or regulators (FGFR2, FGFR3, BRAF1, NF1, and ERBB3) were detected in NPC samples.25 Hotspot mutations of KRAS, HRAS, and NRAS genes, promoter hypermethylation of RASAL, and DAB2 genes are also found.25,128

As an upstream kinase of the p38/MAPK pathway, the overexpression of mitogen-activated protein kinase kinase 6 (MAP2K6) is related to the radioresistance and poor prognosis of patients with NPC.129 In the MAPK/ERK pathway, PAK1 can phosphorylate Raf1 at Ser338 and MEK1 at Ser298, thereby activating the MAPK pathway.130,131 Franck et al. found that the macrocyclic lactone antibiotic ivermectin (IVM) could act as a PAK1 inhibitor to block the phosphorylation process of Raf1 and MEK1, resulting in cytotoxicity to NPC cells.129 After the MAPK pathway activation, it can phosphorylate and activate MAP kinase interacting serine-threonine kinase 1 (MNK1) which has been proved to be overexpressed in many kinds of cancers.133,134 Zhang et al. designed an MNK1 inhibitor compound (12dj) that could inhibit the phosphorylation of downstream eukaryotic initiation factor 4E (eIF4E), thus producing cytotoxicity against NPC cells which is possibly related to the apoptotic cell death subroutine. BLU, a tumor suppressor gene, is found to disrupt cell cycle progression and result in the suppression of tumor growth, which is related to downregulated ERK signaling and the corresponding downstream effector cyclins D1 and B1.136

MiR-483-5p directly decreases death-associated protein kinase 1 (DAPK1) protein expression, increases colony formation of NPC cells, reduces radiation-induced apoptosis and DNA damage, via activating the ERK signal pathway.137 On the contrary, miR-124 can inhibit the occurrence of NPC through the MALAT1/ERK/MAPK axis.138 In addition, Peng et al. found that the suppression of proliferation, invasion, and metastasis of NPC by miR-124 is achieved by regulation of Homo sapiens forkhead box Q1 (Foxq1). Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1 (PIN1), as an important signal regulator, is associated with EBV infection.139 Xu et al.141 found that PIN1 was overexpressed in EBV-associated NPC and AP1 inhibitors Juglone could eliminate the PIN1 effect of EBV-positive NPC cells and PIN1 inhibitors Juglone could eliminate the PIN1 effect of activating the MAPK/JNK pathway, reducing the downstream protein cyclin D1 expression and enhancing the activity of caspase 3, finally inhibiting proliferation. PDZ2 binding kinase (PKB) is a kind of MAPK kinase (MAPKK) that is able to participate in many cellular functions through phosphorylating P38, JNK, and ERK.142-144 Wang et al.145 applied the PKB inhibitor HI-TOPK-032 to NPC cells and found that inhibiting PKB induced the oxidative stress by activating JNK/P38 signals, resulting in the accumulation of reactive oxygen species (ROS) and cell apoptosis.

To sum up, the targeted inhibition of MAP2K6, PAK1, MNK1, FGFR2, PIN1, PKB, and the targeted activation of DAPK1, BLU, miR-124 in the targeted therapy of NPC could be a potential therapeutic strategy through the MAPK pathway.

**STAT3 pathway**

Signal transducer and activator of transcription 3 (STAT3), a transcription factor encoded by the STAT3 gene, is a member of the STAT protein family.146 STAT3 is phosphorylated by Janus kinase (JAK) to have the role of activator of transcription and mediate a series of signal cascade reactions.

In vivo, miRNA is involved in the regulation of the STAT3. Currently a study showed that miR-29a can inhibit the 3′-UTR of STAT3 mRNA, reduce the level of STAT3, phosphorylated STAT3 and Bcl-2, inhibit cell proliferation, and enhance the level of apoptosis and drug sensitivity.147 Li et al.148 found that sulfaphane can upregulate the level of miR-124-3p in NPC cells, inhibit the 3′-UTR of STAT3 mRNA, enhance cell apoptosis, and inhibit cell proliferation. In addition, miR-124-3p was found to be upregulated in UCA1 gene knockout NPC cells and inhibition of the proliferation, invasion, and migration of NPC cells by miR-124-3p was achieved by inhibiting the expression of integrin β1 (ITGB1).149 EBV has previously been proposed to be particularly relevant to the onset of NPC. A quintessential example should be cited that is LMP1, which may contribute to the development of NPC through the STAT3 signal pathway. Ding et al.140 revealed that LMP1 can promote the activation of STAT3, protein kinase C (PKC), NF-κB, and API in EBV-associated NPC cell NPC cells, thus activating Pim1 and promoting the proliferation of NPC cells.

He et al.150 showed that the downregulation of Raf kinase inhibitory protein (RKIP) can activate the signal transduction of the STAT3 pathway, enhance the migration and invasion ability of NPC cells in vitro, besides it can also enhance the metastasis and EMT in vivo. Furthermore, Huang et al.151 found that miR-181a can enhance radiosensitivity by inhibiting RKIP.

Eph receptors, a member of the receptor tyrosine kinase family (RTKs), participate in the regulation of the STAT3 signal pathway. Eph2 is often overexpressed in human malignant tumors, accompanied by the absence of Ephrin-A1, which can promote tumor invasion and metastasis, induce EMT, and maintain the cancer stem cell characteristics.152 JAK also has an essential role in the regulation of the STAT3 signal pathway. It is reported that IL-6 can activate IncRNA DANC in the STAT3 signal pathway, forming a positive feedback loop, enhancing the invasion and proliferation ability of NPC cells in vitro.153 Liu et al.154 indicated that ovadolidolide could significantly inhibit the JAK2/STAT3 signal pathway, upregulate the level of Bax and Slug and decrease the level of Bcl-xL, c-Myc, and cyclin D1, which significantly reduces the cancer stem cell characteristics of NPC cells together with survival, proliferation, invasion, migration, and EMT inhibited, and promote the apoptosis of NPC cells.

