The role of ERK5 signaling in colorectal cancer

Koji Taniguchi1,2,3, Petrus R. de Jong4

1Laboratory of Gene Regulation and Signal Transduction, Departments of Pharmacology and Pathology, University of California, San Diego, La Jolla, California 92093, USA
2Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan
3Department of Microbiology and Immunology, Keio University School of Medicine, Tokyo 160-8582, Japan
4Sanford Burnham Prebys Medical Discovery Institute, NCI-Designated Cancer Center, La Jolla, CA 92037, USA

Correspondence: Petrus R. de Jong
E-mail: r.dejong.usa@gmail.com
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The mitogen-activated protein kinase (MAPK) family includes ERK1/2, p38, JNK and ERK5. The role of MAPKs in colorectal cancer (CRC) is well-established, in particular ERK1/2. Abnormal activation of receptor tyrosine kinases or gain-of-function mutations in critical upstream transducers, including KRAS and BRAF, are responsible for MAPK-mediated tumor progression in CRC. Compared to ERK1/2, the role of ERK5 in CRC development has been underrated. Here we discuss recent evidence for the involvement of ERK5 signaling in the development and progression of CRC, as well as its putative role in resistance to targeted therapy.

Keywords: Colorectal cancer; ERK5; KRAS; Mitogen-activated protein kinase; Targeted therapy

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Colorectal cancer and MAPK signaling

Colorectal cancer (CRC) will be diagnosed in 134,000 persons in the United States in 2016, with an estimated 50,000 people dying from this disease [1]. The adenoma-to-carcinoma sequence that underlies CRC progression constitutes a complex interplay of genetic mutations, epigenetic changes and loss of chromosomal integrity [2]. The Cancer Genome Atlas (TCGA) project confirmed an important etiological role for loss-of-function mutations in adenomatous polyposis coli (APC), TP53 and SMAD4, in addition to gain-of-function mutations in PIK3CA, BRAF and KRAS. These mutations are representative of the main dysregulated pathways in CRC, including the WNT/β-catenin, transforming growth factor (TGF)-β, p53, phosphoinositide 3-kinase (PI3K) and receptor tyrosine kinase (RTK)-RAS pathways [3]. The latter entails the signaling axis that starts at the plasma membrane with ligand-induced activation of RTKs or by gain-of-function mutation of the receptor, followed by the activation of RAS, RAF and the three-tiered mitogen-activated protein kinase (MAPK) pathway (Figure 1A) [4]. Within this signaling axis, activating mutations of KRAS, NRAS or BRAF are found in 55% of CRC patients of the non-hypermutated type [3]. Recently, four consensus molecular subtypes (CMSs) of CRC were established, which confirmed general activation of RTK and MAPK signaling pathways in the CMS1 and CMS3 subtypes [5]. Introduction of the oncogenic KRASG12D mutation in intestinal organoids allows for reconstitution of additional inactivating mutations in tumor suppressors such
as APC, TP53, and SMAD4, resulting in a progressively more invasive carcinoma phenotype [6]. Thus, pharmacological inhibition of the RTK-RAS-MAPK pathway is a rational approach for targeted therapy in major subgroups of CRC patients. One major obstacle in the clinical management of CRC has been resistance to therapy, demonstrated by the 5% response rate to BRAF inhibitors in CRC patients, compared to a 50-80% response rate observed in melanoma patients [7]. Complex signaling pathway rewiring and acquired mutations in the RTK, KRAS and MAPK pathways in CRC cells are responsible for this resistance [8].

**Targeting the MEK/ERK linchpin**

The ERK1/2 MAPK and PI3K/AKT signaling modules are activated downstream of activated RTK, oncogenic RAS, or constitutively active RAF proteins in CRC (Figure 1B) [9]. Within the MAPK family, the MEK1/2-ERK1/2 signaling module appears to serve as a critical linchpin for the transduction of mitogenic signals through RAS and RAF, while the p38 and JNK modules are mainly involved in stress and inflammatory responses [10]. Upregulation of p-ERK1/2 levels has been observed in tumor lysates and tissues from CRC patients, particularly in subjects with oncogenic BRAF mutations [11]. This has resulted in the prolific development of MEK1/2 inhibitors (MEKi), such as PD0325901 (Pfizer), BAY86-9766 (Bayer), trametinib (GSK1120212; GlaxoSmithKline), selumetinib (AZD6244; AstraZeneca), pimasertib (AS703026; Merck), MEK162 (Novartis) and GDC-0973 (Genentech). However, the application of MEKi as single agents has generally not resulted in clinical efficacy, partially due to feedback activation of downstream pathways or activation of parallel signaling modules, such as the PI3K-AKT-mTOR pathway [4]. This phenomenon can be explained by the strong negative regulation ERK1/2 exerts on upstream kinases, mediated by inhibitory phosphorylation of EGFR, SOS, RAF and MEK1 [12]. Thus, upon pharmacological inhibition of the MEK1/2-ERK1/2 module, loss of negative regulation of upstream transducers results in strong activation of pathways downstream of RTKs and mutated RAS or RAF, reactivating mitogenic transcription [12]. Dual targeting of MEK1/2 and PI3K pathways is now evaluated in Phase I and Phase II trials in CRC. While the results of Phase III are awaited, it is worth to note that safety
and tolerability issues (e.g. skin rash, liver and gastrointestinal symptoms) have been frequently reported [13].

