

Potential of casein kinase I in digestive cancer screening

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INTRODUCTION

Casein kinase I (CKI) family is a group of monomeric serine/threonine protein kinases that are ubiquitously found in all eukaryotic organisms. Seven members (encoded by distinct genes) have been identified so far in mammals: α , β , γ_1 , γ_2 , γ_3 , δ , and ϵ . Other eukaryotes seem to have more CKI proteins, for example, *Drosophila* has 8 and *Caenorhabditis elegans* has 8^{7[1,2]}.

CKI family members are involved in many diverse and important cellular functions, such as regulation of membrane transport, cell division, DNA repair, circadian rhythms, apoptosis and cellular differentiation^[3,4]. Mutations and deregulation of CKI expression and activity has been linked to various diseases including neurodegenerative disorders such as Alzheimer's and Parkinson's disease, sleeping disorders and cancer. Recent findings in some digestive cancers provide additional evidence about their critical roles in carcinogenesis and their potential utilization in cancer prevention and therapy.

PREVALENCE OF DIGESTIVE CANCERS IN THE UNITED STATES

According to the data from the American Cancer Society^[5], about 19% of cancer incidence in the United States takes place in the digestive system. Among various digestive cancers (i.e. esophageal cancer, gastric cancer, intestinal cancer, colorectal cancer, liver cancer, gallbladder cancer, pancreatic cancer, etc), colorectal cancer represents > 50% and therefore is the most prevalent one. Despite the high numbers, both incidence and mortality rates for colorectal cancer have been declining steadily since 1975. These declines are mostly thanks to the powerful screening, which makes it possible to remove polyps before they become malignant, or to remove cancerous cells before they develop metastases^[5]. Pancreatic cancer

Abstract

Casein kinase I is a group of ubiquitous Serine/Threonine kinases that have been implicated in both normal cellular functions and several pathological conditions including Alzheimer's disease and cancer. Recent findings in colon and pancreatic cancer have brought tremendous attention to these molecules as potential therapeutic targets in treatment of digestive cancers. In this review, we summarize up to date what is known about this family of kinases and their involvement in carcinogenesis and other pathological conditions. Our emphasis is on their implications in digestive cancers and their potential for cancer screening and therapy.

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Key words: Casein kinase I; Colon cancer; Pancreatic cancer; Gastric cancer; Biomarkers

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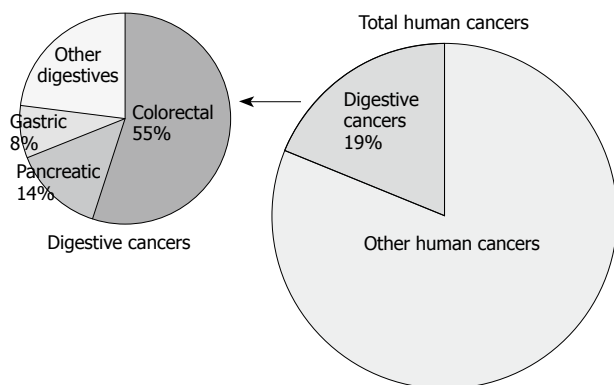


Figure 1 Prevalence and distribution of digestive cancers in USA population. Digestive cancers make up 19% of all cancers in the United States. Colorectal cancer, pancreatic cancer and gastric cancer are the three most common and/or most deadly among them.

is another most deadly digestive cancer, with 34 290 expected deaths in the United States alone for 2008. According to the annual report from the American Cancer Society, only 5% of patients have 5-year survival rate^[5]. This is largely due to an insufficiency of early diagnoses and a high resistance to chemotherapy. The comparison between these two cancers in their survival rates signifies the importance of identifying biomarkers that can help in the screening process as well as in the development of therapeutic strategies. Recent findings on CKI ϵ and CKI δ in colon, pancreatic and gastric cancer, which together comprise the three most common digestive cancers in the United States (Figure 1), suggest that the CKI members may hold promise both as screening markers and as therapeutic targets.

BIOCHEMISTRY AND REGULATION OF CKI

The CKI family members have the highest homology in their kinase domains (53%-98% identical) and differ from most other protein kinases by the presence of the sequence Serine-Isoleucine-Asparagine instead of Alanine-Proline-Glutamate in kinase domain VIII^[6]. Outside their kinase domains, CKI α and CKI β are 76% identical, while the CKI γ isoforms are approximate 50% identical in their C-terminal tails. CKI δ and CKI ϵ have long C-terminal extensions with approximate 53% identity.

