Mini-Review

Scaffold Proteins in Gastrointestinal Tumors as a Shortcut to Oncoprotein Activation

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Keywords

Gastrointestinal tumors · Mitogen-activated protein kinase · p53 · Scaffold proteins

Abstract

Background: The development of cancer involves uncontrolled cell proliferation, and multiple signaling pathways that regulate cell proliferation have been found to be dysregulated in cancers. Extracellular signal-regulated protein kinase (ERK) is one of three major subtypes in the mitogen-activated protein kinase (MAPK) families. The MAPK/ERK pathway (RAS/RAF1/MEK/ERK) plays an important part in promoting cell proliferation in response to growth factors, thereby serving as a driving signal in gastrointestinal (GI) tumors. In contrast, the p53 tumor suppressor functions as a “guardian of the genome” and stops cell proliferation when oncogenic signaling is activated. **Summary:** Both pathways constrain each other in healthy GI epithelium, facilitating controlled proliferation that is essential for tissue repair and regeneration. However, in GI tumors, the MAPK/ERK and p53 pathways are commonly dysregulated, in part due to abnormal posttranslational modifications. Hyperphosphorylation of the ERK protein causes sustained activation of cell proliferation, whereas hypoacetylation of the p53 protein impairs its transcriptional function and blocks cell apoptosis. Multiple scaffold proteins have been found to regulate the posttranslational modifications of ERK and p53 proteins in GI tumors. **Key Message:** Abnormal expression of scaffold proteins may contribute to the dysregulation of the MAPK and p53 signaling pathways and thereby contribute to the development of GI tumors. **Practical Implications:** Scaffold proteins are potential biomarkers and therapeutic targets in GI tumors.

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Mitogen-Activated Protein Kinase Signaling Plays Crucial Roles in Gastrointestinal Tumors

The human genome encodes three major subtypes of mitogen-activated protein kinases (MAPKs): extracellular signal-regulated protein kinase (ERK), c-jun N-terminal kinase, and MAPK14. In a previous work, we systematically discussed the roles of MAPK pathways in colorectal cancer [1]. The ERK signaling pathway plays a central role in regulating cell proliferation as well as triggering transcription factor activation and histone modification in response to growth factor stimulation. A phosphorylation cascade is transduced from the small guanosinetriphosphatase (GTPase) RAS to RAF1, MAPK/ERK kinase (MEK), and ERK, which consequently phosphorylates different effector proteins such as Elk1, Myc, and Rsk, regulating a panel of cellular activities such as proliferation, apoptosis, differentiation, angiogenesis, and metastasis (Fig. 1).

In the canonical ERK signaling pathway, the activating mutation of RAS and amplification of the *RAS/RAF/MEK* genes are thought to be the main cause of ERK hyperactivation in gastrointestinal (GI) tumors. However, a discrepancy has been found between the widespread activation of ERK (in approximately 90% of GI tumors) and limited genetic alterations of upstream elements (in <50% of GI tumors) [2]. Therefore, how ERK is activated without significant alteration of *RAS*, *RAF*, and *MEK* is a major question in the field. This discrepancy also raises a question as to the potential efficacy of inhibitors of RAS/ERK signaling in treating GI tumors.

Synbindin Is a Scaffold Protein of ERK Signaling on the Golgi Apparatus

We have previously identified synbindin (TRAPPC4), which is a subunit of the transport protein particle (TRAPP) complex responsible for endoplasmic reticulum-Golgi transportation [3], as an important regulator of ERK signaling in GI tumors [4, 5]. Synbindin physically binds to ERK and MEK proteins on the Golgi apparatus, facilitating ERK phosphorylation by MEK. This phosphorylation activates Rsk and Elk1, leading to increased cell proliferation and migration [3]. The synbindin protein contains an atypical PDZ domain and a longin domain [6]. Truncation of the longin domain abolished the interaction between synbindin and ERK [3], and mutation of the ERK DEF domain produced the same result [2]. Therefore, we concluded that the synbindin-longin domain binds the DEF domain of ERK. The expression level of synbindin is strongly correlated with the phosphorylation of ERK protein in gastric cancer tissues [2], which elucidates a noncanonical mechanism for ERK hyperactivation in gastric cancers. Targeting synbindin significantly decreased ERK phosphorylation in a nude mouse model, and tumor growth was also significantly inhibited in the synbindin-inhibited group [3]. These findings highlight the potential therapeutic significance of synbindin in gastric cancer, presumably in complementation with RAS or epidermal growth factor receptor inhibitors.

