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RETRACTED ARTICLE: Upregulation of IncRNA HAGLROS enhances the development of nasopharyngeal carcinoma via modulating miR-100/ATG14 axis-mediated PI3K/AKT/mTOR signals

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**ABSTRACT**

We planned to dig the significant role of long noncoding RNA HAGLROS in nasopharyngeal carcinoma (NPC) and the latent mechanism. The levels of HAGLROS in NPC tissues and cells were determined, followed by correlation analysis of HAGLROS level and clinicopathological features of patients suffered with NPC. The impacts of HAGLROS dysregulation on NPC cell viability, apoptosis, and the expression of apoptotic proteins and autophagy-related symbols were investigated. Moreover, we explored whether HAGLROS modulated the expression of autophagy-related gene 14 (ATG14) by competitively sponging miR-100, and then regulated the briskness of PI3K/AKT/mTOR signals in NPC development. The levels of HAGLROS in NPC tissues and cell was very high. High level of HAGLROS indicated a short overall survival in NPC patients. Depressing of HAGLROS lessened NPC cell viability, enhanced apoptosis and reduced autophagy. Besides, HAGLROS negative controlled miR-100 and consequently targeted ATG14 expression, thus modulating NPC cell viability, apoptosis, and autophagy. Besides, dysregulation of HAGLROS/miR-100/ATG14 axis was correlated to the briskness of PI3K/AKT/mTOR signals in NPC cells. Our results indicate that of the augment of HAGLROS contributes to NPC development via modulating miR-100/ATG14 axis-mediated PI3K/AKT/mTOR signals. Our study will offer a comprehensive basis for better illustrating the pathogenesis of NPC.

**HIGHLIGHTS**

1. HAGLROS expression was upregulated in NPC tissues and cells.
2. High expression of HAGLROS indicated a short overall survival in NPC patients.
3. Silencing of HAGLROS promoted apoptosis and inhibited autophagy of NPC cells.
4. HAGLROS regulated ATG14 expression in NPC cells via sponging miR-100.
5. HAGLROS/miR-100/ATG14 axis regulated NPC development via PI3K/AKT/mTOR pathway.

**Introduction**

Nasopharyngeal carcinoma (NPC) is a multifaceted malignant tumor, making its diagnosis challenging [1]. Despite initial radical treatment, approximately 30% of NPC patients develop metastasis or recurrence [2]. Once metastasis occurs, the prognosis results of patients suffered with NPC are poor [3,4]. Statistical data presents that the 5-year survival rate of patients suffered with NPC has not attain the estimated expectation for improvement although great progresses have been made in molecularly targeted methods of NPC treatment [5]. The poor prognosis and lack of targeted therapies for NPC patients have fostered a major effort to prove neoteric diagnostic biomarkers for the early diagnosis and to explore reliable therapeutic targets for NPC patients.

Epidemiologic evidence has suggested that genetic suscep-

coding RNAs (lncRNAs), reported as a group of endogenous non-protein coding RNAs, longer than 200 nt in length and lack of coding-protein potential [7]. Growing studies have pointed out that plenty of dysregulated IncRNAs are involved in progression of various diseases [8–10]. Dysregulation of IncRNAs has been found to be the tumour suppressor genes or oncogenes in multiple tumours [11,12]. Recently, IncRNAs have been recognized as key players in the progression of NPC [13–15]. However, the potential molecular mechanisms of NPC mediated by IncRNAs still remain incompletely clarified.

HAGLROS, a 699 bp IncRNA, is recently reported to take part in the malignant processes of plenty of cancers, containing gastric cancer [16], osteosarcoma [17], colorectal cancer [18], hepatocellular carcinoma [19]. Despite these, the role of HAGLROS in NPC remains incompletely investigated, and the latent roles need to be disclosed. To confirm whether HAGLROS was involved in NPC, we firstly determined the level of HAGLROS in NPC tissues and cells and then dig the
association between HAGLROS level and clinicopathological features of patients with NPC. Moreover, HAGLROS could regulate mRNA expression by targeting miRNAs on the identify of a competing endogenous RNA (ceRNA), thus playing crucial roles in several cancers [18, 19]. We explored whether HAGLROS modulated the expression of autophagy-related gene 14 (ATG14) by competitively sponging miR-100, and then activating PI3K/AKT/mTOR signals in NPC development. Our study will offer a comprehensive basis for better illustrating the pathogenesis of NPC and pave a new angle of view for its treatment.

