The role of long non-coding RNA GAS5 in cancers

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Abstract: Long non-coding RNAs (lncRNAs) have shown potential as a biomarker in the diagnosis and prognosis in multiple cancers. LncRNAs are dysregulated in various cancers, playing either oncogenic or tumor suppressive roles. Emerging evidences have proved that the growth arrest-specific 5 (GAS5) lncRNA can function as a tumor suppressor in several cancers. LncRNA GAS5 is downregulated in many types of cancer, regulating cellular processes such as cell proliferation, apoptosis and invasion. The low level of GAS5 expression often elevates capacity of proliferation and predicts poorer prognosis in some cancers. This review aims to summarize the recent published literature on the biogenesis, regulation mechanism and function of GAS5 in different types of cancers and explore its potential for cancer diagnosis, prognosis and treatment.

Keywords: lncRNA, GAS5, tumor suppressor, tumor

Introduction

The incidence of cancers has been increasing over the years.1 It is universally acknowledged that cancer is a major health issue. Early detection of cancer can greatly increase the probability for curative therapy. Therefore, the search for new diagnostic and prognostic biomarkers and effective therapies goes on.

Advances in high-throughput sequencing have led to the discovery of novel non-coding RNAs (ncRNAs) in recent years. Long non-coding RNAs (lncRNAs) are a group of RNA molecules longer than 200 nucleotides in length that are distinct from known categories of structural RNAs.2 LncRNAs, initially thought to be non-functional,3 were found to play important roles in many human diseases, especially in malignancies.4–10 Increasing evidence suggests that some lncRNAs have important roles in carcinogenesis and cancer progression, and may serve as diagnostic and/or prognostic biomarkers for some cancers.11

The growth arrest-specific 5 (GAS5) gene can encode ncRNAs of various sizes including a lncRNA.12 GAS5 is a tumor suppressor gene located at chromosome 1q25 and was first discovered in 1988 by screening highly expressed genes in growth-arrest cells.13,14 Downregulation of GAS5 expression is observed in many cancers. Clinical outcomes such as lymph node metastasis, tumor recurrence and overall survival are correlated with the expression of GAS5 in various cancers.15–17

The level of GAS5 expression regulates apoptosis, proliferation, invasion, epithelial–mesenchymal transition and metastasis of cancer cells,18–21 and may have prognostic value in various clinical scenarios.22–25 Although the precise molecular mechanism remains unclear, lncRNA GAS5 certainly plays an important role in cancers.
role in carcinogenesis and tumor progression. In our review, we will discuss the potential molecular mechanisms, recent research and clinical assessment of IncRNA GAS5 in cancer. An improved understanding of IncRNA GAS5 in cancer may provide new insights and inspiration for future clinical application.

Gene structure of GAS5

GAS5 belongs to the 5'-terminal oligopyrimidine (5'-TOP) gene family, which includes all ribosomal proteins, protein synthesis elongation factors as well as many genes without ribosome-related functions.26 GAS5 is a non-protein-coding gene located at chromosome 1q25.1 [molecular location: Chromosome 1, NC_000001.11 (173,863,899 to 173,868,882, complement)] and composed of 12 exons which constitute a short open reading frame that does not encode a protein. Some parts of the introns with highly conserved regions are loci of some small nucleolar RNAs (snoRNAs). The essential biological activities of GAS5 may depend, at least in part, on introns that encode multiple snoRNAs27 and on IncRNA GAS5/snoRNA-derived PIWI-interacting RNA (piRNA).12

Molecular mechanisms of IncRNA GAS5

About 7 years ago, 4 main types of mechanisms of action for IncRNA were summarized: 1) signals for transcription, 2) decoys for transcription factors, 3) guides of transcription factors and 4) scaffolds for protein complexes that epigenetically modify chromatin.28 More recently, the mechanisms of action were expanded to include: 1) IncRNA transcription-dependent activation or repression of neighboring genes, 2) inter-chromosomal interactions, 3) formation of nuclear structures (ie, paraspeckles) or R-loops, 4) IncRNAs acting as sponges of miRNAs, 5) regulating post-transcriptional mRNA decay and 6) regulating the cellular localization of RNA-binding proteins or DNA-binding proteins.29