Synbindin Is Regulated by the OCT1 Transcription Factor

To clarify the regulatory mechanism of synbindin expression, we searched for transcription factors that may bind to the promoter region of the *synbindin* gene. We found that the transcription factor OCT1 (POU2F1) had multiple binding sites within the synbindin promoter. This was experimentally confirmed by chromatin immunoprecipitation and luciferase reporter assays. Ectopic expression of OCT1 increased the transcription of synbindin in gastric cancer cells, and knockdown of OCT1 with specific small interfering RNAs caused a substantial decrease in synbindin expression (Fig. 1). The expression of OCT1 and synbindin

Fig. 1. MAPK pathways and the effects of the OCT1-synbindin axis on ERK signaling. OCT1 transactivates synbindin, which binds both MEK and ERK on the Golgi apparatus, facilitating the activation of Elk1, Myc, and Rsk. Adapted from Fang and Richardson [1], Qian et al. [2], and Kong et al. [3]. P, phosphate; Pi, intracellular organic phosphate.

was strongly correlated in gastric cancer tissues, and OCT1 expression displayed a significant correlation with ERK phosphorylation. Importantly, gene copy amplification, messenger RNA upregulation, and protein overexpression of OCT1 all were significantly associated with poor survival of gastric cancer patients [2]. Moreover, the prognostic significance of OCT1 was confirmed in independent patient cohorts. Although OCT1 has been found to have similar transcription targets as OCT4 (a key factor for generating induced pluripotent stem cells), OCT1 does not replace OCT4 in induced pluripotent stem cell generation [7, 8]. Instead, OCT1 has been reported to be a determinant of cancer stem cells and somatic stem cells [9]. Interestingly, ERK signaling is also commonly activated in cancer stem cells [10]. These findings support a scenario wherein OCT1 may drive cancer progression by enhancing the ERK signaling pathway. Therefore, synbindin together with its upstream regulator OCT1 may be a promising prognostic biomarker signature and therapeutic target for gastric cancer.

Other Scaffold Proteins in the MAPK Signaling Pathway

In addition to synbindin, previous studies have revealed multiple crucial scaffold proteins in the MAPK pathway (Table 1). Kinase suppressor of Ras1 (KSR1), known as a classical scaffold protein, exerts significant control over MAPK/ERK signaling. When there is no

Table 1. Outlines of some scaffold proteins conferring spatiotemporal modulation of the MAPK signaling in GI tumors

Scaffold proteins	Sketches
Synbindin	Synbindin binds to ERK and MEK on the Golgi apparatus [3], facilitating ERK phosphorylation by MEK, which activates Rsk and Elk1 [3], leading to increased cell proliferation and migration. The expression level of synbindin is strongly correlated with the phosphorylation of ERK protein in gastric cancer tissues [2].
KSR1	KSR1 constitutively ties to MEK1/2 inactively in cytosolic compartment. Once activated, the complex relocates to the membrane, interacting with Raf and ERK1/2 and phosphorylating the cascade [11]. KSR1 has been found to be overexpressed in gastric cancers, and it is suggested that KSR1 is involved in oncogenesis and prognosis, anticancer drug sensitivity, and chemoresistance in GI tumors [13, 15].
IQGAP1	IQGAP1 links with B-RAF, MEK1/2, and ERK1/2 through different domains (IQ, WW), resulting in sequential phosphorylation and hyperactivation of ERK1/2 [17]. Studies imply that the level of IQGAP1 is related to the prognosis in gastric cancer [18] and the treatment response in rectal adenocarcinomas [19].
Paxillin	Paxillin physically interacts with MEK to induce the activation of ERK when stimulated by hepatocyte growth factor, exerting control over cell motility [11]. In cancers, paxillin is inferred to be concerned with tumor progression and 5-fluorouracil resistance in colorectal cancer patients, due to the result that overexpression of paxillin influences the stability of Bcl-2 via MAPK signaling [51, 52].
Sef	Similar expression to FGF (Sef) regulates the MAPK network through coordination with MEK to hyperactivate ERK on the Golgi apparatus and prevent ERK shuttling from nuclear to cytosolic compartments [11, 53]. The decrease in Sef might cause accumulation of active MEK in nuclear compartment and thereby upregulation of gene transcription. As a result, Sef might be an early signal for tumorigenesis [54].