The CKI kinases appear to have similar substrate specificity *in vitro*^[7], and substrate selection is thought to be regulated *in vivo* via subcellular localization and docking sites in specific substrates. One consensus phosphorylation site is p-Serine/Threonine-X-X-Serine/Threonine, where X refers to any amino acid^[8,9]. This CKI consensus site requires priming by another kinase. CKI also phosphorylates an unprimed site, which optimally contains a cluster of acidic amino acids N-terminal to the target including an acidic residue at *n*-3 and a hydrophobic region C-terminal to the target^[7,10]. A single acidic residue in the *n*-3 position is not sufficient for CKI phosphorylation. In contrast, in several important

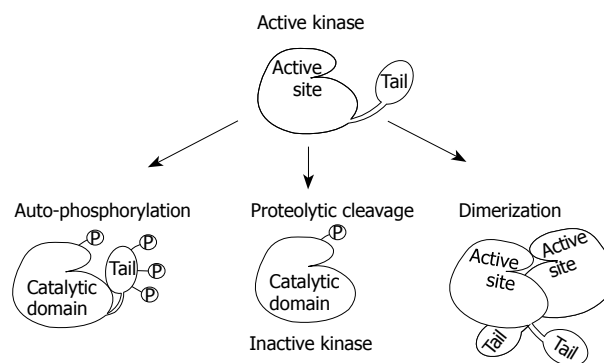


Figure 2 Models of CKI ϵ/δ regulation. CKI ϵ/δ auto-phosphorylation at their C-terminal tail inhibits their kinase activity through a conformational change that places the tail over the active site, therefore blocking it from potential substrates. Dephosphorylation by phosphatases is the most accepted mechanism for their activation *in vivo*. In addition, removal of the tail by proteolytic cleavage or mutations has been used *in vitro* to activate these kinases; however, actual removal of the tail *in vivo* may have additional effects on proper function. Finally, CKI ϵ/δ dimerization at the active site could inactivate these kinases by physically blocking substrates from entering this region.

targets, such as β -catenin^[11,12], CKI does not require *n*-3 priming, albeit less efficiently than the optimal sites^[13].

In general, CKI kinases are constitutively active. The long C-terminal extensions of CKI δ and CKI ϵ , however, are autophosphorylated, and this phosphorylation inhibits the activity of the kinase domain, although *in vivo* phosphatases keep it constitutively active in many cases^[14]. Removal of inhibition by proteolytic cleavage or dephosphorylation of the tail is therefore required to fully activate these kinases^[15]. Studies of regulation of CKI ϵ and CKI δ in the canonical Wnt signaling pathway indicate that dephosphorylation, rather than proteolytic cleavage, is most likely the way *in vivo* for their activation^[16,17]. This was evidenced by the fact that the activity of wild-type (WT) CKI ϵ , but not of the constitutively active mutant form (MM2) in which all inhibitory phosphorylation sites are altered, was increased 5-fold upon Wnt ligand stimulation^[16]. On the contrary, removal of the inhibitory tail actually impaired its proper function *in vivo* due to failure to form a secondary axis, even though its activity was intact *in vitro*^[17]. Consistent with dephosphorylation as a preferential mode of activation *in vivo*, Takano *et al.*^[18] have identified a naturally occurring CKI ϵ variant (S408N), in which one of the putative auto-inhibitory phosphorylation sites is mutated, leading to increased kinase activity. This was shown to have a protective effect against familial advanced sleep phase syndrome due to its ability to phosphorylate clock proteins, resulting in an elongated circadian rhythm^[18].

Even though dephosphorylation has emerged as the predominant mode of activation for CKI ϵ and CKI δ , structural analysis of CKI δ may have revealed an additional mechanism through which CKI ϵ/δ could be regulated *in vivo* (Figure 2). Since Rivers *et al.*^[14] showed that, at the physiological level, constant phosphatase activity appears to keep CKI ϵ and CKI δ in a hypo-phosphorylated state most of the time, which would presumably keep them active, this additional

mode of negative regulation could help minimize their activity even in a dephosphorylated state. Based on X-ray crystallography of CKI δ Δ 317 and molecular replacement from the published crystal structure of the *Schizosaccharomyces pombe* homolog Cki1 (Cki1 Δ 298)^[19,20], Longenecker *et al.*^[19] compared the 3-dimensional (3D) structure of the core protein against its amino acid sequence. Through this more accurate comparison they were able to notice a broad surface across the catalytic domain, which is highly conserved between CKI ϵ and CKI δ . Conservation in this region suggests a possible dimerization site, which could block their activations by physically covering the active site. Therefore, dimerization has also been proposed as an alternative mechanism of negative regulation for CKI ϵ and CKI δ ^[4]. In support of this mechanism, preliminary structural analysis indicates that the CKI ϵ point mutations previously identified in tumor tissue from six breast cancer patients with ductal carcinoma in situ (DCIS) are clustered within this putative dimerization domain (internal communication E. Brumovska and L. Trantirek)^[21]. Moreover, based on their position within the 3D structure of the protein, it appears that accumulation of at least three of these point mutations in the same protein would be sufficient to disrupt dimerization (internal communication E. Brumovska and L. Trantirek). These findings could both explain why five of those six patients had multiple mutations in that region, and also suggest that inhibition of dimerization may contribute to tumorigenesis in patients with DCIS through increased overall kinase activity, providing support for dimerization as an additional mechanism of regulation *in vivo*.

