



## Review Article

# The role of hydrogen peroxide-inducible clone-5 in tumor progression

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### ABSTRACT

The poor prognosis of cancers such as hepatocellular carcinoma is due to high recurrence rate mainly caused by metastasis. Target therapy aiming at critical signal molecules within these pathways is one of the promising strategies for the prevention of metastasis. Hydrogen peroxide-inducible clone-5 (Hic-5), which belongs to the paxillin superfamily, is emerging as a potential target along the metastatic signaling pathway. Hic-5 and paxillin share similar structural features; however, there are a lot of different biochemical properties between them, including tissue-specific distribution, regulation of gene expression, critical signal cascade, and the impacts on cellular phenotypes. This review focus on the recent studies of Hic-5 related to its impacts on signal transduction and transcription responsible for tumor progression. Hic-5 may regulate mitogen-activated protein kinase cascade for cell migration and invasion in various systems. Hic-5 can mediate transforming growth factor- $\beta$ 1-induced epithelial-mesenchymal transition (EMT) via RhoA- and Src-dependent signaling. Moreover, Hic-5 plays a central role in a positive feedback Hic-5-NADPH oxidase-ROS-JNK signal cascade. This sustained signaling is required for regulating EMT-related genes including E-cadherin, Snail, MMP9, and Zeb-1. In addition, Hic-5 can be a transcription coregulatory factor for a lot of nuclear receptors. Owing to the critical role of Hic-5 in signal transduction and transcription responsible for tumor progression, it can be a potential therapeutic target for the prevention of tumor metastasis.

**KEYWORDS:** *Hydrogen peroxide-inducible clone-5, Metastasis, Target therapy*

### INTRODUCTION

Tumor metastasis is responsible for the high recurrence rate and poor prognosis of cancers such as hepatocellular carcinoma (HCC) [1]. Metastasis is initiated by the epithelial-mesenchymal transition (EMT) which facilitates migration and invasion of primary tumor. This will promote the entrance of tumor cells into the blood vessel (intravasation). The tumor cells that survive in the circulation will move out of the blood vessel (extravasation) and finally colonize at the metastatic loci. The tumor microenvironment contains a lot of growth factors and cytokines such as hepatocyte growth factor (HGF) [2] and transforming growth factor- $\beta$  (TGF- $\beta$ ) [3] which are able to trigger the progression of a lot of tumors such as HCC via various molecular pathways. Targeting the critical molecules in the signal pathways of metastasis is one of the promising strategies of antitumor progression. Recently, hydrogen peroxide-inducible clone-5 (Hic-5), which belongs to the paxillin superfamily, is emerging as a potential target along the metastatic signaling pathway. This review focuses on the recent studies of Hic-5 related to its impacts on signal transduction and transcription responsible for tumor progression.

### PHYSIOLOGICAL FUNCTION OF HYDROGEN PEROXIDE-INDUCIBLE CLONE-5 AS A FOCAL ADHESION ADAPTOR

In the past decades, paxillin is known to be the most critical adaptor molecular on focal adhesion (FA) responsible for mediating signal crosstalk between integrin and metastatic factors [3]. Among the members in paxillin superfamily, Hic-5 is the most homologous to paxillin [4]. Given the structure similarity between Hic-5 and paxillin, they share many biological features, including the localization at FA and similar FA-binding factors such as protein tyrosine kinase 2 $\beta$  (Pyk2) and FA kinase [4]. Importantly, Hic-5 and paxillin can respond to the changes in integrin-extracellular matrix (ECM) and receptor tyrosine kinase-mediated signaling, leading to EMT and cell migration [5]. However, there are also a lot of different biochemical properties between them including tissue-specific

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distribution, regulations of critical signal cascade and gene expression, and the impacts on cellular phenotypes [5].