Adapted from Witzel et al. [11].

stimulus, KSR1 stays inactive in combination with MEK1/2 in cytosolic compartment. Once stimulated by the distinct upstream effector, the conjugate starts to relocate to the membrane and recruit RAF and ERK1/2, and thus the downstream components get activated. Sequentially, the phosphorylated ERKs dimerize and head for the nucleus, modulating transcription [11]. The ERK dimers, simultaneously, phosphorylate B-RAF and KSR1 as a negative feedback, putting an end to the reaction [12]. The expression of KSR1 has been found to vary in different carcinomas [13]. A study observed that compared to the expression in the matched normal tissues, ERK1/2, p-ERK1/2, KSR1, and p-KSR1 were distinctly overexpressed in gastric cancer tissues, in association with TNM stage and the lymph metastasis [14]. Besides, the expressions of ERK1/2, p-ERK1/2, KSR1, and p-KSR1 are significantly related with each other, indicating that KSR1 might play a role in the oncogenesis and progression of gastric neoplasms [15]. Additionally, the KSR1-mediated ERK1/2 signaling pathway is inferred to be somehow associated with anticancer drug sensitivity and chemoresistance in GI tumors [13, 15].

IQ motif-containing GTPase activating protein (IQGAP) is another vital scaffold protein participating in the MAPK signaling pathway. The IQGAP family contains three subtypes in human cells, namely IQGAP1, IQGAP2, and IQGAP3 [16]. Among them, IQGAP1 is considered the most important one, as it has been found to take part in numerous cellular activities. Here

we focus on its role in signaling events causing tumorigenesis. IQGAP1 interacts with three protein kinases (B-RAF, MEK1/2, and ERK1/2) through different domains (IQ, WW), in turn phosphorylating the kinases [17]. Since IQGAP1 can hyperactivate ERK, the regulator is presumed to be potentially carcinogenic. Correspondingly, this highly conserved protein has been found to be overexpressed in various carcinomas. Previous research implied that upregulation of IQGAP1 might contribute to poor survival in gastric cancer [18], and both protein expression and localization might impair the treatment response in rectal adenocarcinomas [19].

The Multifaceted Regulation of p53 in GI Tumors

Although ERK signaling drives the proliferation of cells, the presence of an intact p53 signaling network prevents malignant transformation due to its stringent control of genomic stability [20]. It has long been known that RAS and p53 mutations have strong synergistic effects on tumorigenesis [21, 22]. The function of p53 is commonly lost in cancers, but on a genetic level, the *TP53* gene is mutated in approximately half of all cancers [23]. We previously demonstrated that mutant p53 gains oncogenic function by regulating gastric adenocarcinoma-associated, positive CD44 regulator, long intergenic noncoding (GAPLINC) RNA, and the *CD44* oncogene [24]. However, the data suggest that when p53 is not mutated, its inactivation in cancer involves additional regulatory pathways. Such a possible regulator is the E3 ubiquitin ligase MDM2, which has long been recognized as a negative regulator of p53 by targeting p53 for degradation by the proteasome [25, 26]. However, wild-type p53 protein is often found to be highly expressed in cancers where it does not appear to exert a strong tumor-suppressive function [27–29]. How p53 is inactivated in these cases is poorly understood. It has recently been suggested that the function of p53 as a transcription factor is impaired by inefficient acetylation modification [30, 31]. However, the factors that control the status of p53 acetylation are unclear, especially in GI tumors.

