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LETTER

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A summary of our serial study: mechanism of invasion and metastasis in nasopharyngeal carcinoma

Yizhi Ge[1†], Zhenyu Yan[1,2†], Mingyu Du[1], Luxi Qian[1], Fanyu Peng[1], Dan Zong[1*] and Xia He[1,2,3*]

Abstract

Purpose The patients who have nasopharyngeal carcinoma (NPC) have high possibility to metastases. Therefore, it is of great practical significance to explore the molecular mechanism of invasion and metastases in NPC.

Methods Herein summarizes some molecular mechanisms reported by our previous investigation.

Results Recent studies have reported the crucial roles of noncoding RNAs (ncRNAs) in tumor progression. In this letter, we summarize some newfound non-coding RNAs (miRNAs, lncRNAs, circRNAs) and coding RNAs, which could regulate invasion or metastasis in NPC by downstream genes.

Conclusion We elaborated on the clinical and therapeutic implications of partial putative markers for the treatment of invasion and metastasis in NPC.

Keywords Nasopharyngeal carcinoma, Invasion, Metastasis, Mechanism

1 Introduction

Nasopharyngeal carcinoma (NPC) is a unique head and neck cancer with highly malignant phenotype. Recently, with the wide application of imagological examination, image quality has been improved. However, about 20% of NPC patients still have local–regional recurrence and distant metastasis, which affects the therapeutic efficacy of patients with NPC. Therefore, the invasion and metastasis mechanisms of in NPC needs to be elucidated.

The invasion and metastasis of tumor is an orderly and complex biological process. The mechanism involves in the the activation and inhibition of related signaling pathways by promoting or suppressing corresponding function. Currently, few drugs are developed for targeted NPC therapy, and more than 70% of treatment failures are due to metastasis. Therefore, strengthening the research on invasion and metastasis-related molecules, and providing a scientific basis for the screening of biomarkers, which would be helpful for evaluating the risk and identifying therapeutic targets [1].

In recent years, the molecular mechanism of invasion and metastasis of NPC has been expanded from coding RNAs to some non-coding RNAs (ncRNAs) with important regulatory functions. These studies elucidate the molecular process of invasion and metastasis at multiple levels. Herein, we summarized the molecular mechanisms reported in our previous investigations.

[†]Yizhi Ge and Zhenyu Yan contributed equally to this work.

*Correspondence:
Dan Zong
zongdan910@sina.com
Xia He
hexiabm@163.com
[1] Jiangsu Cancer Hospital & Jiangsu Institute of Cancer Research & The Affiliated Cancer Hospital of Nanjing Medical University, 42 Baiziting Road, Xuanwu District, Nanjing, Jiangsu 210009, China
[2] The Fourth School of Clinical Medicine, Nanjing Medical University, Nanjing 210000, Jiangsu, China
[3] Xuzhou Medical University, Xuzhou 221000, Jiangsu, China

2 ncRNAs

2.1 miRNA

MicroRNAs (miRNAs) belong to ncRNAs that play an important role in cellular biological process. Most miRNA genes exist in a single-stranded form consisting of about 22 nucleotides (nt). Targeting 3' untranslated regions (3' UTR) of specific mRNAs is the main function of miRNAs [2].

We put forward the specific roles and mechanisms of miR-140 in NPC for the first time, which was markedly down-regulated in NPC tissues. Our research proved that miR-140 regulates the cell migration and invasion in NPC cell lines by negatively targeting CXCR4 (Table 1, number 1). miR-449b-3p, as a tumor suppressor gene, is down-expressed in NPC samples and inhibited cell invasion and migration (Table 1, number 2). EMT is regarded as the classic signal pathway of metastasis mechanism. miR-449b-3p regulated the EMT related proteins expression, which reflected the EMT related phenotype changes in NPC. Subsequently, the miR-449b-3p/ADAM17/NF-κB axis was identified. Our studies also explored other miRNAs that could regulate invasion or metastasis in different pathways. MiR-432 acts by binding to the 3′-UTR of E2F3 directly in NPC (Table 1, number 3). Our study indicated that miR-34a regulates TGF-β/SMAD4 signal axis (Table 1, number 4). Other signal axes, such as miR-154-5p/KIF14, miR-203a-3p/LASP1, miR-98/STAT3, miR-122/ PI3K/AKT, and miR-184/ Notch2 (Table 1, number 5–9) were also explored. In summary, miRNAs could be considered as tumor suppressors or promoters that target certain cancer-related genes in malignant NPC. Through the bioinformatics data platform under the background of big data our center built, we explored a new precision diagnosis strategy suitable for the characteristics of Chinese NPC patients. Should high-promising EBV miRNA biomarkers being discovered, it may benefit minimally invasive detection of NPC in the future.

