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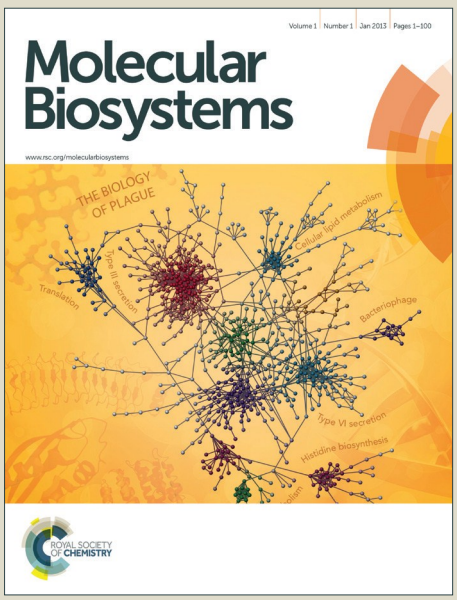

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HOTTIP: a critical oncogenic long non-coding RNA in human cancers

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Abstract

Long non-coding RNAs (lncRNAs) , which represent a novel group of non-protein-coding RNAs and are commonly defined as RNA moleculars larger than 200 nucleotides in length, have been shown to get involved in diverse biological processes, such as cell growth, apoptosis, migration and invasion. In addition, aberrant expression of lncRNAs has discovered in human tumors, where they function as either oncogenes or tumor suppressor genes. Recently, tumorigenic effects of one specific lncRNA, termed as 'HOXA transcript at the distal tip' (HOTTIP) on the initiation and progression of human cancer has been widely reported. Increasing data has showed that dysregulation of HOTTIP was associated with various malignancy including hepatocellular carcinoma, pancreatic cancer, gastric cancer and colorectal cancer, which affected survival and prognosis of cancer patients. Here, we focus on the current knowledge of HOTTIP in various cancers and illustrates the corresponding mechanism and biological function of HOTTIP during tumor development.

Key words: lncRNA; HOTTIP; cancer; tumorigenic; prognostic

Introduction

As a major public health problem across the whole world, cancer has always been the foucs of both the research and clinical studies.[1] Based on two most recent data on cancer statistics estimates, about

1,685,210 new cancer cases and 595,690 cancer deaths would occur in United States in 2016.[2] and in 2015, about 4292,000 new cancer cases and 2814,000 cancer deaths are projected to occur in China.[3] Despite of current advances in the chemotherapy and molecular targeting therapy, cancer remains a leading cause of death and constitutes an enormous burden worldwide.[4, 5] To reduce cancer-related mortality, it is essential for the identification of new diagnostic methods and prognostic biomarkers or potential therapeutic targets.

Thanks to the recent advances in sequencing technologies and large-scale genome sequencing projects, more and more long non-coding RNAs (lncRNAs) have been recently identified in the genome of numerous cancers.[6–9] Technically, lncRNAs are classically defined as RNA transcripts longer than 200 nucleotides in length with no or limited protein-coding capacity.[10] According to the GENCODE analysis (www.gencodegenes.org) of the last version of the Ensembl human genome annotation (GRch38, version 24 from August 2015), 28,031 transcripts originating from 15,941 genes can be identified as lncRNAs.[11] Multiple studies have reported that lncRNAs participate in various aspects of cell biology and potentially contribute to tumor development.[12–14] Although the number of articles about lncRNAs or cancer-related lncRNAs have greatly increased (Fig 1), only a small portion of lncRNAs have been well characterized and little of the underlying molecular mechanism was investigated. Among these lncRNAs, the lncRNA termed as 'HOXA transcript at the distal tip' (HOTTIP) has drawn increasing attention among cancer-related lncRNAs, which have been demonstrated to regulate the genes by various mechanisms, including epigenetic modifications, lncRNA-miRNA and lncRNA-protein interactions.[15–17] We and other researchers have demonstrated that lncRNA HOTTIP are significantly upregulated in various types of human cancer and thereby of high diagnostic value for screening and great clinical value for cancer therapy.[18–22] In this review, we review the current studies about regulation mechanisms and functions of lncRNA HOTTIP in the development and progression of human cancers.