Materials and methods

Patient tissues

A total of 88 patients who were suffered with NPC undergoing biopsy of the nasopharynx at our hospital from March 2015 to March 2017 was chosen in this study. The total enrolled patients were with easy surgery history such as without radiotherapy or chemotherapy. The diagnosis of NPC was pathologically confirmed based on the criteria of World Health Organization (WHO). The tumor tissues and the matched non-tumor tissues were achieved during surgery. Afterwards, the obtained samples were immediately snap frozen in liquid nitrogen and then stored at −80 °C. This research obtained the approval of local ethics committee, and all chosen patients were informed with consent before experiment.

Cell culture test

The human NPC cell lines SUNE1, HK1, 5-8F and 6-10B were available from ATCC and then fostered in Roswell Park Memorial Institute (RPMI) 1640 medium (Sangon Biotech, Shanghai, China) containing 5% fetal bovine serum (FBS; Hyclone, Logan, UT). An immortalized nasopharyngeal epithelial cell NP69 was fostered in Keratinocyte-SFM containing bovine pituitary extract (Sangon Biotech). These cells were then fostered at 37 °C with 5% CO₂.

Transient transfection

The siRNAs for HAGLROS (si-HAGLROS#1 or #2) (100 nM, GenePharma, China) was introduced into 6-10B or HK1 cells to silence HAGLROS, and siNC was chosen as the negative control (NC) for si-HAGLROS; the overexpressed plasmids for HAGLROS (pc-HAGLROS, GenePharma) was transfected into cells to overexpress HAGLROS, and pcDNA3.1 was employed as the NC for pc-HAGLROS; miR-100 mimic (50 nM), mimics control (50 nM), and miR-100 inhibitor (150 nM), and inhibitor NC (150 nM) were purchased from GenePharma and also inserted into 6-10B or HK1 cell lines to detect the level of miR-100. Moreover, pEX-ATG14 and shRNA for ATG14 (sh-ATG14) provided by GenePharma were also transfected into cells to overexpress and knock down ATG14, respectively. pEX2 and sh-NC were chosen as the NC. Cell transfections were conducted using Lipofectamine 2000 reagent (Sangon Biotech), and different transfected cells were obtained for the next experiments after 48 h of transfection.

RT-qPCR

We isolated the tissues and cells resourced total RNA using Trizol reagent (Takara, Dalian, China), afterwards, M-MLV Reverse Transcriptase kit (Takara) was chosen for cDNA synthesis. Subsequently, real-time qPCR was conducted through Rotor-Gene RG-3000A (Corbett Life Science, Sidney, Australia) following the protocol of a standard SYBR Green PCR kit (Takara). U6 and β-actin were chosen as controls for miRNAs and RNAs, respectively, and the 2⁻ΔΔCT analytic method was selected for relative quantitation of gene levels.

Cell viability detection by MTT assay

We seeded cells in a 96-well plate (2 × 10³ cells). After an overnight incubation and subsequent transfection for indicated times, MTT (20 μL, 5 mg/mL; Sangon Biotech) solution was mixed with cells in each well and frosted at 37 °C for 4 h. After centrifugation, 150 μL of dimethyl sulfoxide was supplemented with cells for dissolving the formazan precipitates. MRX II absorbance reader (DYNEX Technologies, Chantilly, Virginia, USA) was chosen for calculating the cell viability at 470 nm.

Detection of apoptotic cells

We frosted cells in each group after different transfection, then cells were washed twice with PBS buffer and then re-suspended in 100 μL binding buffer. Double-staining of Annexin V and propidium iodide (PI) was carried out based on the introductions of the Annexin V-FITC Apoptosis Detection Kit (Sangon Biotech). Afterwards, cells were frosted for 1 h, then BD LSRII Flow Cytometer System (BD Biosciences) and FACSDiva Software were chosen for assessing the apoptotic cells.