The biological processes regulated by GAS5 are summarized in Figure 1. Specifically for GAS5, review of the literature supports the following mechanisms:

Transcriptional regulation through acting as a decoy

Glucocorticoid hormones can accelerate catabolism, modulate the immune response and cell survival when the body is under internal or external stress.30,31 Kino et al discovered that IncRNA GAS5 can act as a decoy for glucocorticoid receptor (GR).32 IncRNA GAS5 can bind to the DNA-binding domain of GR and titrate down the amount of GR that is available to bind glucocorticoid response elements (GREs) in the genomic DNA. Although this RNA and protein interaction between IncRNA GAS5 and GR blocks the binding between GRE and GR in the context of growth arrest and starvation, its relevance in cancer cells is unclear.

Transcriptional regulation through histone methylation/demethylation

In bladder cancer cells, IncRNA GAS5 can directly interact with transcription factor E2F4 and increase its binding to the promoter region of enhancer of zeste homolog 2 (EZH2), a histone-lysine N-methyltransferase gene.33 Consequently, transcription of miR-101 was upregulated and bladder cancer cell growth was suppressed. IncRNA

![Figure 1](https://www.dovepress.com/)

Figure 1 The biological processes regulated by IncRNA GAS5.
GAS5 can also indirectly regulate gene expression through its small RNA derivatives. For instance, GAS5-derived piRNA induces histone H3 lysine 4 methylation and histone H3 lysine 27 demethylation and resulted in increased transcription of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), a proapoptotic protein. The GAS5-derived piRNA binds to PIWIL1/4 proteins, which interact with WDR5 to recruit hCOMPASS-like complexes containing MLL3 and UTX (KDM6A), constituting a molecular mechanism relevant to tumor suppression. Nevertheless, it is very challenging to determine which biological effect of GAS5 is attributable to the intact lncRNA, its small RNA derivatives or both.

**Mirna sponge function**

GAS5 can function as a competing endogenous RNA (ceRNA) to regulate signaling pathways and biological functions. Bioinformatics analysis of complementary regions of GAS5 to microRNAs identified 690 candidates, among which 234 microRNAs showed statistically significant binding. In the context of cancer, LncRNA GAS5 has been shown to bind to miR-21, miR-221, miR-222, miR-135b, miR-23a and miR-103 et al.

**miR-21**: LncRNA GAS5 can suppress the expression of miR-21, an oncogene in various solid tumors and lymphoma expression. Fibroblast factor1 (FGF1) is a mediator of the GAS5/miR-21 axis that regulates proliferation and apoptosis. In NSCLC, suppression of GAS5 expression can lead to chemoresistance to cisplatin because LncRNA GAS5 competes with phosphatase and tension homolog (PTEN) for miR-21 binding.

**miR-221**: GAS5 can inhibit the expression of miR-221 and suppress cell proliferation, invasion and migration as well.

**miR-222**: It can also suppress tumor by downregulating miR-222 resulted in reduced proliferation and invasion in glioma.

**miR-135b**: The overexpression of GAS5 can inhibit tumor growth and enhance radiosensitivity by downregulating miR-135b expression in non-small cell lung cancer.

**miR-23a**: In non-small cell lung cancer, GAS5 can directly interact with miR-23a and then reduce the expression of miR-23a which can promote cell proliferation and invasion.

**miR-103**: The aberrant expression of miR-103 was found to promote colorectal cancer by downregulating the expression of PTEN and regulate growth and invasion of endometrial cancer cells through downregulating the suppressor of TIMP-3. GAS5 can act as an inhibitor of miR-103 and then enhance the expression of PTEN to promote cancer apoptosis.