Discovery of the HOTTIP

The mammalian HOXA locus consists of a cluster of 11 HOX genes with a graded expression pattern along body appendages from proximal (close to the main body) to distal (appendage tip).[23] HOTTIP was originally identified in anatomically distal human fibroblasts such as those from the hand, foot or

foreskin, and the HOTTIP gene was located at the homeobox A (HOXA) locus (chromosomal locus 7p15.2) which encodes a 3764bp transcript (Fig 2). Therefore, the lincRNA was termed 'HOXA transcript at the distal tip' (HOTTIP).[24] It has been confirmed that HOTTIP could directly interact with the Trithorax protein WDR5 inducing an open DNA-chromatin configuration to target WDR5/MLL complexes driving histone H3 lysine 4 trimethylation and thus regulating the transcription of 5' end HOXA locus genes.[24] Interestingly, recent research also found that there is a positive correlation between the expression of HOTTIP and HOX genes in tumors and normal tissue.[18, 19, 25-27] In short, HOTTIP can coordinately activate HOX genes by the recruitment of histone-modifying enzymes. then regarding tumor suppressor genes silencing.

HOTTIP in human cancers

Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is the third cause of cancer-related death and the sixth most common cancer worldwide. However, the treatments for HCC are limited, and most of them are only available to the early stage. At an advanced stage, this cancer is associated with a poor prognosis due to frequent cancer metastasis, tumour recurrence and lack of curative treatment.[28, 29] Therefore, New targets for non-conventional treatments will help to accelerate research on the molecular pathogenesis of HCC.

Quagliata et al.[18] first report that the lncRNA HOTTIP is significantly up-regulated in hepatocellular carcinoma (HCC) specimens (including 52 HCC liver needle biopsies and matched non-neoplastic counterpart.). Moreover, they also observed the marked up-regulation of HOXA13 in HCC. High HOTTIP and HOXA13 expression is associated with metastasis and dismal prognosis in HCC. Tsang et al.[30] also demonstrated that the expression level of HOTTIP was significantly higher in primary HCCs than that in the corresponding non-tumourous livers. To confirm the frequent up-regulation of HOTTIP in HCCs, they further examined the expression of HOTTIP in a larger cohort of 70 pairs of HCCs by qRT-PCR. The results showed that HOTTIP was overexpressed in 81.4% (57/70) of the HCC patients, even in early stage of HCC formation. In addition, they also observed that HOTTIP enhanced HCC tumour growth and metastasis both in vitro and in vivo. Accumulated evidence demonstrated that miRNAs may directly interact with lncRNAs and silencing their expression.[31-33] They found that miR-125b may serve as a post-transcriptional regulator of HOTTIP in HCC, loss of miR-125b

expression might contribute to the frequent up-regulation of HOTTIP in human HCC. Intriguingly, Ge et al.[34] identified lncRNA HOTTIP as a novel target of fMiR-192 and miR-204 and HOXA genes, glutaminase (GLS1) was identified as a potential downstream target of the miR-192/-204-HOTTIP axis in HCC.

Taken together, HOTTIP promoted HCC cell proliferation and metastasis. And HOTTIP might act as an oncogene in HCC, suggesting its potential utilities as a prognostic marker and a novel therapeutic target. Further studies are needed to investigate other possible targets and mechanism that underlie regulatory behaviors.

Pancreatic cancer

Pancreatic cancer (PC) is the fourth leading cause of cancer-related deaths in both men and women in Western societies and is characterized by a poor prognosis, as only 7.7% of patients survive 5 years or more after diagnosis.[35, 36] As the early diagnosis of pancreatic cancer is difficult, patients are frequently at an intermediate or advanced stage when diagnosed. In these cases the prognosis is very disappointing with a 5-year survival rate of only 2%.[37, 38] Moreover, the mechanism of pathogenesis in pancreatic cancer is not completely understood, and there are currently no effective therapies. It is, therefore, crucial to identify novel molecular bio-marker or therapeutic targets in this deadly disease.