## THE ROLE OF HYDROGEN PEROXIDE-INDUCIBLE CLONE-5 IN TUMOR PROGRESSION

Hic-5 can be induced by a lot of tumor progressive factors such as TGF- $\beta$  [4], HGF, [5] and reactive oxygen species (ROS) [6]. Previously, Hic-5 was reported to mediate TGF- $\beta$ 1-induced EMT and cell migration of mammary epithelial cells MCF10A via the repression of E-cadherin [7]. Pignatelli *et al.* further demonstrated that Hic-5 was able to promote ECM degradation and invasion that facilitate TGF- $\beta$ 1-induced EMT of MCF10A breast cancer cells [8]. Hic-5 can also regulate EMT of ovarian cancer cells in a TGF- $\beta$ -independent manner [9]. In a more detailed cellular mechanistic study in three-dimensional ECM, Hic-5 may regulate the interchange of the amoeboid and mesenchymal phenotypes essential for the plasticity of MCF10A [10]. Recently, our study revealed that Hic-5 could be induced by HGF responsible for HCC progression and may serve as a potential HCC prognosis marker [11]. In addition, Hic-5 was also highly expressed in the cancer-associated fibroblast (CAF), required for deposition and remodeling of the stromal ECM in the tumor environment to promote noncell autonomous progression of breast [12] and colorectal cancer [13]. Moreover, Hic-5 is essential for the formation and organization of rosettes in cancer-associated fibroblasts [14]. Together, Hic-5 plays a critical role in tumor progression and may serve as a prognostic indicator of tumors such as HCC.

## THE MOLECULAR MECHANISMS FOR HYDROGEN PEROXIDE-INDUCIBLE CLONE-5 TO TRIGGER TUMOR PROGRESSION

How Hic-5 can be a critical player in tumor progression greatly relies on its impact on signal transduction and transcription as described below.

### The signaling pathway mediated by hydrogen peroxide-inducible clone-5

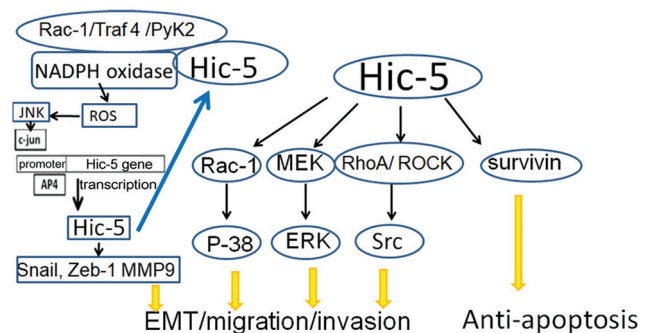
Hic-5 can enhance TGF- $\beta$ -induced signaling by inactivating the inhibitory Smads, such as Smad3 [15] and Smad7, which facilitates cell migration. Hic-5-mediated TGF- $\beta$ -induced EMT was also dependent on RhoA/ROCK1 [7] and Src [8]. Hic-5 may regulate mitogen-activated protein kinase (MAPK) cascade for cell migration and invasion in various systems. For example, Hic-5 induced matrix degradation, cell migration, and invasion via the regulation of Rac1-p-38 (MAPK) pathway [8]. Hic-5 can associate with JNK (MAPK) and its upstream kinase MAPKK4 to trigger the activation of matrix metalloproteinase 2 [16]. In addition, Hic-5 enhances migration via MEK-ERK cascade during endothelial cell migration to lysophosphatidic acid [17]. Recently, it was demonstrated that Hic-5 mediated Src-induced invadopodia rosette formation and organization in active Src-transfected NIH3T3 fibroblasts and cancer-associated fibroblasts [14].

The association of Hic-5 with ROS signaling, which was well known to be critical for tumor progression [18-20], has

been highlighted in recent years. Not only Hic-5 gene expression was found to be induced by ROS [21], as its name suggests, but also it has a great impact on ROS generation. Previously, Hic-5 was found to participate in ROS generation during the migration of endothelial cell [22]. In this context, Hic-5 serves as an adaptor for the assembly of FA complex required for NADPH oxidase-dependent ROS production [22]. In the vascular smooth muscle cells, Hic-5 mediated TGF- $\beta$ -induced activation of NADPH oxidase required for ROS generation and cell adhesion [23]. Our recent report demonstrated that Hic-5 serves as a mediator of the ROS-JNK signaling pathway for HCC progression [11]. Initially, Hic-5 appears to locate both upstream and downstream of ROS-JNK cascade in a patient-derived HCC cell line, HCC329 [11]. Further, we found that Hic-5 plays a central role in the positive feedback Hic-5-NADPH oxidase-ROS-JNK-c-jun molecular circuit in another patient-derived HCC cell line, HCC413 (unpublished result). Briefly, in the upstream, Hic-5 can associate with the regulators of NADPH oxidase including Rac1, Traf4, and Pyk2 which are essential for the activation of NADPH oxidase and ROS generation. In the downstream, we confirm that ROS-JNK pathway, indeed, is responsible for the upregulation of Hic-5 gene expression via the transcriptional factor c-jun coupled with AP4. The Hic-5 thus induced in turn reactivates NADPH oxidase and ROS generation for sustaining JNK signaling. Taken together, a positive feedback Hic-5-Rac1-Traf4-Pyk2-NADPH oxidase-ROS-JNK/c-jun cascade was established for HCC progression [Figure 1].