ERK5: new player in colorectal cancer?

While these combinatorial approaches are in advanced stages of clinical development, the role of another putative resistance pathway has been underappreciated. We recently reported that the combination of MEKi with a small molecule inhibitor of ERK5 was more efficacious in the suppression of human CRC cell line growth in vitro [14], suggesting compensatory activation of ERK5. Big MAP Kinase 1 (BMK1)/ERK5 is a ‘conventional’ member of the MAPK family [15], activated by upstream MEK5 [16], and MEKK2/3 [17] (Figure 1B), and plays an important role in cardiovascular and neuronal development [18]. Activators of the ERK5 pathway include serum stimulation, oxidative stress, laminar shear-stress, hypoxia, hyperosmolarity and RTK ligands such as epidermal growth factor (EGF), nerve growth factor (NGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). ERK5 activation is also reported to drive cell cycling by promoting G1-to-S-phase transition, and induces the expression of pro-survival genes [19]. Thus, the ERK5 MAPK signaling module transduces both pro-proliferative signals and extracellular stress, which functionally places this pathway between the ERK1/2, and p38/JNK MAPK pathways, respectively.

The physiological role of ERK5 in the intestines remains to be charted [20], but its role in cancer has become apparent in recent years. We found that p-ERK5 levels are upregulated upon genetic deletion of Erk1/2 in murine intestinal epithelial cells, or upon pharmacological inhibition of MEK1/2 in human CRC cell lines [14]. To study the role of ERK1/2 signaling in intestinal homeostasis, we crossed Erk1f−/f (global knockout) mice with Erk2fl/fl;Vil-CreERT2 mice, resulting in tamoxifen-inducible deletion of Erk1/2 in intestinal epithelium. These mice showed overt defects in absorptive and secretory cell differentiation and maturation, as well as abnormal epithelial cell migration. Surprisingly, we observed paradoxical hyperproliferation in the intestinal epithelium after ERK1/2 knockout, which was associated with enhanced levels of p-ERK5. We subsequently demonstrated that the combination treatment of MEK1/2 inhibitors with an ERK5 inhibitor more efficaciously blocked CRC cell growth in vitro. These results were recapitulated using siRNA-mediated knockdown of ERK1/2 and/or ERK5 [14].

ERK5 signaling has been associated with pro-proliferative effects in breast, prostate, lung and pancreatic cancer, hepatocellular carcinoma, osteosarcoma, hematological malignancies, as well as CRC [21]. ERK5 activity also protects against tumor necrosis factor (TNF)-α or Fas-mediated apoptosis, and against pro-apoptotic stimuli such as osmotic stress or exposure to cytotoxic agents. Furthermore, ERK5 signaling promotes cell motility, invasion and metastasis, and is a crucial inducer of angiogenesis [22]. These effects are mediated by upstream activation via KRAS, NRAS or BRAF [21], or through ligand-induced activation of RTKs or non-receptor signal transducers such as Src [22]. Interestingly, ERK5 has also been described to be activated downstream of PI3K [23] (Figure 1B). In CRC, a unique mechanism involving miRNA-143/145 may also play a role in the regulation of ERK5 activity. Both miRNA-143/145 act as negative regulators of ERK5 translation, and it has been reported that levels of these microRNAs are dramatically reduced in primary CRC samples and CRC cell lines [24]. The forced overexpression of miRNA-143/145 also prevented tumorigenesis in the Apcmin mouse model, coinciding with reduced ERK5 expression in tumor lysates [25]. Another proposed mechanism for the regulation of ERK5 activity involves putative tumor suppressor SATB2 (special AT-rich sequence binding protein 2). SATB2 was found to suppress ERK5 phosphorylation, coinciding with reduced colony formation, migration, and invasion of CRC cell lines [26]. The regulation of ERK5 signaling by SATB2 may act directly on ERK5, or on its upstream activator MEK5 [27]. There is also ample evidence for a correlation between ERK5 signaling and CRC progression in primary human samples. Immunohistochemical analysis of CRC samples from 335 patients and 80 control tissues showed increasing levels of p-MEK5 in patients with higher TNM stages [28]. This study also showed a significantly lower 5-year overall survival rate of p-MEK5high vs. p-MEK5normal patients [24]. A recent study with 323 CRC patients found increased expression levels of MEK5 and ERK5 compared with control tissues, while expression of ERK5 also correlated with tumor progression. The levels of MEK5 and ERK5 expression were correlated with promotion of cell cycle progression and enhanced cell motility [29]. In a follow-up paper, it was demonstrated that high ERK5 mRNA expression correlated with a worse survival of CRC patients from the TCGA database (151 samples) and GEO metabase (482 samples) [30]. Furthermore, treatment of CRC cell lines with the antimetabolite, 5-fluorouracil (5-FU), resulted in abrogation of the KRAS-MEK5-ERK5 signaling axis. Genetic or pharmacological targeting of MEK5-ERK5 signaling enhanced the cytotoxic effects of 5-FU in vitro. Importantly, in vivo treatment of HCT116 xenografts with a combination of the ERK5 inhibitor (XMD8-92) with 5-FU showed significantly more tumor growth inhibition than either treatment alone [30]. Together, these results and our data...
suggest that ERK5 signaling exerts pro-tumorigenic effects in CRC development and is a potential drug target for this disease.