Li et al. [39] performed gene expression array analysis on eight pancreatic ductal adenocarcinoma (PDAC) tissues and four chronic pancreatitis clinical samples. They found that HOTTIP was one of the most significantly up-regulated lncRNAs in PDAC tissues compared to pancreatic tissues. To further validate these results, they analyzed HOTTIP expression in 90 paired resected samples by qRT-PCR. Compared with adjacent non-tumor tissues, HOTTIP was up-regulated in most PDAC tissues. Moreover, they also found that HOTTIP levels were increased in PDAC cell lines compared with immortalized human pancreatic ductal epithelial cells. Functional and mechanism study indicated that HOTTIP silencing resulted in proliferation arrest by altering cell-cycle progression, and impaired cell invasion by inhibiting epithelial-mesenchymal transition in pancreatic cancer. Since pancreatic cancer is highly resistant to chemotherapy due to the acquisition of drug resistance by pancreatic cancer cells.[40, 41] They further tested whether down-regulation of HOTTIP impaired the resistance of PDAC cells to gemcitabine. Interestingly, the results showed that up-regulation of HOTTIP could enhance the

chemosensitivity of human pancreatic cancer cells to gemcitabine. Similar to hepatocellular carcinoma,[18] their found that HOTTIP and HOXA13 expression was strongly positively correlated in 90 PDAC tissues and in their corresponding adjacent nonneoplastic tissues. By analysis of the relationship between HOXA13 expression in paraffin-embedded PDAC samples and clinicopathological data, they revealed that patients with high HOXA13 expression exhibit increased lymph node metastasis, poor histological differentiation, and decreased overall survival.

Cheng et al.[25] also found that the expression of HOTTIP was higher in PDAC cell lines compared with immortalized human pancreatic ductal epithelial cells. They further demonstrated that silencing of HOTTIP decreased pancreatic cancer cells proliferation, induced apoptosis and decreased migration. However, in contrast to previous studies in hepatocellular carcinoma,[18] HOTTIP does not regulate HOXA13 but participants in regulation of several other HOX genes including HOXA10, HOXB2, HOXA11, HOXA9 and HOXA1 (Fig. 3).

These results suggested that the expression level of HOTTIP was closely related with HOX genes expression. Additionally, HOTTIP expression was elevated in human PDAC cell lines and patient samples compared with controls and functions as an oncogenic lncRNA in pancreatic cancer, suggesting its potential utilities as a prognostic bio-marker and a therapeutic target.

Gastric cancer

Gastric cancer (GC) is one of the most common malignancies in the world, with approximately 951,600 new cases diagnosed in 2012.[2, 42] Although the incidence and mortality of gastric cancer have decreased, most of GC patients are diagnosed with advanced stage and have a poor prognosis.[43] Thus, more sensitive GC bio-markers for improving screening, diagnosis and prognostic evaluation are urgently needed.

The expression level of HOTTIP was remarkably elevated in GC tissues and cell lines compared with that in the normal control.[44, 45] Moreover, high HOTTIP expression was associated with larger tumor size, poorly differentiated, deeper invasion depth, positive lymph node metastasis, advanced TNM stage, and shorter patients overall survival.[44, 45] Further analysis showed that silencing of HOTTIP could inhibit GC cell proliferation, promoted cell apoptosis, and reduced cell invasion and migration.

Another study investigated the underline mechanism of HOTTIP in gastric carcinogenesis. Because the expression level of HOTTIP was closely related with HOXA13 expression,[17, 18] their silenced HOXA13 expression and found that the expression of HOTTIP and insulin growth factor-binding protein 3 (IGFBP-3) genes were decreased. Further mechanism study revealed that the HOXA13-HOTTIP-IGFBP-3 cascade is critical for the gastric tumorigenesis.[46] These findings implicated that HOTTIP may play an important role in GC initiation and progression, and would be a novel prognostic marker and potential therapeutic target for this disease.

Colorectal cancer

Colorectal cancer (CRC) is the third most common malignancy and the fourth most frequent cause of cancer-related deaths worldwide, with particularly high incidence in Western countries.[47, 48] Similar to most other malignancies, lack of molecular bio-markers for tumor cell progression is still one of the most important obstacles challenging CRC therapy.[49] Therefore, new findings on diagnostic and prognostic bio-markers associated with CRC progression and clinical outcome would be of great clinical relevance.

Ren et al.[50] found that the expression of HOTTIP was higher in CRC tissues compared with adjacent normal tissues (in 156 CRC tissues and 21 adjacent non-malignant tissues), and increased lncRNA HOTTIP expression was positively associated with clinical stage, tumor size and distant metastasis in CRC patients. In addition, they also showed that up-regulation of HOTTIP was an unfavorable prognostic factor in CRC patients. Our study also showed that the expression of HOTTIP was higher in CRC tissues than in the adjacent non-tumor tissues, and overexpression of HOTTIP is correlated with an advanced pathological stage and a larger tumor size. Moreover, functional analyses revealed that the silencing HOTTIP expression could affect CRC cell proliferation which induced a significantly increase in the number of cells in the G0/G1 phase and a reduction in the number of cells in the S phase. Further experiments indicate that HOTTIP oncogenic function is partly dependent on repressing p21 expression.[19]

In conclusion, overexpression of HOTTIP may serve as an poor prognosis predictor for CRC patients.