### The impact of hydrogen peroxide-inducible clone-5 on gene transcription

There are two ways for Hic-5 to impact gene expressions in various pathophysiological processes. One of them is the effect on the transcriptional level. Previous studies have demonstrated Hic-5 to be a transcription coregulatory factor of a lot of nuclear receptors [24], such as glucocorticoid receptor (GR) [25,26], androgen receptor [27], and progesterone receptor [28]. For example, Hic-5 may influence genomic occupancy of GR as well as transcription complex assembly, thus serving as an on/off switch for glucocorticoid regulation



**Figure 1:** Hic-5-mediated signaling pathways for tumor progression. Hic-5 can mediate a lot of signaling pathways including the positive feedback Traf4/Pyk2-NADPH oxidase-ROS-JNK signal cascade, regulating the expression of epithelial-mesenchymal transition-related genes (left panel). Hydrogen peroxide-inducible clone-5 can also trigger MEK-ERK, Rac1-p38, or RhoA/ROCK-Src pathway for cell migration and invasion (middle panel) and upregulating survivin for anti-apoptosis (right panel)

of many genes [25]. Furthermore, Hic-5 can be a coactivator of progesterone receptor involved in the regulation of DKK1 and calcitonin expression [28].

The other way for Hic-5 to impact gene expression is based on the signal pathway triggered by Hic-5 as described above. For example, Hic-5 can mediate gene expression of collagen and  $\alpha$ -smooth muscle actin triggered by TGF- $\beta$ /Smad pathway, leading to liver fibrosis [29] or keloids [30]. Moreover, Hic-5 was responsible for transcription of oncogenes such as c-fos [31], and overexpression of Hic-5 could increase the expression of Sp1 and survivin, which inhibited the cell apoptosis, thereby reducing the cell damage induced by hypoxia [32]. In our study, we found that Hic-5-mediated NADPH oxidase-ROS-JNK/c-jun signal cascade can regulate the expressions of EMT-related genes including the suppression of E-cadherin and upregulation of transcription factors Snail and Zeb-1 and a matrix degradation enzyme MMP9. Finally, in the tumor environment, Hic-5 promoted the expression of lysyl oxidase and collagen I in CAFs, which is responsible for orchestrating or generating a tumor-promoting stroma [13].

### **HYDROGEN PEROXIDE-INDUCIBLE CLONE-5 CAN BE REGARDED AS A PROMISING THERAPEUTIC TARGET**

Given the critical role of Hic-5 in mediating the signal transduction and transcription, the suppression of Hic-5 may benefit the prevention of disease progression. Previously, Hic-5 was regarded as a novel factor in vascular remodeling and can be a potential therapeutic target for vascular disorders [33]. Moreover, *in vivo* Hic-5 knockdown by siRNA repressed CCl<sub>4</sub>-induced liver fibrosis in mice [29]. The feasibility that Hic-5 can be a molecular target for blocking tumor progression was also emerging. Previously, Hic-5 has been suggested to be a possible molecular target for the treatment of melanoma [34]. Recently, we also found significant suppression of intrahepatic metastasis of a liver-transplanted patient-derived HCC cell in mice injected peritoneally with chemically modified Hic-5 siRNA (unpublished result).

In addition, one advantage of using Hic-5 as a target is its limited expression in normal tissue compared with paxillin [6]. Thus, target therapy aiming at Hic-5 may cause fewer side effects than paxillin.

### **CONCLUSION AND PERSPECTIVE**

As an adaptor on the FA, Hic-5 can mediate a lot of signaling pathways including the positive feedback Traf4/Pyk2-NADPH oxidase-ROS-JNK signal cascade, regulating the expression of EMT-related genes. Hic-5 can also trigger MEK-ERK, Rac1-p38, or RhoA/ROCK-*Src* pathway for cell migration and invasion and upregulating survivin for anti-apoptosis [Figure 1]. In future, it is worthy of investigating whether Hic-5 can be an ideal therapeutic target for the prevention of tumor progression.

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### **Conflicts of interest**

There are no conflicts of interest.

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