RESEARCH ARTICLE

The potential role of the paxillin paralog Hic-5 in progression of hepatocellular carcinoma

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ABSTRACT

Hepatocellular carcinoma (HCC) is one of the most common causes of death from cancer worldwide. The poor prognosis of HCC is due to high recurrence rate mainly caused by intrahepatic metastasis. Paxillin was known to be a central adaptor protein for mediating focal adhesion (FA) signal required for HCC progression. However, target therapy aiming at paxillin seems unfeasible due to its ubiquitous tissue expression and essential biological functions. Within the paxillin superfamily, hydrogen peroxide inducible clone-5 (Hic-5) is the most homologous to paxillin. This review summarises the recent findings relevant to the differential biochemical and biological roles of Hic-5 and paxillin. Given the structure similarity between Hic-5 and paxillin, Hic-5 shares many of the characteristics of paxillin, including the localization of Hic-5 at focal adhesions and similar FA binding factors. However, some of the regulatory mechanisms and molecular functions of Hic-5 are rather different from those of paxillin. These might explain the differential roles of both adaptors in regulating various pathophysiological processes. Interestingly, both adaptors might play distinct but complementary roles in tumor progression. Due to the more limited tissue distribution of Hic-5, it can be a more suitable therapeutic target for preventing HCC progression.

Key words: Hepatocellular carcinoma; Metastasis; Paxillin; Hic-5; focal adhesions.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common causes of death from cancer worldwide. The mortality rate is very high in China, Taiwan and Southeast Asia[1]. Recently, the incidence of HCC was increasing in countries such as Japan, Italy, France, Switzerland, United Kingdom and the United States[2]. The poor prognosis of HCC is due to high recurrence rate mainly caused by intrahepatic metastasis (about 80%) or extrahepatic metastasis (about 20%)[3]. Therefore, prevention of metastasis is critical for HCC management. To address the issue, the suitable molecular targets within the molecular pathways leading to HCC metastasis are needed to be identified.

Tumor metastasis occurs *via* complicated processes, including epithelial mesenchymal transition (EMT), migration and invasion of primary tumor, followed by intravasation, extravasation and colonization at the metastatic loci. The tumor microenvironment in HCC contains a lot of metastatic factors produced by interaction of primary cell with inflammatory cells, stromal cell and extracellular matrix [4, 5]. Some of the metastatic factors including transforming growth factorβ (TGFβ) [6], hepatocyte growth factor (HGF) [7, 8], vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF) [9] and integrin engagement [10-15], are capable of triggering HCC progression. A lot of signaling components in the FA such as RacGTPase [16, 17], focal adhesion kinase (FAK) [18-22], Src [23], Pyk2 [24] and paxillin [25] are known to be responsible for mediating HCC progression triggered by these metastatic factors.

Among the aforementioned FA signal components, Paxillin is responsible for mediating signal cross talk between integrin and metastatic factors [27]. The role of paxillin in tumor progression of HCC has been demonstrated in a lot of studies. Paxillin phosphorylation at Ser178 mediated by Jun N-terminal kinase (JNK) was involved in the HGF [30] and P21-activated protein kinase (PAK) triggered tumor progression of HCC [31]. Recently, paxillin was found to be a mediator for the actopaxin-triggered HCC metastasis [25].

Within the paxillin superfamily, Hic-5 (hydrogen peroxide inducible clone-5), is the most homologous to paxillin. Hic-5 was initially identified as one of the TGFβ1 and hydrogen peroxide-inducible genes [32]. As paxillin, Hic 5 is also an adaptor molecule essential for triggering progression of tumors [33, 34] including HCC [35]. This review summarizes the recent findings relevant to differential biochemical and biological roles between Hic-5 and paxillin in mediating a lot of cellular phenotypes. Specifically the complementary role of both adaptors in tumor progression is addressed. Moreover, the possibility of Hic-5 to be a promising therapeutic target for prevention of HCC metastasis is highlighted.