Wnt/β-catenin pathway

Wnt/β-catenin signal pathway disorder is closely related to the occurrence and development of cancers.155 As a class of secreted glycoproteins, Wnt can bind to the receptors on the cell membrane and regulate the content of β-catenin in cells. β-catenin combines with T cell factor (TCF)/lymphoid enhancing factor (LEF) family in the nucleus, regulating the expression of the downstream genes.156 The promoter region of Wnt inhibitory factor 1 and SOX1 have been reported to be frequently hypermethylated in NPC which is in connection with the Wnt/β-catenin pathway activation.

β-catenin is a key molecule in the Wnt/β-catenin signal pathway.159 Li et al.160 found that β-catenin could promote the expression of manganese superoxide dismutase (MnSOD), thereby reducing the level of ROS and increasing the resistance of anoikis in NPC cells. A small nuclear protein Chibby is able to inhibit reducing the level of ROS and increasing the resistance of anoikis in NPC cells. A small nuclear protein Chibby is able to inhibit β-catenin transcriptional activation through binding to its carboxyl terminus and decrease β-catenin content in the nucleus by cooperating with 14-3-3 adaptor proteins to transport it out of the nucleus, thereby inhibiting cell proliferation.161 MiR-34c directly suppresses β-catenin expression, thereby inhibiting proliferation, EMT, and radiosensitivity of NPC cells.162 Calpain small subunit 1 (Capn4), a small regulatory subunit of the Calpain family, has been shown to be highly expressed in a variety of cancers.155-165 MiR-
The overexpression of VEGF in tumor tissue is closely related to the increase in angiogenesis, proliferation, and metastasis. Neuruplin-1 (NRP-1) is a VEGF receptor, which is co-expressed with VEGF in many tumor cells. It has been reported that NRP-1 can promote tumor growth. Using shRNA silencing NRP-1, Sun et al. discovered a vital inhibitory effect on the proliferation of CNE-2Z cells in vitro. Fu et al. found that IncRNA HOX transcript antisense intergenic RNA (Hotair) was significantly upregulated in NPC cells and clinical specimens, which can directly enhance the VEGF transcription activity, or indirectly enhance the VEGFA transcription activity and angiopoietin 2 (Ang2) activity through GRP78, mediating angiogenesis and tumor growth in NPC. Contrarily, miR-495 can directly target the 3′-UTR of GRP78, leading to the radiosensitivity of NPC.

However, in the practical application, the clinical effect of conventional methods is limited as a result of the severe side effects. Li et al. studied the combination of bevacizumab and radiotherapy and chemotherapy and found that this combination treatment had a higher disease relief rate and a lower incidence of side effects, which can be used for further clinical experimental research.

Endoplasmic reticulum stress

Endoplasmic reticulum (ER) is directly linked to the synthesis and folding of membrane and secretory proteins. When cells suffer from physiological stress and pathological stress, the balance between demand for protein folding and ER processing ability can be broken, thus ER stress occurs. In the occurrence of ER stress, a series of signal transduction pathways in cells will be activated, which are collectively referred to as unfolded protein response (UPR).

ER stress is involved in the treatment of tumors. Different chemotherapy drugs can have different effects through the UPR signal pathway, as for etoposide, activation of the UPR signal pathway can increase the cell resistance, while it may enhance cisplatin sensitivity and induce apoptosis.

It was also reported that the combination treatment of lenvatinib and iodine-131 increased the expression of ATF-6, IER1 RERK, CHOP, JNK, p38, and caspase 3 in NPC cells, indicating that the treatment-induced apoptosis of NPC cells by upregulating ER stress. Recently, Pan et al. found that the use of a curcumin compound (B63) can promote cell apoptosis, inhibit cell proliferation, and arrest the cell at the G2/M stage. The level of CHOP, ATF-4, and XBP-1 protein was significantly upregulated, suggesting that the activation of ER stress pathway may have a significant role in the anti-tumor effect of B63. Lin et al. revealed that Tetrandrine (TET) treatment of NPC cells increased apoptosis, upregulated the expression level of calpain 1, calpain 2, caspase 12, 1RE-1a, 1RE-1 β, GADD153, Glycogen-regulated protein 78 (GRP78), ATF-6a, and ATF-6, indicating that TET induced cell apoptosis through ER stress.

Signal pathways in NPC, including the NF-κB pathway, MAPK pathway, STAT3 pathway, Wnt/β-catenin pathway and ER stress pathway can regulate their downstream genes’ expression, thereby influencing the biological behaviors of NPC cells. In each pathway, some potential targets and corresponding drugs mentioned in this review are shown in Fig. 2.