Western blot assay

We lysed cells using cell lysis buffer (Beyotime, Haimen, China) for extraction of total protein. The total isolated proteins (30 μg per lane) were separated on 12% SDS-polyacrylamide gels and transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Primary antibodies to β-actin, ATG14, apoptotic proteins (including Bcl-2, Bax, cleaved-caspased-3, pro-caspased-3, cleaved-caspadased-9, pro-caspased-9), autophagy markers (including LC3II, LC3I, Beclin-1, and P62), and PI3K/AKT/mTOR pathway-related proteins (including PTEN, PI3K/p-PI3K, AKT/p-AKT, and mTOR/ p-mTOR) (Abcam, Cambridge, UK) were diluted to 1:1000 before use and then used to incubate the membranes overnight at 4 °C. After further incubation with the recommended secondary antibodies (1:5000, Abcam), the signals were uncovered using an Odyssey Infrared Imaging System (LI-COR, Lincoln, NE, USA). β-actin was chosen as the control.
**Dual-luciferase reporter assay**

The pMIR-HAGLROS-wt/mut and pMIR-ATG14-3′-UTR-wt/mut (Sangon Biotech) were obtained and then transfected into 6-10B cells, together with miR-100 mimic or mimic NC. After 48 h of co-transfection, luciferase activity of reporter vectors was evaluated with Dual-Luciferase Reporter Assay System (E1910, Promega, WI, USA).

**RNA binding protein immunoprecipitation (RIP)**

For investigation of the association of HAGLROS vs. miR-100, RIP assay, including preparation of lysate and magnetic beads, immunoprecipitation, RNA purification and qPCR analysis, was also carried out following the manufacturer’s instructions of RNA-Binding Protein Immunoprecipitation Kit (17e700, Millipore, USA). AntiAgo2 (Millipore, USA) was employed to amplify HAGLROS and miR-100, with normal mouse Anti-IgG (Millipore, USA) as a NC.

**Statistical analysis**

We carried out all experiments independently with 3 times repeat. Statistical analysis for obtained data was conducted by SPSS Statistics 20.0 software (IBM, Armonk, NY, USA). The data are shown as the mean ± standard deviation (SD). Student’s t-test was chosen for analysing differences between groups. Differences in HAGLROS expression between NPC tissues and matched non-tumour tissues were calculated using Wilcoxon test. The association between HAGLROS level and clinical characteristics of NPC patients were calculated by chi-square test. The Kaplan–Meier method was chosen for survival curves, followed by the log-rank test to estimate the statistical differences. Pearson correlation analysis was conducted to estimate the association of HAGLROS level vs. miR-100 level. A value of $p < .05$ was chosen to be statistically significant.

**Results**

**Correlation between HAGLROS level and clinicopathological features of patients with NPC**

HAGLROS is pointed out as a highly expressed factor in several cancers, such as osteosarcoma [17] and gastric cancer [20]. However, whether HAGLROS is also dysregulated in NPC remains unknown. In this study, we firstly detected the levels of HAGLROS in NPC tissues and analyzed the association of HAGLROS vs. the clinicopathological features of patients suffered with NPC. HAGLROS was distinctly enhanced in NPC tissues relative to that in the adjacent non-tumor tissues (ANTT) ($p < .05$, Figure 1(A)). Moreover, the augmented level of HAGLROS was positive associated with pathological stage (Figure 1(A)). Our findings indicated that HAGLROS may promote the NPC progress and is correlated with disease severity. In addition, based on the mean expression levels HAGLROS, 40 patients with NPC were separated as high-HAGLROS level group and the remaining 48 patients were separated as low-HAGLROS level group. The results of survival analysis showed that the HAGLROS expression was negatively associated with the overall survival time of NPC cases ($p = .0171$, Figure 1(B)). In addition, HAGLROS was discovered to be significantly associated with T stage, clinical stage and locoregional recurrence ($p < .05$, Table 1).