Scaffold Proteins That Regulate p53 Acetylation and Activation

Recently, we reported that ArhGAP30 is a determinant of p53 acetylation in colorectal cancer. The ArhGAP30 protein functions as a scaffold protein, binding to the C-terminal of p53 and to P300 acetyltransferase. ArhGAP30 promoted P300-mediated acetylation of p53 K382 residue, and mutation of K382 blocked the effect of ArhGAP30 on p53 function. Importantly, the expression of ArhGAP30 and p53 acetylation was strongly correlated in colorectal cancer tissues, suggesting that ArhGAP30 is a major determinant of p53 acetylation and functional activation in colorectal cancer (Fig. 2) [5]. Of note, we previously reported that some other RhoGAPs (such as ArhGAP11A) may promote the tetramerization of p53 and increase its transcription activity [32]. Therefore, the RhoGAP family seems to have multifaceted roles in p53 function, which are independent of their canonical functions as GTPase activation proteins.

Tribbles 1 (TRIB1), a homologous scaffold protein of Tribbles [33], is a multifunctional regulator of several cellular signaling events. Of note, studies have shed light on its carcinogenic potency for targeting the cancer suppressor p53 [34]. Coordinating with histone deacetylase 1 (HDAC1), TRIB1 facilitates the HDAC1-dependent deacetylation of p53 and thereby suppresses the distinct DNA binding of p53, which consequently results in a decrease in p53-induced cell apoptosis and enhances the incidence of cancer [34]. However, the particular loci where TRIB1 binds p53 and HDAC1 remain to be clarified. Accordingly, the data reveal

Fig. 2. The ArhGAP30 scaffold protein facilitates P300-mediated acetylation of p53 and its functional activation, and RhoGAP promotes the assembly of p53 monomers into functional tetramers. TRIB1 physically ties p53, exerts control over the HDAC1-dependent deacetylation of p53, and thereby suppresses the binding efficacy of the particular DNA and p53. Adapted from Xu et al. [32], Miyajima et al. [34], and Wang et al. [44].

that TRIB1 is upregulated in a variety of carcinomas, including colorectal and esophageal cancer [35]. Given these findings, TRIB1 is probably a novel target for the diagnosis and treatment of GI neoplasms.

Scaffold Proteins That Regulate p53 Ubiquitination

Wild-type p53 turns inactive via the ubiquitin-proteasome-mediated degradation pathway. This tightly regulated process is mainly associated with MDM2, a major E3 ubiquitin ligase for p53, more exactly with its CTD domain [36] in some conditions. Previous studies have suggested that ubiquitination and degradation of p53 can be upregulated when Twist1 [37], YY1 [38], and gankyrin [39] bind to MDM2, while ARF can be an inhibitor downregulating the ubiquitination of MDM2 [40]. Focal adhesion kinase (FAK) is a multirole tyrosine kinase involved in various cellular events, ranging from cell motility to adhesion and metastasis activities. Strikingly, FAK has been discovered to act as a kinase-independent mechanism, especially as a scaffold protein physically interacting with p53. It is proposed that FAK targets p53 through a distinct region in the N-terminal named FERM, which has three domains termed F1 (binding to p53), F2 (conferring nuclear localization), and F3 (binding to MDM2) [14], while the matched site of p53 is a 7-amino-acid region (amino acids 65–71) in the amino terminal [41]. Both p53 and FAK can shuttle in or out of the nucleus. In the absence of adhesion or in circumstance of cellular stress, FAK runs into the nuclear compartment and interacts with p53 and MDM2; thus, the FAK-p53-MDM2 complex confers ubiquitination and degradation of p53, inducing malignant cell proliferation and apoptosis resistance [14, 42]. However, the intricate relationship between FAK and p53 in cytoplasm is incompletely understood yet. Interestingly,

Fig. 3. Prognostic significance of the scaffold proteins synbindin (a) and ArhGAP30 (b) in gastrointestinal tumors. Kaplan-Meier survival analysis was used to compare the prognosis of different patient groups. Adapted from Kong et al. [3] and Wang et al. [44].

p53 can suppress the FAK promoter directly, forming a dynamic feedback loop [43]. Given the fact that the distribution of FAK is abundant in numerous malignant tumors including colon cancer and that the level of FAK is obviously higher in tumors with mutant p53 than in tumors with wild-type p53 [43], the question might be raised as to how to make full use of the bidirectional regulation between FAK and p53, cell survival, and apoptosis and whether it is possible to translate this mechanism into anticancer therapy [42].