Some other signal pathways related to the targeted therapy of NPC

Zhao et al. found that the Notch signal pathway may have a new function of conferring radioresistance on CNE-1 and CNE-2 cells, which is mediated by miR-20a-5p and neuronal PAS domain protein 2 (NPAS2). MiR-20a-5p can promote the invasion, metastasis, and radioresistance of NPC cells by inhibiting GTPases Rab27B, an important role in the development and metastasis of tumors. As a suppressor and promoter in various tumors, TGF-β not only has inhibitive roles in tumorigenesis but also induces EMT and tumor.
The expression of E-cadherin, N-cadherin, twist, and snail can be regulated by the TGF-β pathway activated by annexin A2, which mediates the EMT process of NPC TW01 cells.197 Wang et al.198 found that berberine can reduce the activation of the TGF-β signal pathway induced by specificity protein 1 (Sp1) in CNE-2 cells, thus enhancing radiosensitivity. Nicotine negatively regulates miR-296-3p which directly targets the Ras/Braf/Erk/Mek/c-Myc pathway mediated by oncogenic protein MK2, promoting cytoplasmic transposition of c-Myc and the chemotherapy resistance, cell cycle progression, and EMT process of NPC.68 HIF-1 signal pathway is mainly regulated by the stability and activity of HIF-1α, and the overexpression of HIF-1α can lead to poor prognosis of NPC.199 Chen et al.200 constructed a kind of nanoparticle that can load HIF-1α siRNA, which has an inhibitive effect on tumor growth in CNE-2 tumor models. AMPK/mTOR/HIF-1 pathway can regulate the angiogenesis and glycolysis of NPC, and the microRNA-BART1-5P encoded by EBV acts on the α1 catalytic subunit of AMPK to regulate tumor metabolism and angiogenesis.201 The aberrant regulation of store-operated Ca²⁺ entry (SOCE) is highly associated with the process of NPC. The Ca²⁺ channel blocker skf96365 can inhibit the colony formation and increase the cell death rate by interfering with the Ca²⁺ signal induced by EGF, and induce the apoptosis and cell cycle arrest of G2/M and S-phase in CNE-2 and HONE-1 cells through caspase pathway.202 NaBu can promote SOCE in 5-8F and 6-10B cells to increase Ca²⁺ in flow and induce apoptosis in NPC cells.203 In addition, the overexpression of WW domain-containing oxidoreductase (WWOX) gene can also promote apoptosis by accumulating the cleavage of Caspase 3.204

**NON-CODING RNA RELATED TO TARGETED THERAPY OF NPC**

Long non-coding RNA (IncRNA) and microRNA (miRNA) are highly associated with various tumors. In the past 5 years, the mechanism of IncRNA and miRNA in the progression, radioresistance, drug resistance, and angiogenesis of NPC has been studied.184,205–207 The role of IncRNAs and miRNAs in different NPC cell lines and the corresponding mechanisms have been, respectively, listed in Tables 1 and 2.

The anti-cancer effect

Recent studies have found that some microRNAs and IncRNAs in tumor tissues are downregulated and show negative regulation of pathological activities of NPC.

IncRNA cancer susceptibility candidate 2 (CASC2) could inhibit proliferation, induce apoptosis through inhibiting the activation of miR-18a-5p/RB binding protein 8 (RBBP8) axis,208 MiR-324-3p can inhibit the invasion of tumor cells through its suppression of Homo sapiens GLI family zinc-finger 3 (GLI3) gene expression.209 High expressions of miR-7 inhibit the
proliferation and invasion of NPC cells by downregulating the expression of S-phase kinase-associated protein 2 (Skp2) which reduces the ubiquitination degradation of its targets such as p21, p57, and E-cadherin.\(^{210}\) MiR-212 directly targets and inhibits the expression of SOX4, a transcription factor at its downstream promoter.\(^{219}\) LINC00460 is highly associated with the EMT in NPC tissues compared to the normal nasopharyngeal epithelium, could promote proliferation and inhibit apoptosis in NPC via sponging miR-145 and UCA1.\(^{205}\) As a member of the CASC family, lncRNA CASC9 could promote proliferation and inhibit apoptosis in NPC via sponging miR-150.\(^{214}\) MiR-185-3p and miR-324-3p regulate the growth and apoptosis of NPC, which can be partially achieved by targeting the 3′-UTR of SMAD7.\(^{217}\)

### Table 1. Potential long non-coding RNA targets and related molecular mechanisms in NPC

| lncRNA | Cell lines | Roles | Effects on NPC cells | Corresponding signal pathway | References |
|--------|------------|-------|----------------------|-----------------------------|------------|
| ANRIL  | 5-8F, CNE-1, CNE-2 and HONE-1 | Oncogene | ↑Proliferation, ↓Apoptosis, ↓Radiosensitivity | [ANRIL]/[miR-125a] | 205 |
| XIST   | CNE-1, CNE-2 | Oncogene | ↑Proliferation, ↓Radiosensitivity | [XIST]/[miR-29c] | 206 |
| NEAT1  | HK-1, CNE-1, CNE-2 subclone S18 and SUNE-1 subclone 5-8F | Oncogene | ↑Proliferation, ↓Cisplatin resistance, ↓Angiogenesis | [NEAT1]/[let-7a-5p]/[lrsf-1] Ras/MAFk signal pathway | 207 |
| Hotair | CNE-1, CNE-2 | Oncogene | ↑Proliferation, ↓Radiosensitivity | [Hotair]/VEGFA or ↑ GRP78/ ↑Ang2 | 184 |
| CASC2  | SUNE-1, SUNE-2, 6-10B | Tumor suppressor gene | ↑Proliferation, ↓Apoptosis, ↓Migration, ↑Biomarker of poor prognosis | [CASC2]/[miR-18a-5p]/[RRBP8] axis | 185 |
| CASC9  | CNE-1 | Oncogene | ↑Proliferation, ↑Glycolysis, ↓Biomarker of poor prognosis | HIF-1 signal pathway | 218 |
| FAM225A| CNE-1, CNE-2, HONE-1, SUNE-1, HNE-1, 5-8F, 6-10B, C666-1 and HK-1 | Oncogene | ↑Proliferation, ↑Invagination | [miR-S90-3p, miR-1275]/[lTGB3/ FAK/P3K/Akt pathway | 70 |
| UCA1   | 5-8F, CNE-2 | Oncogene | ↑Tumor promoter, ↑Proliferation, ↑Mutation, ↓Invagination | [UCA1]/[miR-145]/[ADAM 17] | 206 |
| LINC00460 | 5-8F | Oncogene | ↑Metastasis, ↑Invagination, ↑EMT | [LINC00460]/[miR-30a-3p]/[Rap1A] | 207 |
| ANCR   | CNE-1, CNE-2, SUNE-1, C666-1, HONE-1 and HNE-1 | Oncogene | ↑Proliferation, ↓Radiosensitivity, ↓Caspase pathway | [ANCR]/[PTEN] | 226 |
| MALATA1| 5-8F, CNE-2 | Oncogene | ↑Radiosensitivity, ↑Activity of cancer stem cells | MALAT1/miR-1/Slug axis | 227 |
| PVT1   | 5-8F, CNE-2 | Oncogene | ↑Radiosensitivity, ↓Apoptosis, ↑Biomarker of poor prognosis | ATM–p53 pathway | 228 |