**Impacts of HAGLROS dysregulation on NPC cell viability, cell apoptosis and autophagy**

To investigate the impacts of HAGLROS on NPC cell biological processes, the HAGLROS level in several NPC cell lines was grabbed. HAGLROS was highly accelerated in NPC cell lines, including SUNE1, HK1, 5-8F and 6-10B, relative to that in normal nasopharyngeal epithelial cell NP69 ($p < .05$, Figure 1(C)). 6-10B cells were chosen for the subsequent experiments due to the highest HAGLROS expression among these NPC cell lines. Subsequently, after using approaches of pc-HAGLROS or si-HAGLROS (#1 or #2) transfection, HAGLROS expression was significantly enhanced or silenced in 6-10B cells ($p < .05$, Figure 1(D)). si-HAGLROS#2 had stronger inhibitory effects than si-HAGLROS#1 and was chosen for the following tests. Also, MTT assay pointed out that silencing of HAGLROS by transfection of si-HAGLROS#2 dramatically depressed 6-10B cell viability, whereas overexpression of HAGLROS by transfection of pc-HAGLROS changeover the above effects ($p < .05$, Figure 1(E)). Flow cytometry showed that silencing of HAGLROS remarkably promoted the apoptosis of 6-10B cells ($p < .001$); and western blot revealed that silencing of HAGLROS promoted the levels of several apoptotic proteins including Bax, cleaved-caspase-3 and cleaved-caspase-9, but depressed the level of anti-apoptotic protein Bcl-2 (Figure 1(F)). While no obvious effects on 6-10B cell apoptosis was observed when HAGLROS was overexpressed. Moreover, western blot revealed that silencing of HAGLROS significantly decreased the levels of LC3II/LC3I and Beclin-1, but increased P62 ($p < .05$, Figure 1(G)), deducting that silencing of HAGLROS inhibited autophagy of 6-10B cells. Overexpression of HAGLROS resulted in the opposite results on autophagy-related symbols in 6-10B cells.

**Correlation between HAGLROS and miR-100**

It is reported that IncRNAs could regulate the expression of miRNAs on the identify of ceRNA [21,22]. Previous findings have revealed that HAGLROS was involved in disease progression via targeting miR-100 [18,23]. We hence grabbed the association of HAGLROS vs. miR-100 in NPC. miR-100 was dramatically depressed in pc-HAGLROS group and surprised accelerated in si-HAGLROS#2 group in comparison to that in their respective NC group ($p < .01$, Figure 2(A)), suggesting that there was a reverse correlation between miR-100 and HAGLROS. To verify their relationship, we carried out further tests and the data revealed that HAGLROS could target miR-100 ($p < .05$, Figure 2(B–C)), in accordance with the above prediction. Moreover, compared to ANNT, miR-100 was gradually decreased in NPC tissues with the severity of pathological stage ($p < .05$, Figure 2(D)). Depressed miR-100 was also discovered in NPC cell lines, including SUNE1, HK1, 5-8F...
Figure 1. HAGLROS expression is upregulated in NPC tissues and high expression of HAGLROS indicated a short overall survival in NPC patients. A: Expression of HAGLROS was detected by qPCR in NPC and non-tumour samples. B: Kaplan–Meier survival analysis of HAGLROS expression for overall survival. Silencing of HAGLROS decreased cell viability, promoted apoptosis and inhibited autophagy of 6-10B cells. C: Expression of HAGLROS was detected by qPCR in NPC cells and normal nasopharyngeal epithelial cell NP69. D: Expression of HAGLROS was detected by qPCR in 6-10B cells after transfection with pc-HAGLROS and si-HAGLROS (#1 or #2), respectively. E: 6-10B cell viability was measured by MTT assay after transfection. F: 6-10B cell apoptosis was detected by flow cytometry after transfection, and the expression of apoptotic proteins was determined by western blot. G: Expression of autophagy markers was determined by western blot in 6-10B cells after transfection. Data are expressed as mean ± SD. *p < .05, **p < .01, and ***p < .001.
and 6-10B, relative to NP69 cells (p < .05, Figure 2(E)). Besides, Pearson correlation analysis confirmed that there existed a downside association of HAGLROS vs. miR-100 (R = 0.7026, p < .001, Figure 2(F)).

**HAGLROS regulates apoptosis and autophagy of 6-10B cells via miR-100**

To disclose the possible roles of HAGLROS and miR-100 in NPC, 6-10B cells were co-transfected with si-HAGLROS#2 and miR-100 inhibitor, followed by detection of the combined impacts of HAGLROS silencing and miR-100 depressor on cell viability, apoptosis, and autophagy. As presented in Figure 3(A), miR-100 was markedly accelerated/depressed in 6-10B cells by transfection with miR-100 mimic/miR-100 inhibitor compared to their respective NC, respectively (p < .001). Subsequent experiments showed that HAGLROS silencing and miR-100 inhibition simultaneously significantly reversed the impacts of HAGLROS silencing on the viability (p < .05, Figure 3(B)) and apoptosis (p < .01, Figure 3(C)) of 6-10B cells, as well as the levels of apoptotic proteins (Figure 3(C)) and autophagy symbols (p < .05, Figure 3(D)). All of the findings revealed that HAGLROS could regulate tumour apoptosis and autophagy by negatively regulating miR-100.