**miR-196a-5p**: GAS5 was proved to suppress the proliferation, migration and invasion of glioma stem cells by binding to miR-196a-5p, an onco-miRNA contribute to glioma pathogenesis.

**miR-23**: GAS5 can inhibit gastric cancer by regulating miR-23 as well.

**miR-203a**: GAS5 can negatively regulate the expression of miR-203a and keep miR-203a away from its target gene TIMP2 which can inhibit cancer cell metastasis in osteosarcoma.

**miR-137**: In melanoma, GAS5 can positively regulate miR-137 and then promote cell proliferation, migration and invasion.

**miR-196a and miR-205**: Overexpression of GAS5 can inhibit cell proliferation and invasion by binding to miR-196a and miR-205 in cervical cancer to suppress cell growth through aplasia Ras homolog member I (ARHI).

**Signaling pathway associated with LncRNA GAS5**

It was reported that the inhibition of mammalian target of rapamycin (mTOR) pathway depends on GAS5. In prostate cancer, GAS5 was proved to inhibit cancer proliferation and progression by targeting miR-103 through the protein kinase B (AKT)/mTOR signaling pathway. Overexpression of GAS5 was proved to inhibit the miR-222 expression and then suppressed cell proliferation in gastric cancer through phosphatase and tensin homolog (PTEN)/phosphorylated protein kinase B (Akt)/phosphorylated mammalian target of rapamycin (mTOR) pathway as well.

GAS5 is a downstream target of Notch pathway in breast cancer. Notch1 can promote breast cancer cells proliferation by modulating GAS5. In NSCLC cells, GAS5 overexpression was negatively correlated with the expression of EGFR pathway. GAS5 was associated with embryonic stem cells (CSCs) self-renewal by regulating NODAL signaling. In pancreatic cancer cells, GAS5 can inhibit the expression of miR-181c-5p and then prohibit cell chemoresistance through suppressing Hippo signaling. We summarized the function of GAS5 in various cancers in Figure 2.
Function in various cancers

Gastrointestinal cancer

Colorectal cancer (CRC) was the third common cancer in the world. It was reported that the expression of GAS5 in CRC was lower than those in normal tissue. Meanwhile, the low expression of GAS5 was significantly associated with large tumor size, low grade, advanced TNM stage, higher local recurrence rate and distant metastasis rate. The lower expression of GAS5 was associated with poor overall survival. Univariate and multivariate analysis further revealed that GAS5 was an independent prognostic factor for CRC. The overexpression of GAS5 can inhibit proliferation, migration and invasion in CRC. Yang et al have concluded that the overexpression of GAS5 can induce G0/G1 cell cycle arrest and apoptosis. Thus GAS5 may become a novel prognostic marker and a potential target for CRC in the future.

Previous studies have found that lncRNA GAS5 was downregulated in gastric cancer. Meanwhile, the downregulated level was significantly associated with larger tumor size and advanced pathologic stage. Patients with lower expression of GAS5 have poorer disease-free survival and overall survival than those with higher GAS5 expression. Vivo and vitro experiments further demonstrated that the GAS5 can upregulate proliferation and induce apoptosis in gastric cancer.

It was reported that GAS5 expression was lower in pancreatic cancer than normal tissue. And overexpression of GAS5 can inhibit cell proliferation in pancreatic cancer by decreasing G0/G1 phase and increasing S phase. Downregulation of GAS5 was associated with chemoresistance in pancreatic cancer as well. Moreover, the overexpression of GAS5 can inhibit pancreatic cancer cell tumorigenesis in vivo and suppress tumor metastasis.

In hepatocellular carcinoma, overexpression of GAS5 could promote prognosis, suppress proliferation and invasion of hepatoma cells. Low expression of GAS5 was closely related with differentiation and poor overall survival.

Wang et al have found that GAS5 was downregulated in esophageal cancer (EC) and could inhibit the growth of EC. However, Li et al reported that contrast to other cancers, the GAS5 was overexpressed in EC. GAS5 is proved to promote proliferation, metastasis and inhibited apoptosis in EC by regulating miR-301a. The concrete role and regulation function of GAS5 in EC still need further study.