*↑* means upregulation, *↓* means downregulation, EMT epithelial–mesenchymal transition, ANRIL CDKN2B antisense RNA 1, XIST X inactive-specific transcript, Rsf-1 remodeling and spacing factor 1, VEGFA vascular endothelial growth factor A, GRP78 glucose-regulated protein 78, Ang2 angiogenin 2, CASC2 cancer susceptibility candidate 2, RBBP8 retinoblastoma binding protein 8, HIF-1 hypoxia induced factor 1, ITGB3 integrin j3, UCA1 urothelial carcinoma-associated 1, Rap1A Ras-related protein 1A, ANCR, antidifferentiation non-coding RNA, PTEN phosphatase and tensin homolog, MALAT1 metastasis-associated lung adenocarcinoma transcript 1

Promotion of cancer

Conversely, some miRNAs and lncRNAs are upregulated in cancer tissues and facilitate pathological activities related to NPC, including inhibition of apoptosis, promotion of tumor cell proliferation, invasion, and metastasis. LncRNA ANRIL, the expression of which is upregulated in NPC tissues compared to the normal nasopharyngeal epithelium, could promote proliferation and inhibit apoptosis in NPC via sponging miR-125a.\(^{206}\) As a member of the CASC family, lncRNA CASC9 could activate HIF-1α, thus facilitating the tumorigenesis and glycolysis of NPC cells.\(^{218}\) The expression of oncogene ADAM 17 could be increased after lncRNA urothelial carcinoma-associated 1 (UCA1) sponges miR-145 and UCA1 in NPC is regarded as a tumor promoter.\(^{219}\) LINC00460 is highly associated with the EMT in NPC cells via binding miR-30a-3p to regulate the expression of Ras-related protein 1A (Rap1A).\(^{220}\) What’s more, patients whose infiltrating lymphocytes are characterized by high expression of lncRNA AFAP1-AS1 incline to distant metastasis and poor prognosis, especially with positive PD1.\(^{221}\) No effective dinitrosopiperazine...
### Table 2. Functions and mechanism of miRNAs in NPC cells

| MiRNA | Cell lines | Roles | Functions | Mechanism | References |
|-------|------------|-------|-----------|-----------|------------|
| miR-26b | CNE-2, HNE-1 | Tumor suppressor gene | Plk4/1 | FOXD3/ | 225 |
| miR-7 | CNE-1, CNE-2 | Tumor suppressor gene | Plk4/1 | miR-7/ | 210 |
| miR-16 | CNE-1, CNE-2 | Tumor suppressor gene | Plk4/1 | miR-16/ | 77 |
| miR-1 | 5-8F, CNE-2 | Tumor suppressor gene | Plk4/1 | MALAT1/ | 227 |
| miR-124 | 6-10B | Tumor suppressor gene | Plk4/1 | miR-124/ | 138,139,114,167 |
| miR-148b | CNE-2, C666-1 | Tumor suppressor gene | Plk4/1 | miR-148b/ | 314 |
| miR-149 | 6-10B, 5-8F | Oncogene | Plk4/1 | miR-149/ | 222 |
| miR-150 | CNE-2, HONE-1 | Tumor suppressor gene | Plk4/1 | miR-150/ | 215 |
| miR-181a | CNE-2-IR, CNE-2 | Oncogene | Plk4/1 | miR-181a/ | 151 |
| miR-212 | 6-10B, CNE-2 | Tumor suppressor gene | Plk4/1 | miR-212/ | 211 |
| miR-324-3p | 5-8F | Tumor suppressor gene | Plk4/1 | miR-324-3p/ | 209 |
| miR-504 | CNE-2-IR, HK-1-IR | Oncogene | Plk4/1 | miR-504/ | 231 |
| miR-18a-5p | NPC cells | Oncogene | Plk4/1 | miR-18a-5p/ | 208 |
| miR-495 | 5-8F, 5-8F-IR | Tumor suppressor gene | Plk4/1 | miR-495/ | 185 |
| miR-138-5p | HONE-1, HK-1 | Tumor suppressor gene | Plk4/1 | miR-138-5p/ | 229 |
| miR-29a | CNE-2R, CNE-2 | Tumor suppressor gene | Plk4/1 | miR-29a-3p/ | 79 |
| miR-20a-5p | CNE-1, CNE-2 | Oncogene | Plk4/1 | miR-20a-5p/ | 195 |
| miR-29c | HNE-1, CNE-2 | Tumor suppressor gene | Plk4/1 | miR-29c/ | 216 |
| miR-19b-3p | CNE-1, CNE-2 | Oncogene | Plk4/1 | miR-19b-3p/ | 118 |
| miR-142-3p | CNE-2, SUNE-1 | Tumor suppressor gene | Plk4/1 | miR-142-3p/ | 63 |
| miR-124-3p | SUNE-1, C666-1 | Tumor suppressor gene | Plk4/1 | miR-124-3p/ | 149 |
| miR-152 | CNE-2 | Tumor suppressor gene | Plk4/1 | miR-152/ | 62 |
| miR-101 | CNE-2, 5-8F | Tumor suppressor gene | Plk4/1 | miR-101/ | 214 |
| miR-483-5p | CNE-1, 5-8F | Oncogene | Plk4/1 | miR-483-5p/ | 137 |
| miR-432 | CNE-2, 5-8F | Tumor suppressor gene | Plk4/1 | miR-432/ | 212 |
| miR-185-3p, miR-324-3p | CNE-2 | Tumor suppressor gene | Plk4/1 | miR-185-3p, miR-324-3p/ | 217 |
| miR-92a | 5-8F, 6-10B | Oncogene | Plk4/1 | miR-92a/ | 74 |
| miR-506 | CNE-2, 5-8F | Tumor suppressor gene | Plk4/1 | miR-506/ | 172 |
| miR-378 | 5-8F, 6-10B | Oncogene | Plk4/1 | miR-378/ | 315 |
| miR-9 | 5-8F, CNE-1 | Tumor suppressor gene | Plk4/1 | miR-9/ | 72 |