The pleiotropic effects of ERK5 signaling in different phases of oncogenesis has led to the development of a variety of small molecular inhibitors of either MEK5 (e.g. BIX02188, BIX02189, Compound 6) or ERK5 (XMD8-92, XMD17-109) [31]. Despite their potency, these compounds display a variety of off-target effects and more specific compounds have been developed [32, 33]. These established and novel ERK5 inhibitors may be useful for CRC treatment, as we found that this pathway is upregulated in intestinal epithelial cells upon abrogation of ERK1/2 signaling [14]. Validation of the results obtained with older generations of MEK5/ERK5 inhibitors should therefore be performed using these novel compounds.

Notably, our results are at odds with a recent publication by Lochhead et al. who showed that inhibition of ERK5 in cancer cells with oncogenic KRAS or BRAF mutations did not inhibit cell proliferation, even in combination with MEK1/2 inhibitors [34]. Despite some differences, we concur with their findings that CRC cell lines with KRAS or BRAF mutations seem to preferentially activate the ERK1/2 pathway. This was also observed in intestinal organoids with reconstituted loss of APC and expression of oncogenic KRAS [14]. In fact, most CRC cell lines tested showed very low or undetectable levels of p-ERK5. Pharmacological inhibition of MEK1/2 induced markedly increased levels of p-ERK5 in these cell lines [14]. Lochhead et al. reported that they did not find suppression of cell proliferation in CRC cell lines HCT116 (KRAS mutant) or HT-29 (BRAF mutant) by pharmacological inhibition of the MEK5/ERK5 axis. To this end, the authors employed the selective MEK5 inhibitor BIX02189 and measured [3H]thymidine incorporation (i.e., DNA synthesis) as a surrogate marker for cell proliferation, while they measured inhibition of the targeted pathway with a MEF2D luciferase reporter construct for pharmacodynamic monitoring. Inhibition of MEF2D reporter activity occurred at low micromolar concentrations of BIX02189, while inhibition of cell proliferation was not observed with doses below 10 µM [34]. In our experiments, we used ERK5 inhibitor XMD8-92 and assessed cell proliferation (i.e., cell density) with a tetrazolium-based metabolic assay [14]. The differences in the type of assay and the compound used to target ERK5 activity may have resulted in different outcomes in these experiments. Since off-target effects can always play a role in pharmacological treatments, the use of siRNA at low nanomolar concentrations is necessary to validate the biological significance of the targeted pathway. The siRNA-mediated knockdown of signal transducers may also reveal kinase-independent roles of these enzymes. We found that siRNA-mediated knockdown of ERK5 resulted in 30-50% reduced cell proliferation in all four different CRC cell lines tested, including HCT116 [14]. In contrast, Lochhead et al. reported that knockdown of ERK5 in HCT116 cells did not affect cell proliferation, as measured by [3H]thymidine incorporation. To address the importance of ERK5 signaling in CRC initiation and development, we propose that genetic targeting of ERK5 by using shRNA or CRISPR with patient-derived xenografts (PDXs) might shed more light on this topic. This would reveal the role of cell-autonomous ERK5 signaling in CRC development. In parallel, treatment of established CRC-PDXs with novel classes of ERK5 inhibitors would be able to test the translational potential of ERK5 inhibition. At the same time, this pharmacological in vivo approach would evaluate the effects of ERK5 inhibition in the tumor microenvironment in CRC.

Concluding remarks

There is ample evidence from both experimental models and human CRC samples for an association between upregulation of ERK5 activity in the pathogenesis and progression of this disease. However, the causative role of ERK5 signaling in CRC remains controversial. Conclusive experiments with in vivo PDX models are currently lacking to establish unambiguous causality with regard to ERK5 activity and CRC development. Such experiments may lead the way for novel drug combinations to potentially overcome the resistance to MEKi in CRC.

Conflicting interests

The authors have declared that no conflict of interests exist.

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Abbreviations
5-FU: 5-fluorouracil; BMK1: Big MAP kinase 1; CMS: Consensus molecular subtype; CRC: Colorectal cancer; EGF: Epidermal growth factor; FGF, Fibroblast growth factor; GPCR: G-protein coupled receptor; MAPK: Mitogen-activated protein kinase; MEK: MEK inhibitor; NGF: Nerve growth factor; PDGF: Platelet-derived growth factor; PDX: Patient-derived xenograft; PI3K: Phosphoinositide 3-kinase; RTK: Receptor tyrosine kinase; TGF-α: Tumor necrosis factor alpha; VEGF: Vascular endothelial growth factor.

References