Malignant pleural mesothelioma (MPM)

MPM is a malignant cancer with poor prognosis. Some lncRNAs have clinical significance like predicting metastasis and overall survival. Previous studies have shown that GAS5 expression is lower in MPM cells and its expression could be regulated by drugs inducing growth arrest in MPM. The high level of GAS5 could increase promoter activity. Moreover, GAS5 could control cell cycles in a glucocorticoid receptor-decoy way. As a result, GAS5 plays an important role in MPM and could be a targeted agent in the future.

Urological malignancies

Renal cell carcinoma (RCC) is a common carcinoma with poor prognosis. Hui ping et al provide the first evidence that GAS5 expression was lower in RCC cells and the...
overexpression of \textit{GAS5} can inhibit cell proliferation, induce cell apoptosis, arrest cell cycle, progress cell death and inhibit invasion. Therefore, the decreased expression of \textit{GAS5} was associated with tumorigenesis and progression.\textsuperscript{67,68}

In bladder cancer, accumulating studies have found that overexpression of \textit{GAS5} can promote apoptosis in drug-induced resistance. The upregulated expression of \textit{GAS5} can promote apoptosis by affecting GA induced apoptosis and inhibiting EZH2 transcription.\textsuperscript{33} The level of \textit{GAS5} was lower in bladder cancer tissues and the low expression of \textit{GAS5} was positively related to higher pathological grades and could be a prognosis for disease-free survival of bladder cancer patients. Enhancement of \textit{GAS5} can also reduce the chemotherapy resistance to doxorubicin via Bcl2.\textsuperscript{69}

Prostate cancer is the most prevalent malignancy in male patients. Mouse xenograft models were used to explore the \textit{GAS5} effects on prostate cancer. Overexpression of \textit{GAS5} can significantly inhibit prostate cancer cell proliferation and tumor growth in vitro and vivo.\textsuperscript{53}

\section*{Breast cancer}
\textit{GAS5} expression is significantly downregulated in breast cancer cells. The overexpression of \textit{GAS5} can induce or facilitated apoptosis in breast cancer cells and produce an increase in sensitivity to treatments by several different pathways.\textsuperscript{18} Moreover, \textit{GAS5} can promote the apoptosis of triple negative and estrogen receptor-positive breast cancer cells. Pickard et al have reported that the use of mTOR inhibitors may enhance \textit{GAS5} levels to suppress cancer growth as well.\textsuperscript{70} The plasma level of \textit{GAS5} changes after surgery. The preoperative level of \textit{GAS5} can reflect the active degree of proliferation in breast cancer. Thus, the plasma \textit{GAS5} can be a biomarker to assess the prognosis evaluation after surgery.\textsuperscript{16} The expression of \textit{GAS5} was decreased in breast cancer cell from patients treated with trastuzumab and proved to contribute to trastuzumab resistance.\textsuperscript{71} As a result, \textit{GAS5} plays an important role in regulating breast cancer cells.

\section*{Lung cancer}
Lung cancer is a common malignant cancer with the highest mortality in China. Compared with common tissues, the expression of \textit{GAS5} was decreased in non-small cell lung cancer (NSCLC). Moreover, \textit{GAS5} was declined in early stage before surgery compared with healthy control patients.\textsuperscript{23} The overexpression of \textit{GAS5} can inhibit NSCLC cell proliferation, promote apoptosis and improve radiosensitivity of NSCLC cells.\textsuperscript{45} Decreased expression of \textit{GAS5} is correlated with advanced TNM stages and larger tumor size. It can regulate NSCLC chemoresistance to cisplatin-based therapy as well.\textsuperscript{42} Moreover, \textit{GAS5} was found to be overexpressed in epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) sensitive cell lines compared with the resistant cell lines.\textsuperscript{56} It is associated with the resistance of EGFR-TKIs. In the future, it can be a potential agent to deal with resistance of EGFR-TKIs.