Comparison of the biochemical properties between Hic-5 and paxillin

Differential structure and binding properties

Paxillin comprises numerous discrete structural domains for scaffolding FA components in response to integrin engagement and growth factors [36]. The C-terminal half of paxillin contains four LIM domains, serving as binding sites for several structural and regulatory proteins such as tubulin and the protein tyrosine phosphatase (PTP) PEST. The N-terminus of paxillin is composed of five

leucine- and aspartate-rich LD motifs (LD1-LD5), with multiple tyrosine, serine and threonine phosphorylation sites for recruiting a lot of signal molecules including Src tyrosine kinase, focal adhesion kinase (FAK), receptor for activated C kinase 1(RACK1), JNK, p-38 and Abl. Hic-5 shares the same 11-exon genomic organization as paxillin with minor differences in the number of LD domains in the N-terminal region (five for paxillin and four for HIC-5) [36].

Given the structure similarity between Hic-5 and paxillin, Hic-5 shares many of the characteristics of paxillin, including the localization at FA and similar FA binding factors such as protein tyrosine kinase 2 beta (PYK2), c-Src tyrosine kinase (Csk), FAK, Arf GAP1 (GIT-1) [37] and PTP-PEST [38]. However, there are paxillin interacting FA components such as Crk and Src which can not bind Hic5. One distinct binding activity of Hic-5 was ascribed to its LIM domain by which Hic-5 may form LIM-LIM hetero-oligomers with LIM-only proteins such as PINCH or CRP2 [41]. In contrast, LIM4 of paxillin cannot form oligomers and does not interact with PINCH or CRP2 [41].

Differential phosphorylation pattern involved in FA signaling

The phosphorylation patterns of Hic-5 and paxillin induced by extracellular stimuli are also rather different. Upon integrin engagement, paxillin becomes tyrosine phosphorylated, primarily on tyrosine residues 31 and 118 (Y31 and Y118, respectively at LD1), in a FAK- and Src-dependent manner, resulting in activation of a lot of critical signal cascades for cell spreading and motility [42, 43]. In contrast, Hic-5 does not exhibit the aforementioned phosphorylation patterns probably due to lack of cognate tyrosine residues. However, phosphorylation of Hic-5 may occur through PYK2 following hyperosmotic stress [47] and platelet activation [48].

In addition to the discrepancies of the molecular structure, interaction and phosphorylation pattern for FA signaling as described above, there are a lot of different biochemical properties between both Hic-5 and paxillin including tissue specific distribution, regulation of gene expression, interaction with critical signal cascade, and the impacts on cellular phenotypes.

Differential tissue specific distribution between paxillin and Hic-5

Tissue expression of paxillin is broader than that of Hic-5

Whereas paxillin is ubiquitously expressed in most tissue and cell types, Hic-5 is enriched only in certain tissue such as smooth muscle (in particular the vasculature), large intestine and uterus and relatively high in the lung and spleen [33]. This implicates that the biological roles of paxillin are broader than Hic-5. Indeed, paxillin ablation causing early embryonic lethality, while Hic-5 knockout exhibits only very mild vascular defects [33].

Differential pattern of inducible gene expression between paxillin and Hic-5

One distinct regulatory mechanism of Hic-5 is the inducible gene expression by a lot of extracellular stimuli. In the most early studies, Hic-5 gene expression was found to be induced by reactive oxygen species (ROS) [59], as its name suggests. Hic-5 expression can also be induced during TGFβ1-induced senescence of

osteoblastic cell line [32], angiotensin II-induced abdominal aortic aneurysm (AAA) development [60], methylmercury-induced ER stress [61] and Escherichia coli-induced prostatic inflammation [62]. Also, epithelial expression of Hic-5 in mouse and human prostate tissues was elevated after castration, leading to epithelial regression through the repression of c-myc gene [63]. In contrast, the evidence regarding inducible gene expression of paxillin was very rare, with only one report demonstrating that paxillin mRNA is induced by TGFβ as shown on a retrovirus-mediated gene trap screen [64].