And knockdown of HOTTIP impaired CRC cells proliferation and induced apoptosis. However, a further larger sample size and molecular mechanism investigation is needed to support these results.

Lung cancer

Lung cancer is the most common cancer in the world and the leading cause of cancer death among males in both developing and developed countries.[1, 51] The major drawbacks in lung cancer treatment are the predominantly late diagnosis and fast onset of resistance to chemotherapy.[52, 53]

The expression of HOTTIP was significantly elevated in lung cancer tissues compared with adjacent normal tissues. Moreover, knockdown of HOTTIP in A549 cells and NCIH446 cells inhibited proliferation. Consistent with this, depletion of HOTTIP suppressed tumor growth in a mouse model of lung cancer. Western blot analysis demonstrated that cell cycle regulators Cdc25C, Cyclin B1 and Cyclin D1 were decreased upon knockdown of HOTTIP. Pro-apoptotic factor Bad was increased, whereas anti-apoptotic factors Bcl-2 and Bcl-xL were decreased after HOTTIP down-regulation.[26] Since platinum-based chemotherapy is first-line treatment for lung cancer chemotherapy,[54, 55] Gong et al.[56] aim to explore the association of SNPs in well-characterized lung cancer-related lncRNAs with susceptibility and platinum-based chemotherapy response of lung cancer. Their found that patients with HOTTIP rs5883064 allele and rs1859168 allele were remarkably associated with lung cancer susceptibility or platinum-based chemotherapy response.

In summary, these results suggested that HOTTIP may act as an oncogene and may be potential biomarkers to predict lung cancer risk and platinum-based chemotherapy response in lung cancer patients.

Other human cancers

Osteosarcoma is the most common primary bone cancer in children and adolescents.[57, 58] Li et al. [22]verified that HOTTIP was overexpression in osteosarcoma (OS) tissues compared with adjacent non-tumor tissues, and elevated HOTTIP expression was associated with advanced clinical stage and distant metastasis. Furthermore, they showed that lncRNA HOTTIP could be considered an independent prognostic factor in OS patients. Additionally, knockdown of HOTTIP suppressed OS cell proliferation, migration and invasion in vitro.

Zhang et al.[59] uncovered that in prostate cancer cells HOTTIP knockdown decreased the cells proliferation and induced cells apoptosis. They further demonstrated that several target proteins involved in the HOTTIP affecting cell cycle, including cell cycle inhibitors Cyclin D1, antiapoptotic protein Bcl-2 and proapoptotic protein bax. Similar to gastric and liver cancer, there is a positively correlated between HOTTIP and HOXA13, and HOXA13 may serve as a downstream target of HOTTIIP involving in prostate cancer cells proloferation.

Taken together, dysregulation of HOTTIP was involved in the progression and development of OS and prostate cancer. Moreover, HOTTIP also may be a promising diagnostic and therapeutic target for those deadly diseases.

Future directions

There is no doubt that the up-regulation of HOTTIP affects various cancer-related aggressive phenotype, signaling pathways and has a critical role in cancer development,[18, 19, 27, 60] and the dysregulated HOTTIP found in tumors suggest that it may represent effective targets for diagnostic, prognostic, and therapeutic purposes. However, since lncRNA HOTTIP research is still in its infancy, there are several unresolved issues using HOTTIP as clinical biomarkers for the diagnosis and treatment of cancer. While we known that knockdown of HOTTIP can suppress cancer cell proliferation, invasion and migration and promoted cancer cell apoptosis, the molecular mechanism of HOTTIP remains to be further studied. Therefore, a deeper investigation of HOTTIP and a better understanding of its detailed molecular mechanism are needed to facilitate their applications to the diagnosis and treatment of cancer.