\section*{Other cancers}
Acute myeloid leukemia is a common hematologic cancer with genetically heterogeneous. It was found that overexpression of \textit{GAS5} was associated with shorter overall survival.\textsuperscript{72} In mantle cell lymphoma, targeting the control of \textit{GAS5} may significantly improve survival.\textsuperscript{73}

The expression of \textit{GAS5} was downregulated in melanoma tissue compared with normal tissues. It was reported that expression of \textit{GAS5} was related to distant metastases and TNM stage in melanoma by regulating miR-17 transcription. In the future, \textit{GAS5} may be a potential target for the treatment of melanoma.\textsuperscript{49}

\textit{GAS5} was proved to be downregulated in glioma. It was reported that \textit{GAS5} can suppress glioma stem cells (GSCs) and further promote apoptosis.\textsuperscript{46} The lower expression of \textit{GAS5} was significantly related to increased rate of death, recurrence and progression.\textsuperscript{74}

Osteosarcoma is a common malignancy with a high incidence of death in children and young adults. The expression of \textit{GAS5} was found significantly decreased in osteosarcoma tissues and cells. \textit{GAS5} can suppress cell growth, proliferation and epithelial–mesenchymal transition in osteosarcoma.\textsuperscript{75}

In thyroid cancer, the expression of \textit{GAS5} was lower than benign tumor tissues. The expression of \textit{GAS5} was significantly related to tumor stage, lymph node metastasis, the multiple cancer foci of thyroid cancer, disease-free survival and overall survival.\textsuperscript{76}

In head and neck cancer patients treated with radical chemoradiotherapy (CRT), the expression of \textit{GAS5} was lower in patients achieved complete response than those with partial response and progressive disease. Thus, \textit{GAS5} can be a prognostic biomarker for head and neck cancer in CRT therapy.\textsuperscript{25}

Patients with low expression of \textit{GAS5} was reported to have poorer disease-free survival and overall survival than those with higher level in ovarian cancer. And the overexpression
of GAS5 can suppress ovarian cancer cell proliferation and promote apoptosis.\textsuperscript{77} GAS5 can act as a tumor suppressor lncRNA in endometrial cancer as well.\textsuperscript{40}

In patients with cervical cancer, GAS5 expression level was associated with FIGO stage, metastatic parameter, clinical staging and overall survival. The downregulation of GAS5 was proved to enhance cell proliferation and invasion.\textsuperscript{24,78} Moreover, the expression of GAS5 can influence cisplatin resistance in cervical cancer by regulating the phosphorylation of Akt.\textsuperscript{79}

## Challenges and future perspectives

Cancer is a major health issue often associated with gene mutation. It is increasingly acknowledged that not only the change of the protein-coding genes but also non-protein-coding genes can contribute to different cancers. With lncRNA GAS5 becomes a hot topic, much evidence indicates that lncRNA GAS5 represents a potent tumor suppressor and is aberrantly expressed in various cancers. The suppressive function is involved in multiple pathways including proliferation, metastasis, invasion and CSCs. LncRNA GAS5 can interact with miRNAs and then regulate different genes to regulate the related pathways (Table 1). It has been found that LncRNA GAS5 is lower expressed in a variety of cancers including CRC, breast cancer, gastric cancer, hepatocellular carcinoma, osteosarcoma, esophageal carcinoma and pancreatic cancer. In tumors, lower expression of lncRNA GAS5 is significantly associated with clinicopathological features such as TNM stage, histological grade, tumor size and distant metastasis (Table 1). Moreover, the expression of lncRNA GAS5 can affect the survival and prognosis of some cancers. The exact mechanism of lncRNA GAS5 action is not completely known. The molecular mechanism of lncRNA GAS5 in cancer progression involves in Gr, TRIB3, c-Myc, eIF4E, EZH2, TRAIL, CDK6, FGF1, PTEN, ARHI, CXCR4, (AKT)/mTOR, EGFR and NODAL pathway.