GENERAL PATHOLOGY OF CKI

CKI members (α , γ , δ , and ϵ) have been implicated in several pathological conditions, including sleep disorders^[18,22], Alzheimer's Disease (AD)^[23] and cancers^[4,21,24,25], particularly through identification of specific point mutations and/or changes in expression. Among them, CKI ϵ and δ emerge as the most critical and positive players in these diseases. The recent findings on their active involvement in hyperphosphorylation of tau protein and accumulation of toxic peptide Amyloid β , two primary characteristics of AD^[26,27], further support this notion.

Similarly, the connection between CKI ϵ / δ and cancers has been strengthened through identification of their targets in promotion of cell proliferation and/or inhibition of apoptosis, both of which can contribute to tumorigenesis. For instance, CKI ϵ , δ as well as γ are found to be positive regulators of the canonical Wnt signaling pathway, which promotes cell proliferation through activation of proto-oncogenes like c-myc and cyclin D1, and which is also up-regulated in several malignancies, particularly colon cancer^[4,28,29]. Moreover, our recent study showed that CKI ϵ is a positive regulator of the Akt pathway^[30], which is also activated in several cancers, including colon cancer^[31] and breast cancer^[32]. In addition, Akt signaling was also shown to contribute to

resistance to a wide range of chemo-therapeutic drugs, making it a very attractive target for cancer treatment research^[33-37]. Following up on the suggestion that CKI ϵ may contribute to breast cancer based on both changes in protein expression and accumulation of CKI ϵ point-mutations in tumor samples from DCIS patients^[21], we found that CKI ϵ up-regulates Akt in breast cancer cell lines in an independent manner of Phosphate and tensin homologue deleted on chromosome ten (PTEN)^[30], the major inhibitor of the Akt pathway^[38,39]. Moreover, inhibition with the CKI ϵ / δ inhibitor IC261 was able to block phosphorylation of both Akt and its downstream target Glycogen Synthase Kinase 3 β (GSK3 β), suggesting that CKI ϵ function is required for Akt activity^[30]. A similar effect with IC261 was also seen in Hs578T breast cancer cells, in which the Akt pathway is normally up-regulated regardless PTEN, suggesting that, at least in breast cancer, CKI ϵ / δ may contribute to a more tumorigenic environment through PTEN-independent Akt activation^[30].

The connection between CKI ϵ / δ and cancer has also been strengthened by their role in down-regulating apoptosis, particularly Fas-mediated apoptosis^[4]. Stimulation by Fas ligand (CD95/APO-1) or agonistic antibodies leads to caspase 8 activation, which can either result in caspase-3 activation or in mitochondria-mediated cell death signaling through Bid cleavage by caspase-8. Desagher *et al.*^[40] showed that over-expression of CKI ϵ stabilizes Bid, resulting in a lower number of apoptotic cells, while inhibition of CKI had the opposite effect. In addition, CKI ϵ / δ have also been suggested to contribute to apoptosis by playing a role in the switch mechanism between canonical and non-canonical Wnt signaling, where they may promote canonical Wnt signaling at the expense of JNK-mediated apoptosis^[41,42]. However, new evidence showing that CKI ϵ and CKI δ can be activated not only by canonical Wnt3a^[16] but also by non-canonical Wnt5a^[43] has raised questions about the latter hypothesis.

CKI IN COLON CANCER

Last year, two independent studies provided strong evidence that CKI ϵ / δ play a role in early stages of tumorigenesis predisposing to colon cancer. Umar *et al.*^[44] used the *Citrobacter rodentium*-induced transmissible murine colonic hyperplasia (TMCH) model, which allows studying changes during these early stages, and showed that CKI ϵ protein levels as well as its activity are increased by 2- to 3-fold after 6 and 12 d of infection, suggesting an association between CKI ϵ up-regulation and colon cancer development. Based on an increase in both Wnt target genes and S45-phosphorylated β -catenin, Umar *et al.*^[44] proposed that CKI ϵ up-regulation contributes to colonic hyperplasia through activation of the Wnt pathway, as well as phosphorylation of β -catenin at S45. The notion that CKI ϵ contributes to colon cancer through up-regulation of canonical Wnt signaling is not surprising, given that the pathway itself is activated in a majority of colon cancers, often through inducing mutations in key components