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Table 1 Functional characterization of the HOTTIP in various tumors

Tumor type	Expression	Functional role	Related gene	Role	Reference
Hepatocellular carcinoma	Upregulation	Proliferation cell viability migration Tumorigenesis	HOXA13, HOXA11 HOXA10, AGO2, GLS1,miR-192, miR-204 miR-125b	Oncogene	18, 30, 34
Gastric cancer	Upregulation	Proliferation migration invasion tumorigenesis	HOXA13, IGFBP-3	Oncogene	27, 61
Colorectal cancer	Upregulation	Proliferation apoptosis	P21	Oncogene	19
pancreatic cancer	Upregulation	Proliferation apoptosis,migration invasion, and chemoresistance	HOXA13, HOXB2, HOXA11, HOXA9, HOXA1, HOXA10, E-cadherin, Vimentin, Snail 1, MMP-3, MMP-2, SMAD3, AURKA, AHNAK, GDF15, SGK1 and CD44	Oncogene	25, 62
		Proliferation apoptosis	Cdc25C, Cyclin B1, Cyclin D1, Bad, Bcl-2 and Bcl-xL		
Lung cancer	Upregulation	Proliferation apoptosis	HOXA13, Bax, Bcl-2 and Cyclin-D1	Oncogene	26
Prostate cancer	Upregulation	Proliferation migration invasion	/	Oncogene	59
Osteosarcoma	Upregulation			Oncogene	22

Table 2 Clinical significance of the HOTTIP in various tumors

Tumor type	Overexpression of HOTTIP	Reference
Hepatocellular carcinoma	Shorter overall survival and positive metastasis	[18, 34]
Gastric cancer	Poorer tumor differentiation, larger tumor size, deeper invasion depth, advanced TNM stages, positive lymph node metastasis and shorter overall survival	[27, 63]
Colorectal cancer	Advanced pathological stage and larger tumor size; Advanced T stage , clinical stage and distant metastasis, shorter overall survival	[19, 50]
pancreatic cancer	Shorter overall survival	[20]
Lung cancer	Higher lung cancer risk	[56]
Osteosarcoma	Advanced clinical stage, distant metastasis and shorter overall survival	[22]
Tongue squamous cell carcinoma	Advanced T stage , clinical stage and distant metastasis, shorter overall survival	[21]

Fig. 1 Increasing articles about lncRNA and Cancer-related lncRNAs. PubMed was searched with key words “lncRNA” or “lncRNA cancer”. (https://www.ncbi.nlm.nih.gov/pubmed, as of 2015).

Fig 2. The HOTTIP locus in humans and the coding potential analyses of HOTTIP transcripts. (a) UCSC Genome Browser (http://genome.ucsc.edu/) view of the 7P15.2 region in humans, which contains the HOTTIP gene. (b) PhyloCSF predicted that HOTTIP has no protein coding potential. The tracks showed the PhyloCSF score for each codon in each of 6 frames. Regions with a score greater than 0 are predicted to be coding, while regions with a score less than 0 are predicted to be noncoding. The protein coding gene GAPDH and the noncoding RNA gene HOTAIR were used as controls. (c) Prediction of HOTTIP structure based on minimum free energy (MFE) and partition function. Color scale indicates the confidence for the prediction for each base with shades of red indicating strong confidence. (http://rna.tbi.univie.ac.at/).

Fig 3. Overview of the known regulatory mechanisms for HOTTIP. (1) The RNA-Binding Proteins (RBPs) including PTB-eIF4AIII-DGCR8-FUS-UPF1 and the WDR5/MLL complex. (2) EMT bio-marker including E-cadherin, Vimentin, Snail 1, MMP-2, MMP-3 and SMAD3. (3) HOXA genes including HOXA1; HOXA2; HOXA6; HOXA10; HOXA11 and HOXA13.

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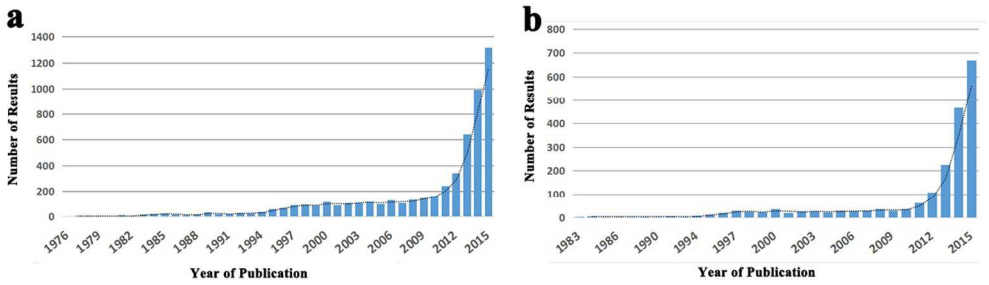