Differential impacts of Hic-5 and paxillin on intracellular signal cascades

The impacts of Hic-5 gene expression on essential signal cascades are more prominent than those of paxillin. Whereas Hic-5 gene expression and nuclear translocation can be induced by ROS as described above, it appears that Hic-5 may also positively regulate ROS generation in the focal adhesion [65]. In this context, Hic-5 serves as an adaptor for association of TRAF4 and $p47^{phox}$ which initiate Rho GTPase activation required for NADP oxidase-dependent-ROS production. The ROS generated in turn targets the redox-sensitive phosphatase PTP-PEST in FA, establishing a positive feedback cycle that facilitates Rac1 activation leading to sustained MAPK activation and cell migration [65]. Similarly, whereas TGFβ was known to be an inducer of Hic-5 [66, 67], TGFβ-induced signaling can also be positively regulated by Hic-5. Previously, Hic-5 was found to promote TGFβ-induced signaling by binding to and inactivating the inhibitory Smads, Smad3 [68] and Smad7 [69] leading to enhanced TGF-β/ Smad2 signaling required for EMT. Also, Hic-5 may bind to Smads 1, 5 and 8, for repressing bone morphogenetic protein (BMP) signaling [70]. In addition, Hic-5 may serve as a scaffold protein that specifically activates the MAPK cascade. For example, in a model for abdominal aortic aneurysm (AAA), Hic-5 interacted specifically with JNK and its upstream kinase MAPKK4 to trigger the downstream signaling [60].

Differential impacts of Hic-5 and paxillin on cellular phenotypes

Given the aforementioned discrepancies of Hic-5 and paxillin in biochemical properties, it is anticipated to find the divergence of both adaptors in regulation of cellular phenotypes such as cytoskeletal organization, cell adhesion, cell migration and cell growth. Whereas paxillin is well known to be required for cell adhesion, Hic-5 may suppress excess changes in cytoskeleton structure by antagonizing paxillin [71, 72]. The regulation of cell growth by paxillin and Hic-5 is also antagonistic. In general, paxillin is responsible for transducing both adhesion-dependent and independent growth signaling [73-75]. On the contrary, Hic-5 serves as one of the fail-safe system for the adhesion dependence of cell growth by negatively regulating the cell cycle positive regulator, cyclin D1 [41].

The role of Hic-5 and paxillin in tumor progression

Distinct but complementary roles of Hic-5 and paxillin in tumor progression

As described in previous section, paxillin is well known to be involved in tumor progression, ascribed to its crucial role in mediating focal adhesion signaling [73]. Recently, the role of Hic-5 tumor progression was also emerging [33, 77, 78]. Both paxillin and Hic-5 expressed in a variety of invasive/metastatic cancers, including

breast, lung, and prostate tumors and are regarded as potential prognostic markers and therapeutic targets [33]. However, due to the differential biochemical and biological properties, the mechanisms for the two adaptors in triggering tumor progression are also rather divergent. Whereas tyrosine phosphorylation of paxillin is essential for mediating EMT and cell migration [79, 80], gene expression of Hic-5 was required for these processes [65, 81]. In the cell culture systems, Hic-5 is highly detectable in mesenchymal cell lines but absent in epithelial cell lines. Moreover, Hic-5 expression can be induced by TGFβ leading to EMT, invadopodia formation, cell migration, and invasion [67]. This is ascribed to that Hic-5 can bind and inactivate the inhibitory Smads to enhance TGF-β receptor signaling as described above [68-70]. In addition, the induction of Hic-5 expression by TGFβ was dependent on RhoA/ROCKI [79]. Furthermore, ectopic expression of Hic-5 is sufficient to promote normal mammary cells to undergo EMT in the absence of TGF-β [67] and stimulate cell migration of NMuMG cells [34]. In contrast, overexpression of paxillin is unable to induce the transition to mesenchymal phenotype [82]. In spite of these discrepancies, it appears that both adaptors may contribute metastatic change in a concerted manner. This notion was supported by the evidence that Hic-5 may cooperate with paxillin to regulate metastasis of breast cancer [78]. Also, the FA structure protein vinculin is able to interact with paxillin and Hic-5 via Rac1 and RhoA, respectively, required for FA turn over and cell migration[83].

The potential role of Hic-5 in HCC progression

The involvement of paxillin in HCC progression is evident as described in the **Introduction section.** Recently, the role of Hic-5 is also emerging. One report demonstrated that the expression and phosphorylation of Hic-5 were upregulated in HCCs overexpressing proline-rich tyrosine kinase 2 (Pyk2) [35], a member of the FAK family known to be involved in HCC metastasis [24, 84, 85]. Also, TGF-β, which can induce Hic-5 for malignant transformation [67], is recently highlighted to be one of the critical metastatic factors triggering HCC progression [86-88]. In our recent report, we found Hic-5 mediated ROS-JNK signaling and serve as a potential therapeutic target for prevention of HCC progression [89]. In this study, Hic-5 positively cross-talks with ROS to trigger sustained ERK (MAPK) signaling for HCC progression induced by hepatocyte growth factor (HGF) (see Scheme in Fig.1). Notably, ROS is also a well-known mediator of tumor progression including HCC [89-91]. Together, these studies implicated that Hic-5 is one of the key factors in HCC progression.