2.2 lncRNA

Long non-coding RNAs (lncRNAs) are involved in the regulation of gene expression and attract great attention in various fields. However, the molecular mechanism of lncRNA involved in invasion and metastasis in NPC remains unclear. The expression of ZNRD1-AS1, a zinc ribbon domain containing 1 antisense 1, is upregulated in NPC cells, tissues and other cancers [3, 4]. ZNRD1-AS1 is overexpressed in 40 NPC tumor samples and 5 normal nasopharyngeal tissues from our clinical center. Moreover, they are positively correlated with advanced TNM stage. And we also demonstrate that ZNRD1-AS1 promotes the invasion and metastasis via the miR-335–ROCK1 axis in NPsC (Table 1, number 10).lncRNA-LUADT1 promotes invasion and metastasis through Hippo/YAP signaling pathway, and it is regulated by miR-1207-5p, showing a competitive relationship. This lnRNA-miRNA-gene-regulated axis strengthens the theory that ncRNA deeply affected the biological process of invasion and metastasis (Table 1, number 11). LncRNA-UCA1/miR-145/ADAM17 (Table 1, number 12–13) is also involved in metastatic mechanisms, and the lncRNA-UCA1 act as a molecular sponge in this pathway. Another lncRNA, lncRNA01133, mediates the biological function of NPC cells by binding to YBX1 (Table 1, number 14). We also explored the lncRNA-CYTOR which led to the promotion of NPC by inducing the up-regulation of ANXA2 by binding to miR-613 competitively, thus (Table 1, number 15). The impact of lncRNAn326322 on NPC was firstly reported by our studies till now (Table 1, number 16). Owing to the lack of an open reading frame, these lncRNAs participate in signaling pathways through the sponge function [5]. The competing endogenous RNAs (ceRNA) hypothesis confirms that lncRNA can regulate sponge microRNAs through post-transcriptional ways, thus affecting the function of microRNAs and increasing the expression of target gene [6–8].

2.3 circRNA

Circular RNAs (circRNAs) are widely expressed in a non-linear state in eukaryotic cells. They have a closed ring structure, which is usually looped by the exon or intron of the host gene. Their expression of circRNAs is relatively stable and they are difficult to degrade by exonuclease, which can be used as an ideal clinical diagnostic marker. Moreover, circRNA could serve as a molecular sponge to adsorb miRNAs, and affect gene expression by regulating splicing and translation. The expression of circFOXM1 is higher in NPC cell lines and is mainly located in the cytoplasm. Consistent with previous studies, we found that circRNAs could act as a "microRNA sponge" to regulate downstream gene expression (Table 1, number 17). Research work demonstrated the sponge function of circFOXM1 and the downstream miR-136-5p-SMAD2 signal axis. Furthermore, advanced clinical stage was associated with this circFOXM1 signal axis, which indicated that circFOXM1 might be a possible target in clinical NPC strategy. CircRNA, hsa-circ-0046263, could sponge miR-133a-5p and upregulate the expression of the downstream targeting gene IGFBP3. Besides, it promotes migration, invasion, and tumor metastasis of NPC cells (Table 1, number 18). Interestingly, IGFBP3 was also a

Table 1 The related articles reported in this manuscript of our previous research