However, several challenges exist in the GAS5 field, including the relatively low level in the plasma compared to the normal tissues. Nowadays, the studies of lncRNA GAS5 are still in preclinical stage, the number of cancer patients involved is limited. The precious concentration of lncRNA GAS5 in the serum of cancer patients and healthy patients has not been established. And the challenge here is the standardization of detection methods worldwide. It is unclear whether GAS5 interact with additional chromatin-modifying enzymes through other molecular mechanisms.

GAS5 can also play an important role in non-cancer disease, such as cardiovascular disease, osteoarthritis, type 2 diabetes, inflammatory bowel disease and autoimmune disease.\textsuperscript{80–84} It still needs more studies to discover the real function in various cancers. Future studies to the precise expression of GAS5 in different diseases and disease progression, regression and response to therapies can be conducted to confirm its potential use as the biomarker in diagnosis and response to therapies.

### Table 1: Related miRNAs in various cancers and lower expression of lncGAS5 is associated with clinical features

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Related miRNA</th>
<th>Clinicopathological features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorectal cancer</td>
<td>miR-103, miR-221</td>
<td>Larger tumor size, low histological grade, advanced TNM stage, poor prognosis, lymph node metastasis, local recurrence rate, distant metastasis rate Accepted FIGO stage and metastatic parameter, poorer overall survival</td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>miR-196a, miR-205</td>
<td>Advanced tumor size, advanced pathologic stage, poorer DFS, poorer OS</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>miR-222, miR-23a</td>
<td>Larger tumor size, advanced pathologic stage, poorer DFS, poorer OS</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>miR-181c-5p</td>
<td>Chemotherapeutic drug resistance, promote tumorigenesis and metastasis</td>
</tr>
<tr>
<td>Hepatocellular cancer</td>
<td>miR-21</td>
<td>Poor prognosis, differentiation, portal vein</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>miR-103</td>
<td>Poor outcome</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>miR-21</td>
<td>Trastuzumab resistance</td>
</tr>
<tr>
<td>NSCLC</td>
<td>miR-23a, miR-135b</td>
<td>Advanced TNM stages and larger tumor size, poor tumor differentiation</td>
</tr>
<tr>
<td>Glioma cancer</td>
<td>miR-196a-5p, miR-222</td>
<td>-</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>miR-203a</td>
<td>-</td>
</tr>
<tr>
<td>Melanoma</td>
<td>miR-137</td>
<td>-</td>
</tr>
<tr>
<td>Bladder cancer</td>
<td>-</td>
<td>Higher pathological grades, poor disease free survival, Enhance the chemotherapy resistance to doxorubicin</td>
</tr>
<tr>
<td>Thyroid cancer</td>
<td>-</td>
<td>Advanced TNM stages, lymph node metastasis, poor DFS, poor OS</td>
</tr>
<tr>
<td>Head and neck cancer</td>
<td>-</td>
<td>Higher complete response rate</td>
</tr>
</tbody>
</table>

**Abbreviations:** NSCLC, non-small cell lung cancer; DFS, disease free survival; OS, overall survival.
The potential of GAS5 for cancer diagnosis has been supported by many studies. It can impact the chemotherapy sensitivity in certain cancer and provides a new strategy for overcoming drug resistance. Nowadays, precise medical treatments such as targeted therapies have the advantage of precise specificity and low toxicity. In future studies, IncRNA GAS5 may serve as a new molecular target for the treatment of cancer.

Conclusion
In conclusion, the discovery of GAS5 has provided new hope for cancer patients. Decreased expression of GAS5 is associated with less proliferation, invasion and metastasis in cancer cells and always predicts advanced TNM stage, high recurrence and poor prognosis in various tumors. We hope to identify novel and sensitive biomarkers and therapeutic targets in cancer patients by understanding the molecular related pathways and clinicopathologic features in different cancers. Future investigation of GAS5 may lead to novel therapeutic strategies.

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Disclosure

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