**ATG14 is targeted by miR-100**

miRNAs are shown to play important roles in disease development via controlling the levels of their targets [24,25]. Using HumanTargetScan, ATG14 was bio-informatically predicted as a latent target of miR-100 (http://www.targetscan.org/cgi-bin/targetscan/vert_71/, Figure 4(A)). Luciferase reporter test told us that miR-100 could directly target ATG14 3'UTR (Figure 4(B)). Besides, ATG14 was markedly depressed after overexpression of miR-100, but markedly increased after inhibition of miR-100 (p < .01, Figure 4(C,D)), implying that ATG14 expression was negatively regulated by miR-100.

**miR-100 regulates apoptosis and autophagy of 6-10B cells through targeting ATG14**

We further confirmed the latent impacts of miR-100 and ATG4 in NPC. As shown in Figure 5(A), ATG14 was firstly overexpressed in 6-10B cells by transfection with pEX-ATG14 Table 1. Correlation between HAGLROS expression and clinicopathological characteristics of patients with nasopharyngeal carcinoma.

| Characteristics         | Cases | Low | High | p value |
|-------------------------|-------|-----|------|---------|
| Gender                  |       |     |      |         |
| Male                    | 68    | 37  | 31   | .346    |
| Female                  | 20    | 11  | 9    |         |
| Age                     |       |     |      |         |
| <50                     | 42    | 22  | 20   | .679    |
| ≥50                     | 46    | 26  | 20   |         |
| T stage                 |       |     |      |         |
| T1-T2                   | 58    | 32  | 26   | .426    |
| T3-T4                   | 30    | 16  | 14   |         |
| N stage                 |       |     |      |         |
| N0-N1                   | 38    | 24  | 14   | .037    |
| N2-N3                   | 50    | 24  | 26   |         |
| Clinical stage          |       |     |      |         |
| I-II                    | 28    | 24  | 4    | .002    |
| III-IV                  | 60    | 24  | 36   |         |
| Distance metastasis     |       |     |      |         |
| Yes                     | 30    | 17  | 13   | .062    |
| No                      | 58    | 31  | 27   |         |
| Locoregional recurrence |       |     |      |         |
| Yes                     | 22    | 8   | 14   | .000    |
| No                      | 66    | 40  | 26   |         |

**Figure 2.** HAGLROS negatively regulated miR-100 expression in 6-10B cells. A: Expression of miR-100 was detected by qPCR in 6-10B cells after transfection with pc-HAGLROS and si-HAGLROS (#1 or #2), respectively. B: The association between HAGLROS and miR-100 was confirmed by dual-luciferase reporter assay. C: The association between HAGLROS and miR-100 was confirmed by RIP assay. D: Expression of miR-100 was detected by qPCR in NPC and non-tumor samples. E: Expression of miR-100 was detected by qPCR in NPC cells and normal nasopharyngeal epithelial cell NP69. F: Correlation between HAGLROS expression and miR-100 expression was analyzed by Pearson correlation analysis. Data are expressed as mean ± SD. *p < .05, **p < .01, and ***p < .001.
Figure 3. HAGLROS regulated cell viability, apoptosis and autophagy of 6-10B cells via sponging miR-100. A: Expression of miR-100 was detected by qPCR in 6-10B cells after transfection with miR-100 mimic, miR-100 inhibitor and their NCs, respectively. B: 6-10B cell viability was measured by MTT assay after cotransfection of si-HAGLROS#2 and miR-100 inhibitor. C: 6-10B cell apoptosis was detected by flow cytometry after cotransfection of si-HAGLROS#2 and miR-100 inhibitor, and the expression of apoptotic proteins was determined by western blot. D: Expression of autophagy markers was determined by western blot in 6-10B cells after cotransfection of si-HAGLROS#2 and miR-100 inhibitor. Data are expressed as mean ± SD. *p < .05, **p < .01, and ***p < .001.