Number	Title	DOI/PMIC	Publish year
1	MicroRNA-140 inhibits tumor progression in nasopharyngeal carcinoma by targeting CXCR4. International journal of clinical and experimental pathology	PMID: 31966622	2017
2	Feedback loop in miR-449b-3p/ADAM17/NF-κB promotes metastasis in nasopharyngeal carcinoma	https://doi.org/10.1002/cam4.2469	2019
3	MicroRNA-432 Suppresses Invasion and Migration via E2F3 in Nasopharyngeal Carcinoma	https://doi.org/10.2147/OTT.S233435	2019
4	MiRNA-34a reversed TGF-β-induced epithelial-mesenchymal transition via suppression of SMAD4 in NPC cells	https://doi.org/10.1016/j.biopha.2018.06.115	2018
5	MiR-154-5p Suppresses Cell Invasion and Migration Through Inhibiting KIF14 in Nasopharyngeal Carcinoma	https://doi.org/10.2147/OTT.S242939	2020
6	MiR-203a-3p suppresses cell proliferation and metastasis through inhibiting LASP1 in nasopharyngeal carcinoma. Journal of experimental & clinical cancer research	https://doi.org/10.1186/s13046-017-0604-3	2017
7	The effects of microRNA-98 inhibits cell proliferation and invasion by targeting STAT3 in nasopharyngeal carcinoma	https://doi.org/10.1016/j.biopha.2017.06.094	2017
8	MiR-122 exerts anti-proliferative and apoptotic effects on nasopharyngeal carcinoma cells via the PI3K/AKT signaling pathway	PMID: 30403591	2018
9	miR-184 Inhibits Tumor Invasion, Migration and Metastasis in Nasopharyngeal Carcinoma by Targeting Notch2	https://doi.org/10.1159/000493459	2018
10	ZNRD1-AS1 Promotes Nasopharyngeal Carcinoma Cell Invasion and Metastasis by Regulating the miR-335-ROCK1 Axis	https://doi.org/10.2147/OTT.S250028	2020
11	Long non-coding RNA LUADT1 promotes nasopharyngeal carcinoma cell proliferation and invasion by downregulating miR-1207-5p	https://doi.org/10.1080/21655979.2021.2001952	2021
12	MiR-145, a microRNA targeting ADAM17, inhibits the invasion and migration of nasopharyngeal carcinoma cells	https://doi.org/10.1016/j.yexcr.2015.08.006	2015
13	Long noncoding RNA UCA1 promotes the proliferation, invasion, and migration of nasopharyngeal carcinoma cells via modulation of miR-145	https://doi.org/10.2147/OTT.S182290	2018
14	Long non-coding RNA LINC01133 mediates nasopharyngeal carcinoma tumorigenesis by binding to YBX1	PMID: 31106003	2019
15	Long noncoding RNA cytoskeleton regulator RNA promotes cell invasion and metastasis by titrating miR-613 to regulate ANXA2 in nasopharyngeal carcinoma	https://doi.org/10.1002/cam4.2778	2020
16	Long non-coding RNA n326322 promotes the proliferation and invasion in nasopharyngeal carcinoma	https://doi.org/10.18632/oncotarget.22828	2017
17	CircFOXM1 acts as a ceRNA to upregulate SMAD2 and promote the progression of nasopharyngeal carcinoma	https://doi.org/10.1002/mgg3.1914	2022
18	Hsa_circ_0046263 functions as a ceRNA to promote nasopharyngeal carcinoma progression by upregulating IGFBP3	PMID: 32703944	2020
19	ZNF488 Enhances the Invasion and Tumorigenesis in Nasopharyngeal Carcinoma Via the Wnt Signaling Pathway Involving Epithelial Mesenchymal Transition	https://doi.org/10.4143/crt.2014.311	2016
20	Downregulating HMGA2 attenuates epithelial-mesenchymal transition-induced invasion and migration in nasopharyngeal cancer cells	https://doi.org/10.1016/j.bbrc.2015.05.068	2015
21	MYC-activated RNA N6-methyladenosine reader IGF2BP3 promotes cell proliferation and metastasis in nasopharyngeal carcinoma	https://doi.org/10.1038/s41420-022-00844-6	2022
22	LncRNA EPB41L4A-AS2 represses Nasopharyngeal Carcinoma Metastasis by binding to YBX1 in the Nucleus and Sponging MiR-107 in the Cytoplasm. International journal of biological sciences	https://doi.org/10.7150/ijbs.55557	2021

downstream targeted gene that crossed over other signal pathways and mechanisms which were described below.