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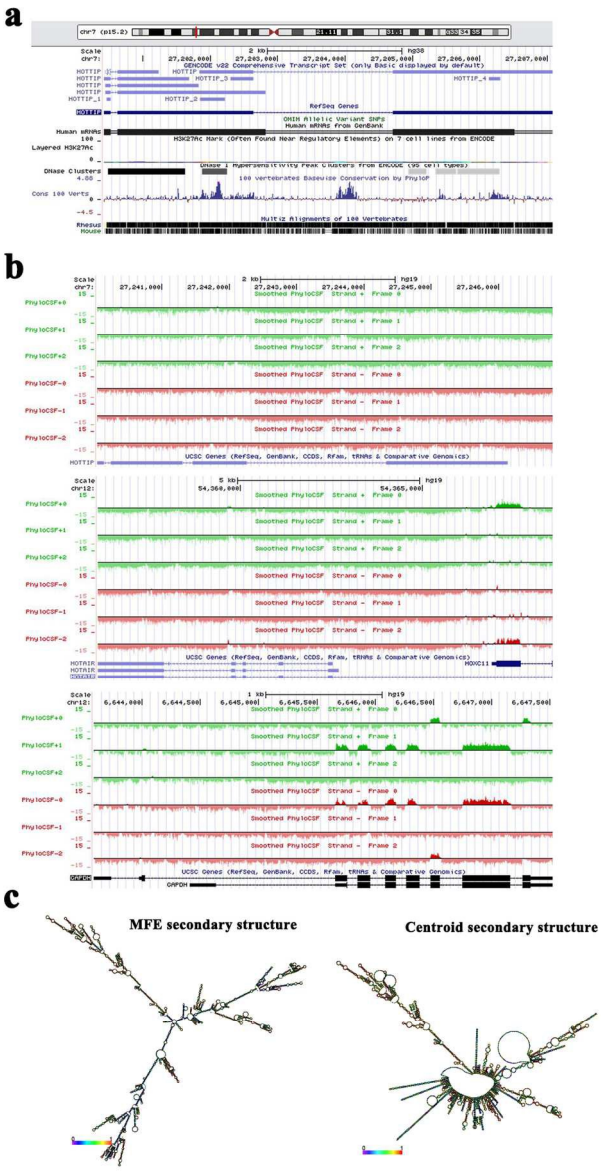

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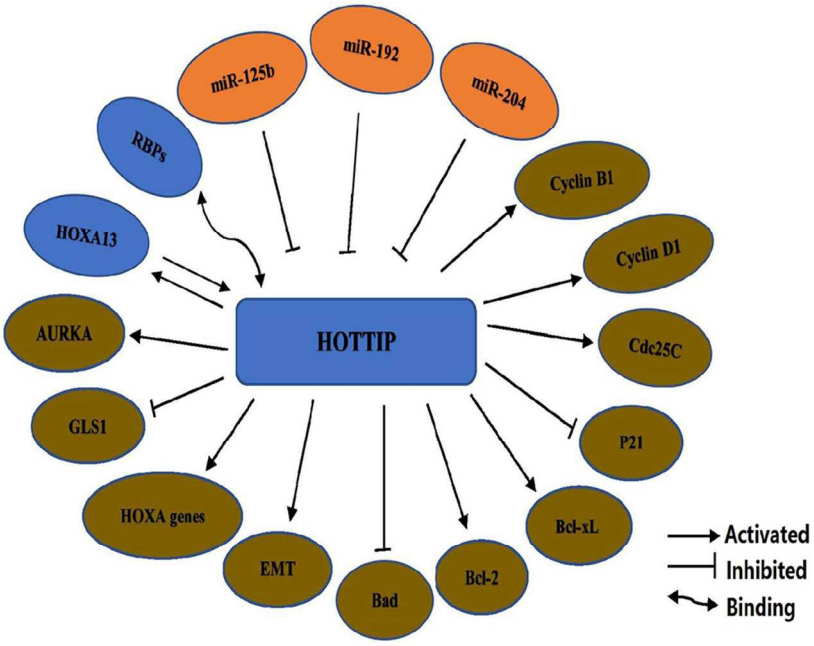

Regulation of HOTTIP

423x352mm (72 x 72 DPI)