"↑" means promoting, "↓" means inhibiting, JAG1 Jagged1, Skp2 S-phase kinase-associated protein 2, FGFR2 fibroblast growth factor receptor 2, MALAT1 metastasis-associated with lung adenocarcinoma transcript 1, MTA2 metastasis-associated gene 2, PKP3 Plakophilin3, CCND1 cyclin D1, CCND2 cyclin D2, CDK2 cyclin-dependent kinase 2, CCNE2 cyclin E2, RIKP Raf kinase inhibitory protein, SOX4 SRY-box transcription factor 4, GLI3 Homo sapiens GLI family zinc-finger 3, NRP1 nuclear respiratory factor 1, RBBP8 RB binding protein 8, GRP78 glucose-regulated protein 78, EIF4EBP1 eukaryotic initiation factor 4E binding protein 1, eIF4E eukaryotic initiation factor 4E, COL1A1 collagen type I alpha 1 chain, Rab27B member RAS oncogene family, HBP1 HMGB-box transcription factor 1, TNFAIP3 TNF alpha-induced protein 3, ZEB2 Zinc-finger E-box binding homeobox 2, ITGB1 integrin beta-1, DNMT1 DNA methyltransferase 1, ITGA3 integrin subunit alpha 3, DAPK1 death-associated protein kinase 1, E2F3 E2F transcription factor 3, SMAD7 SMAD family member 7, PTEN Phosphatase and tensin homolog, LHX2 LIM Homeobox 2, TCF4 transcription factor 4, TOB2 transducer of ERBB2
Radioresistance

The radioresistance of NPC is one of the main reasons for the low efficacy of radiotherapy. It is widely believed that some miRNAs are upregulated or downregulated in radiation-resistant NPC cells and can reduce or enhance the sensitivity of tumor cells to radiation. How non-coding RNAs induce or inhibit the radioresistance of NPC has been shown in Fig. 3. The knockdown of IncRNA X inactive-specific transcript (XIST) could upregulate miR-29c, resulting in the inhibition of DNA damage repair and an increase of the radiosensitivity of NPC cells.236 Antidifferentiation non-coding RNA (ANCR) could regulate PTEN expression epigenetically to promote radioresistance.237 Besides, IncRNA MALAT1 modulates cancer stem cell activity of NPC and induces radioresistance via regulating the miR-1/slug axis.237 LncRNA NEAT1 downregulates remodeling and spacing factor 1 (Rsf-1) expression to activate the Ras-MAPK pathway to mediate NPC resistance to cisplatin through direct interaction with let-7a-5p.14,207 Downregulated expression of IncRNA PVT1 can also induce apoptosis of NPC cells through the DNA damage repair pathway after radiotherapy, thus being regarded as a prognostic indicator.238 By reducing the level of eucharyotic initiation factor 4E binding protein 1 (EIF4EBP1), miR-138-5p increases eIF4E, enhancing the autophagy of NPC cells induced by radiotherapy.239 Mir-101 enhances radiation-induced autophagy and radiation sensitivity of tumour cells by targeting S100A4 (STMN1).240 By inhibiting nuclear respiratory factor 1 (NRF1), miR-504 interferes with mitochondrial-mediated oxidation reaction and enhances radioresistance.231

CLINICAL SIGNIFICANCE

Clinical trials

Failure of NPC treatment mainly includes distant metastasis, recurrence, radiation resistance, and drug tolerance. Targeted therapy blocks signal transmission by targeting specific molecules related to tumor progression in and out of NPC cells, thus inhibiting the occurrence and development of NPC. At present, most of the clinical trials of targeted therapy against NPC are clinical phase I or II trials, which show that different targeted drugs can delay the process of NPC to different degrees and prolong the life of patients.

At present, clinical trials on targeted therapy for NPC are not abundant, which are mainly targeting EGFR and VEGF/VEGFR. Drugs that target EGFR include gefitinib, nimotuzumab, cetuximab, h-R3, and so on while drugs targeting VEGF/VEGFR mainly include axitinib, afiblercept, bevacizumab, sorafenib, sunitinib.