Figure 4. ATG14 was a functional target of miR-100. A: Complementary pairing sequence of miR-100 and ATG14 predicted by HumanTargetScan. B: The target relationship between miR-100 and ATG14 in NPC was confirmed by dual-luciferase reporter assay. C–D: Expression of ATG14 was detected by qPCR and western blot in 6-10B cells after transfection with miR-100 mimic, miR-100 inhibitor and their NCs, respectively. Data are expressed as mean ± SD. *p < .05, and **p < .01.
compared to pEX2 (\(p < .001\)). 6-10B cells were co-transfected with miR-100 mimic and pEX-ATG14, followed by detection of the synergistic impacts of miR-100 overexpression and pc-ATG14 on cell viability, apoptosis, and autophagy. We found that overexpression of miR-100 alone markedly depressed cell viability (\(p < .05\), Figure 5(B)), enhanced apoptosis (\(p < .01\), Figure 5(C)), and inhibited autophagy by regulating autophagy symbols (\(p < .05\), Figure 5(D)), which were markedly changeover after co-transfection of miR-100 mimic and pc-ATG14 (all \(p < .05\), Figure 5(B–D)). We deducted that miR-100 could mediate the malignant behaviours via targeting ATG14 in 6-10B cells.

**Verification experiment in HK1 cells**

HK1 cells were transfected with different vectors to verify the reliability of the above results. HAGLROS, miR-100, and ATG14 in HK1 cells was all depressed by transfecting with si-HAGLROS, miR-100 inhibitor, and sh-ATG14 related to their respective NCs, respectively (all \(p < .01\), Figure 6(A)). Moreover, we found that silencing of HAGLROS remarkably inhibited HK1 cell viability (\(p < .05\), Figure 6(B)), induced apoptosis (\(p < .001\), Figure 6(C)) and inhibited autophagy by retraining the levels of LC3II/LC3I and Beclin-1 but accelerating p62 (\(p < .05\), Figure 6(D)). Moreover, the impacts of silencing of HAGLROS alone in HK1 cells were dramatically counteracted after HAGLROS silencing and miR-100 inhibition synchronously, which were further reversed after knockdown of ATG14 concurrently (all \(p < .05\), Figure 6(B–D)).

**HAGLROS regulates malignant behaviors of NPC cells by PI3K/AKT/mTOR signals**

PI3K/AKT/mTOR signals is frequently activated in diverse biological and pathological processes [26]. We observed that the level of PTEN was markedly depressed while the levels of p-PI3K, p-AKT, and p-mTOR were markedly accelerated after overexpression of HAGLROS alone in 6-10B cells, which were changeover after overexpression of HAGLROS and miR-100 synchronously, and that were reversed again by overexpression of HAGLROS, miR-100, and ATG14 concurrently (Figure 6(E)). Moreover, HAGLROS silencing alone distinctly increased PTEN expression and decreased the levels of p-PI3K, p-AKT, and p-mTOR in HK1 cells, while these impacts was obviously changeover after suppression of HAGLROS and miR-100 synchronously, and that were also reversed again after...
Figure 6. Effects of HAGLROS/miR-100/ATG14 axis on cell viability, apoptosis and autophagy of HK1 cells. A: Expression of HAGLROS, miR-100, and ATG14 was detected by qPCR and western blot in HK1 cells after different transfections. B: HK1 cell viability was measured by MTT assay after different transfections. C: HK1 cell apoptosis was detected by flow cytometry after different transfections. D: Expression of autophagy markers was determined by western blot in HK1 cells after different transfections. E: Effects of HAGLROS/miR-100/ATG14 axis on expression of PI3K/AKT/mTOR pathway-related proteins in both 6-10B and HK1 cells after different transfections. F: The graphical mechanism chart of HAGLROS in mediating cell biological processes of NPC cell lines. Data are expressed as mean ± SD. *p < 0.05, **p < 0.01, and ***p < 0.001.
suppression of HAGLROS, miR-100, and ATG14 concurrently (Figure 6E–F). All of these data showed that the influences of HAGLROS/miR-100/ATG14 axis in NPC cells were possessed by activating PI3K/AKT/mTOR signals.

Discussion

NPC remains to be one of usual head and neck malignancies, displaying a significant geographic distribution with high occurrences in China [27,28]. Several effective diagnostic and therapeutic targets have been identified in other cancers, nevertheless, it remains not unclear in NPC [29,30]. Following the quick development of biological technologies, IncRNAs have been pointed out or testified as crucial regulators in diverse diseases, including various cancers [31]. Though IncRNAs are widely reported participating in plenty of diseases, the key IncRNAs involved in NPC development still remains incomplete grabbed.