Prognostic Significance of Scaffold Proteins

Since the scaffold proteins have been proved to be of significance in cell proliferation and apoptosis, with MAPK and p53 regulating events, a myriad of relevant research has turned to explore the correlation between the distinct scaffold proteins and tumor prognosis. Studies have revealed that low expression of the ArhGAP30 scaffold protein was associated with poor survival of colorectal cancer patients (Fig. 3), which was supported by independent patient cohorts [44]. The prognostic significance of ArhGAP30 was independent of the American Joint Committee on Cancer (AJCC) tumor stage, suggesting that this biomarker may be used to complement traditional prognostic strategies. We also demonstrated that introduction of the *ArhGAP30* gene into tumors could dramatically suppress the growth of colorectal cancer xenografts in nude mice, suggesting that ArhGAP30 could represent a promising therapeutic target. Likewise, synbindin expression was also associated with patient survival in gastric cancer (Fig. 3a).

IQGAP1 has been widely observed in both gastric and colorectal tumors, especially in the deep tissues and invasion fronts of advanced carcinomas [45], which is presumed to be of importance in metastasis and shortened lifespan [46]. Experiments have indicated that tumor susceptibility and aggression may partly rely on the gene dose of IQGAP1 [47], and the absence of IQGAP1 may predict a favorable prognosis [18]. Interestingly, treating tumor-bearing mice with the WW peptide, the only known conserved domain in IQGAP1 bound to ERK, can

remarkably harm MAPK-mutant neoplasms selectively and reduce resistance to certain anti-cancer drugs [47]. What is more, it is hypothesized that IQGAP1 is a key to radiochemotherapy resistance [19]. Meanwhile, the overexpression of KSR1 and p-KSR1 is somehow involved with TNM stage and lymph metastases in gastric cancer [14]. Besides, KSR1 offers resistance to multiple antitumor agents, such as cytochalasin H and tunicamycin, identified through drug screening assays [13], while *Ginkgo biloba* extract (EGb) 761 has been shown to reduce KSR1-induced chemoresistance by virtue of its antioxidant effect [15]. As for FAK, a meta-analysis accomplished in 2016 [48] declared that upregulation of FAK resulted in worse overall survival (average hazard ratio = 2.073), and in particular, poorer overall survival was observed in gastric cancer (average hazard ratio = 2.073), whereas there seemed to be no correlation between FAK and neoplasm staging. In addition, another report considered that the effect of FAK varied in different phenotypes of gastric cancer, probably due to individual factors [49]. However, a previous analysis revealed no marked prognostic significance of FAK in colon adenocarcinomas [50]. To date, there are few studies providing insight into the clinical significance of TRIB1 in GI tumors. Taken together, the multifunctional scaffold proteins should be attached great importance as promising targets for guiding treatment and estimating prognosis, deserving further study.

Conclusions

The discovery of scaffold proteins in the ERK and p53 signaling pathways highlights the complexity of cancer cell signaling. Although these scaffold proteins, such as synbindin and ArhGAP30, have opposite effects on cellular phenotypes (pro- and antiproliferation, respectively), they share some common features that deserve our attention: (1) Scaffold proteins do not directly execute posttranslational modifications on their client proteins, but rather catalyze the modification by modulating protein-protein interactions. (2) The expression of scaffold proteins can strongly affect the extent of posttranslational modification in cancer tissues. (3) The expression level of scaffold proteins may be associated with cancer prognosis, and they may be therapeutic targets. Since the expression of many proteins is tissue-specific, it is unclear whether synbindin and ArhGAP30 are also important in the posttranslational modification of ERK and p53 in other cancers and tissue types. It is worthwhile considering that there may be other scaffold proteins for the p53 and ERK pathways in GI tumors.

We are now extending our research by constructing a gene knockout model of synbindin. In this new system, the roles of synbindin in cancer development can be determined at higher resolution. The effects of synbindin disruption on gene expression and protein posttranslational modifications will be examined by microarray and mass spectrometry. Through this work, we hope to understand the roles of scaffold proteins in a more comprehensive manner.

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Disclosure Statement

All authors declare that they have no conflict of interest regarding this work.

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