In phase II clinical trial, gefitinib is well-tolerated by subjects along with a poor response rate, making it less suitable for clinical treatment.232 In contrast, nimotuzumab in combination with 5-fluorouracil is significantly effective and well-tolerated by subjects in the treatment of recurrent and metastatic NPC.233 In stage N3 NPC, induction chemotherapy and sequential nimotuzumab plus CCRT achieved a positive survival benefit with tolerable toxicity.234 Compared with helical tomotherapy (HT) combined with cetuximab followed by adjuvant chemotherapy (ACT) with docetaxel, HT combined with cetuximab followed by ACT with cisplatin is an effective treatment for locally advanced disease (LANC), which has a high survival rate and few side effects.235 In a phase II trial, cetuximab-radiotherapy has better therapeutic effects but shows more acute adverse effects than cisplatin-chemoradiotherapy.236 h-R3 can enhance the radiosensitivity of locally advanced NPC with good safety, but the long-term efficacy is not effective.237 In patients with NPC, the use of bevacizumab can show a better effect compared with corticosteroids.238 Adding bevacizumab to chemoradiation is an effective and feasible treatment, suggesting that bevacizumab may delay the progression of subclinical disease.239 Axitinib has good control of disease progression and safety in patients with severe pre-treated NPC.240 Afibercept plus docetaxel has achieved preliminary efficacy in Chinese patients with NPC, and this treatment deserves further trials.241 Mk-2206 is an inhibitor of Akt, which has limited activity in the heavily pre-treated group of patients in a multicenter phase II clinical trial.242 Further studies are needed to select appropriate Akt inhibitors to treat NPC.

Famititinib, a tyrosine kinase inhibitor whose targets include VEGFR, platelet-derived growth factor receptor (PDGFR), and stem cell factor receptor (SCFR), has an encouraging anti-cancer profile and tolerability for patients with NPC along with chemoradiation. And it is necessary to expand the sample size to further confirm the efficacy.243 In NPC patients receiving high-dose radiation therapy, the incidence of upper respiratory tract bleeding will become higher after the use of sunitinib.244 Sorafenib has a certain anti-tumor effect and is well-tolerated by the subjects.245 The outcome of the phase II clinical trial has verified sorafenib combined with cisplatin and 5-FU is feasible and tolerable in patients with recurrent or metastatic NPC.246 Combination of endostar with gemcitabine–cisplatin chemotherapy can have good control of cancer progression and improve the prognosis of patients with NPC.247 Besides, endostar combined with IMRT has lower acute toxicity compared with IMRT.248 In clinical trials, the effect of targeted drugs alone is not good, whose PFS is generally less than one year, while targeted drugs combined with chemotherapy or radiotherapy can significantly delay the progress of the tumor, with a great improvement of PFS and OS.

In addition, there are some problems when studying clinical trials in targeted therapy of NPC:

1. The quantity of patients is small, which may cause the inaccuracy of the results.
2. Almost all the targets that occurred in clinical trials of NPC are VEGFR or EGFR and other new drugs targeting miRNAs or other key molecules can be developed.
3. More phase III clinical trials are needed after the drug has passed phase I and phase II clinical trials.

We summarize the current clinical trials related to targeted therapy for NPC, which are presented in Table 3.

Biomarkers and preclinical researches

PKB, mTOR, and PI3K in NPC tissues are highly upregulated and have higher positive rates than in normal nasopharyngeal tissues; Cox regression analysis reveals expressions of PI3K, PKB and mTOR are the major risk factors for the prognosis of NPC.249 High expression of LMP1 is strongly associated with NPC patients’ survival, which indicates LMP1 may be potential prognostic
biomarkers. In a study of NPC epithelial tissue, high expression of HIF-1α is found to be a new independent biomarker for predicting poor prognosis in NPC patients. It is found that the serum BMI-1 antibody in patients is significantly higher than in normal persons, suggesting that BMI-1 antibody may be a potential biomarker of NPC and has diagnostic and prognostic value. CDH6 is overexpressed in the LMP1-positive NPC tissues, which can promote EMT and metastasis of NPC, which may be a therapeutic target of NPC. Multivariable Cox regression analysis shows that BST2 expression can serve as independent prognostic factors and high BST2 expression can predict poor prognosis in patients with locally advanced NPC treated with platinum-based chemoradiotherapy. The expression of NRP-1 is highly upregulated in the NPC tissues and the multivariate analysis indicates that the overexpression of NRP-1 is an independent prognostic factor for NPC patients. Multivariate Cox regression indicates that the elevated MAP2K6 is independently associated with poor prognosis in NPC patients. Phosphorylated MNK1 has a significantly higher expression in NPC tissues compared to the nasopharyngeal epithelium and may be an independent prognostic factor of NPC. The concentration of DAPK1 methylation in serum is significantly higher compared with the normal group and may become a diagnostic biomarker for early NPC.