Fig. 1 Proposed mechanism for Hic-5 to regulate ROS-mediated sustained signaling in a positive feedback circuit

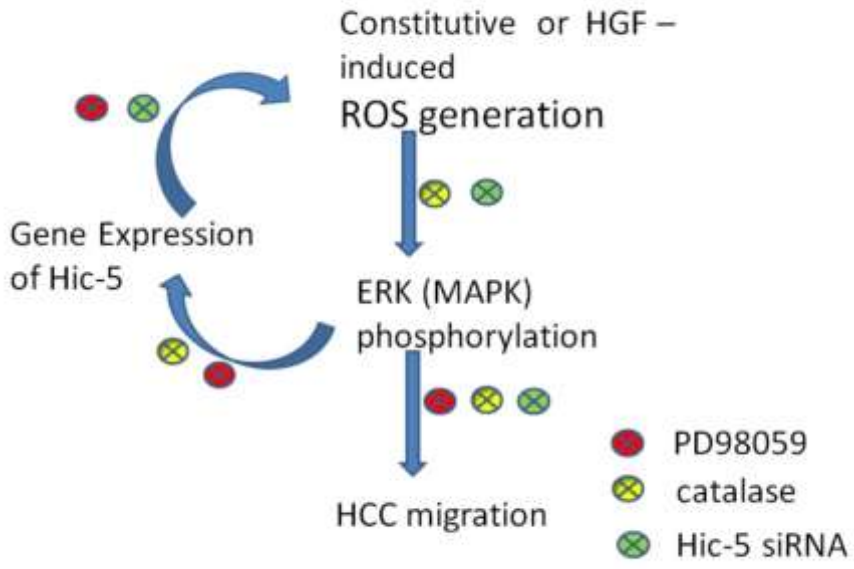

Fig. 1 Constitutive or HGF-induced ROS generation triggers ERK(MAPK) activation, leading to gene expression of Hic-5, which then contribute to sustained ROS generation and signaling transduction required for cell migration of HCC. This signal circuit was delineated by using inhibitors or siRNA against the indicated signal components. Circles with red, yellow and green represent inhibitors of ERK (PD98059), ROS scavenger (catalase) and Hic-5 siRNA, respectively. In the relevant experiments, activities (in case of ERK and ROS) or expression (in case of Hic-5) of each component in the circuit can be suppressed by inhibitors of the two upstream components. Cell migration is prevented by all inhibitors. HGF: hepatocyte growth factor.

Conclusions and perspectives

Since Hic-5 is the most homologous to paxillin, it can be expected that both adaptors share similar biological properties. However, a lot of the regulatory mechanism and molecular function of Hic-5 are not shared by paxillin (summarized in Table 1). Importantly, both adaptors play distinct but complementary role in tumor progression. Due to the more limited distribution of Hic-5, it can be a more promising therapeutic target than paxillin for preventing HCC progression.

Table 1. Comparison of Pathophysiological charateristics of paxillin and Hic-5

Pathophysiological Chrateristics	**Hic-5**	**paxillin**
Tissue distribution	**limited**	**Ubiquitous**
Stimulators	ROS, TGFβ, HGF and others	Multiple growth factors/cytokines /integrin engagement
Regulatory mechanisms	Gene expression (major) /phosphorylation (rare)	Phosphorylation (major)/Gene expression (rare)
Effect on Cell growth	Safe guarding adhesion dependence of cell growth (-)	Anchorage- dependent and independent cell growth (+)
Effects on FA phenotype	EMT/ migration (+)	EMT/migration (+)
	Cell spreading (-)	Cell spreading (+)
	Cytoskeletal change (-)	Cytoskeletal change (+)
Involvement in tumor progression	metastasis of breast cancer (+) HCC progression (+)	metastasis of breast cancer (+) HCC progression (+)

(+), and (-) represent positive and negative regulation, respectively, of the indicated phenotypes by Hic-5 or paxillin; ROS:reactive oxgen species; HCC hepatocellular carcinoma

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