We elaborated the evolution model of NPC from multiple levels, such as circRNA expression microarray from five pairs of NPC tissue samples. This further supported the spatio-temporal heterogeneity of NPC, and the identified biomarkers could be used as a target in the preparation of reagents, kits or tests for the

assessment of NPC metastasis. They can also be applied in drugs screening for NPC metastasis.

3 Coding RNAs

The activation of the Wnt/β-catenin signaling pathway play a role in ZNF488-induced EMT transition (Table 1, number 19). As an oncogene, ZNF488 is important in invasion and tumorigenesis in NPC. HMGA2, which is highly expressed during embryogenesis, is a member of the high-mobility group family (Table 1, number 20). It also has carcinogenicity in the metastatic process due to the TGFβ/Smad3 signaling pathway. This biomarker may greatly contribute to solve the radioresistance and promote radiotherapy sensitivity. It can be considered as a radiosensitization target and become an effective method for the treatment of patients with radiotherapy resistance.

4 N6-Methyladenosine

N6-methyladenosine (m6A) is the most abundant post-transcriptional modification in mRNA [9]. The main regulators of m6A are "writers, erasers, and readers" [10]. The methyltransferase complex is also called writers, while METTL3 is the only catalytic subunits. The "erasers" refer to FTO and ALKBH5, and the "readers" mainly includes YTH domain-family and IGF2BP family. These proteins contribute to the dynamic and reversible process of m6A modifications. IGF2BP3, an m6A readers, remains an unidentified mechanism in NPC. We investigated the basic expression and biological functions of IGF2BP3 in NPC. We first identified an oncogenic axis consisting of MYC-IGF2BP3-KPNA2 (Table 1, number 21). IGF2BP3 is highly expressed in tumor tissues and is related to the advanced stage, which has been reflected in vitro. We demonstrated that the genetic alteration of IGF2BP3 was due to MYC which acted as a transcription factor binding to the promoter region of IGF2BP3 and taking over the activation of mRNA amplification. Given that IGF2BP3 had the characteristics of RBP KPNA2, it was positively expressed in NPC tissues. Our data illustrated that the IGF2BP3 affected stability of KPNA2 mRNA in an m^6A-dependent manner. This hypothesis was also verified in vivo. In conclusion, IGF2BP3 played a critical role in NPC progression by affecting the mRNA stability of KPNA2.

LncRNA EPB41L4A-AS2 is also involved in invasion and metastasis of NPC. lnRNA EPB41L4A-AS2 inhibited the metastasis of NPC by binding to YBX. Simultaneously lncRNA EPB41L4A-AS2 reduced the stability of snail mRNA and reverse EMT via the miR-107-LATS2 axis at the post-transcriptional level (Table 1, number 22).

5 Conclusion

Our research group dedicates to exploring the mechanisms of non-coding RNAs in the invasion and metastasis in NPC. Besides, we discovered and explored other molecules, such as super-lncRNAs and ubiquitinates. We elaborated on the clinical and therapeutic implications of putative biomarkers that for clinical therapy of invasion and metastasis in NPC. A molecular stratification system containing miRNA, circRNA and other biomarkers was established, which is valuable for the establishment of accurate diagnosis system of NPC in our center.

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Authors' contributions
Yizhi Ge and Zhenyu Yan contributed to the data analysis, results interpretation, paper writing, and re-program for the work. Mingyu Du, Luxi Qian, and Fanyu Peng performed the critical revision for this manuscript. Xia He and Dan Zong contributed to research design, data analysis, results interpretation, paper writing, and critical revision of this and all related papers. All authors approved the final version and agreed to be accountable for all aspects of the work.

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All data of this manuscript re-published were authorized by related authors, and the related results could be downloaded online. Further inquiries can be directed to the corresponding authors.

Declarations

Ethics approval and consent to participate
This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication
Not Applicable.

Competing interests
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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