Clinical analysis shows that the high expression of miR-92a is associated with adverse clinicopathological features such as lymph node metastasis and distant metastasis in advanced
| Drug       | Mechanism/target | Trial phase | Treatment schedule | Patients number | Patients characteristics | PFS          | OS            | References |
|------------|------------------|-------------|--------------------|----------------|-------------------------|--------------|---------------|------------|
| Gefitinib  | EGFR             | II          | Single drug        | 19             | Recurrent metastatic NPC| –            | 16 months     | 232        |
| Nimotuzumab| EGFR             | II          | Combined with cisplatin and 5-fluorouracil | 39             | Recurrent metastatic NPC| 7.0 months  | 16.3 months   | 233        |
| Nimotuzumab| EGFR             | II          | Induction chemotherapy, sequential Nimotuzumab plus concurrent chemoradiotherapy | 45             | N3M0                    | 79.5% (3 years) | 85.6 (3 years) | 234        |
| Cetuximab  | EGFR             | II          | Concurrent HT with cetuximab, followed by chemotherapy (docetaxel and cisplatin) | 43             | Stage III and 10 Stage IV | 79.1% (2 years), 72% (3 years) | 93.0% (2 years), 85.7% (3 years) | 235        |
| Cetuximab  | EGFR             | II          | Cetuximab-radiotherapy | 21             | Stage III–IVb          | 95.2% (3 years) | 100% (3 years) | 236        |
| h-R3       | EGFR             | II          | Radiotherapy combined with h-R3 | 35             | Stage III–IVb          | –            | –             | 237        |
| Bevacizumab| VEGF             | II          | –                   | 44             | –                       | –            | 90.9% (2 years) | 238        |
| Bevacizumab| VEGF             | II          | Bevacizumab, corticosteroid-controlled | 112            | –                       | –            | –             | 239        |
| Axitinib   | VEGFR            | II          | Single drug         | 40             | Recurrent or metastatic NPC | 5.0 months | 10.4 months   | 240        |
| Aflibercept| VEGF             | I           | Aflibercept plus docetaxel | 16             | –                       | –            | –             | 241        |
| Mk-2206    | Akt              | II          | Single drug         | 21             | Recurrent or metastatic NPC | 3.5 months | 10 months     | 242        |
| Famitinib  | VEGFR, PDGFR     | I           | Single drug         | 20             | Stage III or IVa-b NPC | 75% (3 years) | –             | 243        |
| Sunitinib  | multi-kinase inhibitor | II          | Single drug         | 14             | Previously received high-dose radiation | 3.5 months | 10.5 months   | 244        |
| Sorafenib  | multi-kinase inhibitor | II          | Single drug         | 28             | –                       | 3.9% (6 months) | 4.2 months    | 245        |
| Sorafenib  | multi-kinase inhibitor | II          | Single drug         | 54             | Recurrent or metastatic NPC | 7.2 months | 11.8 months   | 246        |
| Endostar   | VEGF, VEGFR, PDGFR | II          | Combination of gemcitabine | 30             | –                       | 19.4 months | 90.2% (1 year) | 247        |
| Endostar   | VEGF, VEGFR, PDGFR | –           | Intensity-modulated radiotherapy combined with endostar | 23             | Stage III-IVa          | 100% (2 years) | 100% (2 years) | 248        |

*“–” means not clear* 
PFS: progression-free survival, OS: overall survival
expression of miR-185-3p and miR-324-3p is significantly elevated after treatment and decreases at recurrence or metastasis compared with pretreatment, which reveals miR-124-3p may serve as the prognostic biomarker in NPC.259 The expression of miR-185-3p and miR-324-3p may be vital markers for prediction of low response to RT/CRT.217 Expression of lncRNA Hotair in NPC tissues is higher than in nasopharyngeal tissues and the multivariate analysis reveals that Hotair is a potential biomarker for prognosis of NPC.260 LncRNA ANRIL is overexpressed in NPC tissues, which is related to the clinical stage and can serve as an independent predictor for NPC patients.261 In addition, Annexin A1, CDKN3, OCT4, c-Src, COX-2, TNFAIP3, RERG, PBK, STAT3, DANCRI, miR-29a, β-catenin, c-Myc, and Capn4 can be used as prognostic markers in NPC. Annexin A1 is a potential biomarker for predicting response to RT of NPC and predicting NPC differentiation and prognosis.262,263 High expression of CDKN3 is an independent negative prognostic factor in NPC.264 OCT4 is significantly associated with EMT and can be used as an independent prognostic factor in NPC.265 Elevated serum c-Src levels are associated with poor prognosis in NPC patients.86 In NPC patients, aberrant expression of COX-2 is associated with recurrence and poor prognosis.100 Downregulation of TNFAIP3 is associated with distant metastasis and worse patient prognosis.266 The methylation rates of RERG can serve as novel biomarkers for early detection and screening of NPC.267 High-level PBK in NPC is significantly associated with poor prognosis.145 STAT3 is associated with a relatively good prognosis in NPC patients.268 LncRNA DANCRI can be used as a biomarker for poor prognosis.269 MiR-29a can promote metastasis and invasion of NPC cells, and it would be an ideal prognostic marker.270 Positive expression of β-catenin and c-Myc is negatively correlated with the survival rate of NPC patients, demonstrating they can be used as important prognostic biomarkers.271 Capn4 can promote invasion and metastasis of NPC, which suggests that Capn4 may be an independent prognostic factor in NPC.272

The lack of preclinical models is an important reason that hinders the development of targeted therapies for NPC, and we summarize some meaningful preclinical studies.

PBK can promote the growth of NPC in nude mice and HI-TOPK-032, the specific inhibitor for PBK/TOPK, can significantly reduce the volume and weight of NPC in nude mice without obvious signs of toxicity.145 In Wong et al.’s research, the tumor weight of mice treated with the PI3K-mTOR dual inhibitor PF-0469150210 is significantly reduced when compared with the control group. Besides, the PF-04691502 has minimal effect on the animal’s body weight with no gross toxicity observed throughout the treatment. Brevilin A can inhibit PI3K/Akt/mTOR and STAT3 signaling pathways in vitro and Brevilin A treatment led to no significant weight loss in treated mice. These contributions to the preclinical development of Brevilin A as a chemotherapeutic for NPC.274 Evofosfamide is a hypoxia-activated prodrug that selectively targets hypoxic regions in solid tumors. Since HIF-1α is overexpressed in NPC tissues, the results provide preclinical evidence that Evofosfamide is used as a single drug in combination with DDP to target the selective anoxic fraction of NPC.275 COX-2 can promote the occurrence and recurrence of NPC, and the cellular senescence of fibroblasts in COX-2 knockout mice is significantly increased, suggesting that COX-2 may be a potential indicator to predict NPC recurrence and treatment resistance as well as a target for targeted therapy of NPC.190 The expression of EGFR correlates with β-catenin in NPC patient specimens, and β-catenin is responsible for regulating CSC characteristics of EGFR/Akt activation. The results suggest that targeting β-catenin is a reasonable clinical treatment for NPC with high EGFR or Akt expression.276