In previous studies, HAGLROS was abnormally expressed and was pivotal in the malignant development of many cancers [17–19]. Consistent with the previous studies, we also detected that HAGLROS level in NPC tissues and cells was high. An enhanced level of HAGLROS pointed out a short overall survival in NPC patients. Silencing of HAGLROS retrained NPC cell viability, enhanced apoptosis and depressed autophagy. Hence, we deduced that HAGLROS may also function as an oncogene in NPC development and its enhanced expression could suggest a poor prognosis.

Recently, the ceRNA hypothesis has proposed that plenty of RNA transcripts, such as mRNAs, IncRNAs, and circRNAs, are able to be interacted with each other by competitive binding to the binding sites and therefore exhibit key functions in both normal circumstances and disease conditions [32–34]. Significant advances have been possessed in illustrating the molecular principles of NPC [32]. Various IncRNAs have been proved to play a vital role in NPC development on the identity of ceRNAs [35,36]. Notably, HAGLROS is also shown to act as a ceRNA to sponge several miRNAs, for instance, miR-5095 [19], and miR-100 [18,23]. In line with these, we also discovered that there was a downside association of HAGLROS vs. miR-100. Also, miR-100 was crucial to cancer diagnosis, prognosis, and therapy [37]. Moreover, a previous study has shown that the expression of miR-100 is depressed in 12 of 14 primary human NPC biopsy samples, and the depressed miR-100 results in an accelerated Plk1 level, then promotes NPC progression [38]. As for this research, miR-100 inhibition markedly reversed the impacts of HAGLROS silencing on the viability, apoptosis, and autophagy of NPC cells. Given the potential role of miR-100 in NPC development, we thus deduce that HAGLROS may promote NPC development via negatively regulating miR-100.

Moreover, miRNAs are pivotal in biological and pathological processes via mediating their targets. Our results revealed that ATG14 was targeted by miR-100 in NPC cells. ATG14 is one of critical regulator reported to modulate autophagy initiation [39]. It has been reported that ATG14-induced cytoprotective autophagy is a pivotal factor to mediate the sensitivity of cancer cells to cisplatin-produced apoptosis [40,41]. Mukhopadhyay et al. also demonstrated that ATG14 facilitated lipophagy could induce endoplasmic reticulum stress mediated mitoptosis in cancer cells [42]. The crosstalk between autophagy and apoptosis are recognized as key molecular mechanism involved in the tumor progression [43]. Our study showed that overexpression of ATG14 reversed the impacts of miR-100 upregulation alone on NPC cell viability, apoptosis, and autophagy. Considering the key role of ATG14 in autophagy and apoptosis, we believe that miR-100 may regulate the apoptosis and autophagy processes in NPC cells through targeting ATG14.

Also, our study preliminarily revealed that the impacts of HAGLROS/miR-100/ATG14 axis in NPC cells were possessed by activating PI3K/AKT/mTOR signals. Activation of PI3K/AKT/mTOR signals has been shown to be implicated in cell survival (e.g. cancer) [26] and was also confirmed to be related to the poor prognosis of NPC [44]. Moreover, Lin et al. revealed that capsaicin-induced autophagy and apoptosis in NPC cells by inhibiting the PI3K/AKT/mTOR signals [45]. Wong et al. confirmed that PF-04691502, the PI3K/mTOR inhibitor, might be a latent therapeutic drug in NPC [46]. These data suggest that targeting PI3K/AKT/mTOR signals may be a latent therapeutic strategy for NPC. In addition, dysregulation of HAGLROS/miR-100/ATG14 axis in NPC cells could regulate the activation of PI3K/AKT/mTOR signals. Hence, we speculate that the roles of HAGLROS/miR-100/ATG14 axis in NPC development may be possessed by activating PI3K/AKT/mTOR signals.

Taken together, our results indicate that upregulation of HAGLROS contributes to NPC development via modulating miR-100/ATG14 axis-mediated PI3K/AKT/mTOR signals. This research may offer a meaningful basis for better illustrating the biology of NPC. However, we plan to carry out in vivo animal experiments to verify the discovered role of HAGLROS/miR-100/ATG14 axis in NPC development at another angle.

Disclosure statement

No potential conflict of interest was reported by the authors.

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