**DISCUSSION**

The models for NPC research can be divided into two categories: in vitro models and in vivo models. The former includes the previous two-dimensional models and the current potential of the three-dimensional (3D) models.277 Two-dimensional models are effortless to build and maintained at a low cost.278 However, the lack of crossstalk with fibroblasts, immune cells and endothelial cells indicates that the tumor microenvironment in vivo cannot be entirely simulated, which is not convenient for drug penetration and drug resistance evaluation.277,279,280 The construction of 3D tumor spheroid models for NPC could be accomplished by EB virus-positive C666-1 cells, and also EB virus negative CNE-1 and CNE-2, HONE-1, and SUNE-1.124,171,273,281 Researchers can utilize different matrix to reconstruct tumor microenvironment, which guarantees drug sensitivity, the alterations of signaling pathways and gene expression in tumor cells.277 Benefiting from its simulation of body condition, they ensure the reliability and validity of the experimental results of the targeted drug candidates. However, the study used lapatinib, a tyrosine kinase inhibitor against EGFR, on HONE-1 spheroids exhibited that the drug did not diffuse effectively to core cells of mass.282 Organoids are a group of cells that undergo organ differentiation and directional self-organization of adult stem cells or pluripotent embryonic stem cells in vitro.283 The organoids from patients are called patient-derived organoids (PDO) models, which have the characteristics of high simulation, short culture period, and stable passage.284 It is considered as the most ideal preclinical model at present. Tumor organoids are obtained by using tumor cells harvested from patients to culture masses in vitro, which retains the genetic background and reproduces the microenvironment. PDO models are promising substitutes for patients, which can simulate the treatment response from the aspects of pathology, gene, cell and tumor microenvironment.285 Organoids can also be used as the model of NPC stem cells. The increased expression of tumor stem cell markers in organoids can be considered as the concentration and enrichment of tumor stem cells so that organoids behave similarly to patients on tumor recurrence and treatment resistance. Organoids have advantages in the study of intercellular interaction and immune microenvironment for it stably carry the EB virus for a long time and mimic intercellular communication involving in exosomes.286 The test results of the breast cancer organoids for the HER-2 targeted drug gefitinib were consistent with the patients’ conditions, indicating that the organ model had similar properties to cancer in vivo.287 The construction of in vivo models could be divided into spontaneous models, induced models, transplantation models, and transgenic models.288-292 Spontaneous models are rarely obtained and utilized.292 Induction conforms to the characteristics of tumor dynamics and is often used to screen carcinogens but seldom used in targeted therapy for NPC.293 Transplantation models include subcutaneous xenotransplantation, orthotopic xenotransplantation, and patient-derived tumor xenografts (PDX).293,294 The first two tumors have different injection sites and in terms of the xenograft models, the tumor was in a mouse microenvironment rather than a human one. PDX models maintain the heterogeneity of tumor tissues, thus enabling phase I efficacy evaluation results to be more than 87% similar to clinical results.295,296 Several NPC PDXs are available for research in the past, including C18, C17, and C15.297 However, they passed so long in nude mice that they may have altered the biological characteristics of original tumors. Thus, novel NPC

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SPRINGER NATURE
In recent years, the research and development of drugs targeting IncRNAs for the treatment of NPC are still rare and studies will be needed to develop new strategies based on IncRNAs in the future. MiRNAs have recently been found to be highly or lowly expressed in tumor cells, and some miRNAs have been shown to promote or inhibit the processes of NPC cells in vitro and in vivo. Future NPC treatment can inhibit the proliferation, metastasis, EMT or angiogenesis of tumor cells by inhibiting the relevant miRNAs. Some miRNAs can increase the sensitivity of tumor cells to anti-cancer drugs and can be used in combination with targeted therapeutic drugs to improve the efficacy, while other miRNAs can reduce the radioresistance of NPC cells and can be considered in combination with local IMRT. However, to carry out the above possible therapeutic methods, it is also necessary to continuously study the functions of molecules including proteins, DNA, and RNA on the cytoplasm and membrane of tumor cells and normal cells, gradually complete the relationship network in the tumor and perform cellular and animal experiments on potential targets. In addition, miRNAs have an important role in the maintenance of the tumor microenvironment and communication between tumor cells, and it can be explored to understand what the specific mechanism of miRNA involvement is, which in turn provides a theoretical basis for possible targeted therapy.

In addition to further study in NPC treatment, effective population screening may improve the detection rate of early-stage NPC, which is conducive to the early treatment of NPC. By now, the detection of early-stage NPC is mainly through EBA IgA antibody (EA-IgA), anti-EV capsid antigen (VCA-IgA), anti-EV nuclear antigen 1 (EBNA1-IgA), while the extensive application is restricted by the low sensitivity and specificity. Encouragingly, recent studies have found that plasma EBV DNA detection displays a better outcome on early-stage NPC detection. Besides, with the profound study of vaccine technology and the growing understanding of EBV immunology, an increasing number of vaccine candidates against EBV have been developed. Clearly, thorough studies are needed to provide more insight into early-stage NPC screening and NPC vaccines, which have a huge application foreground in the prevention and treatment of NPC in the future.

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**AUTHOR CONTRIBUTIONS**

Y.K., W.H., X.H., and C.R. contributed to design the study. Y.K., W.H., J.Q., Q.G., and J.H. drafted and critically revised the manuscript. H.X., X.J., L.W., and C.R. discussed and revised the manuscript. All authors read and approved the final manuscript.

**ADDITIONAL INFORMATION**

Competing interests: The authors declare no competing interests.

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