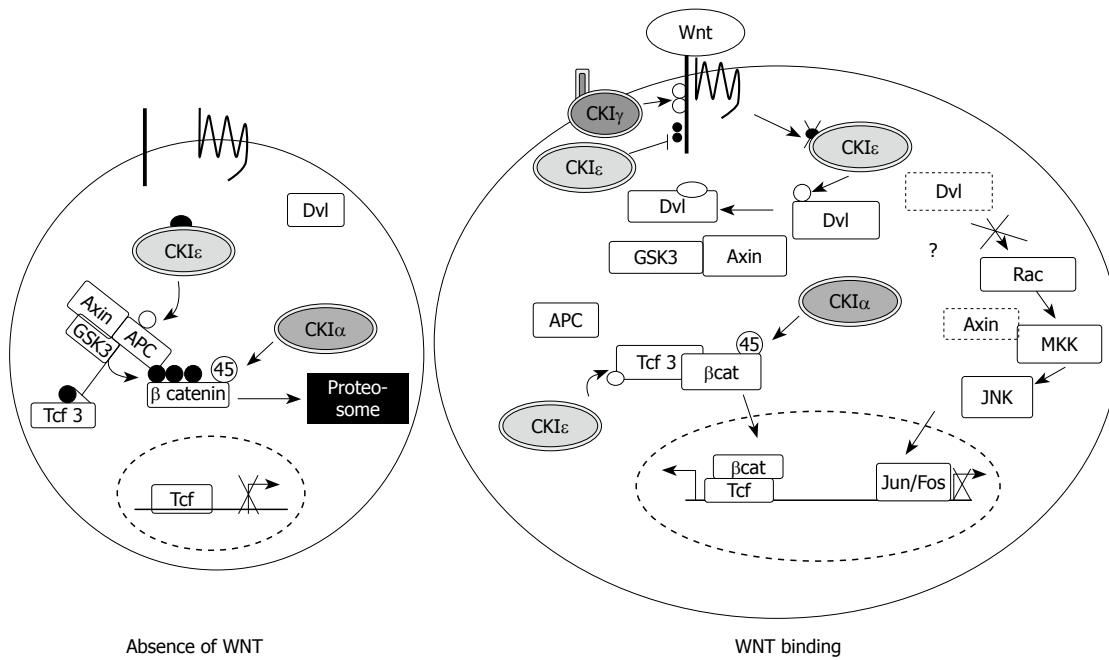


Figure 3 CKI members in canonical Wnt signaling. In the absence of Wnt ligand, β -catenin is phosphorylated by GSK3 β after priming at S45 by CKI α and becomes degraded. In this state, CKI ϵ phosphorylates APC and stabilizes the β -catenin degradation complex. Upon Wnt ligand binding to its receptor, CKI ϵ is fully activated through dephosphorylation and contributes to the disassembly of the β -catenin degradation complex by phosphorylating Dishevelled (Dvl) and facilitating its interaction with Axin and GSK3 β . Therefore, even though CKI α still primes β -catenin at S45, without additional phosphorylation by GSK3 β , β -catenin becomes stabilized and accumulates in the cytoplasm. CKI ϵ also promotes β -catenin stability by phosphorylating Tcf3 which competes against GSK3 β for β -catenin. Stabilized β -catenin then translocates into the nucleus, where it contributes to activation of Wnt target genes. CKI ϵ also negatively regulates the Wnt pathway by phosphorylating the LRP-co-receptor, which is positively phosphorylated by CKI γ . Since epistasis analysis showed that CKI ϵ -mediated phosphorylation of LRP was downstream of its phosphorylation of Dvl, this is most likely a negative feedback loop to quickly deactivate the pathway after propagation of the signal.

such as Adenomatous polyposis coli (APC) and β -catenin^[45]. However, their interpretation of increased β -catenin phosphorylation at S45 is questionable. First, the reported increase in β -catenin phosphorylation at S45 in TMCH is actually only subtle after normalizing to total β -catenin levels and therefore more likely due to “a proportional increase in overall β -catenin abundance”^[44]. Moreover, even though two reports originally attributed priming phosphorylation of β -catenin at S45 to CKI ϵ ^[11,46], Liu *et al*^[12] convincingly showed that it is CKI α , not CKI ϵ , that phosphorylates β -catenin at S45 *in vivo*. They further demonstrated that RNAi against CKI α rather than CKI ϵ , inhibited S45 phosphorylation of β -catenin in 293T cells^[12], making CKI α the most likely CKI member responsible for priming phosphorylation of β -catenin. In the absence of Wnt signaling, GSK3 β can associate with the β -catenin degradation complex and further phosphorylate β -catenin. This additional phosphorylation targets β -catenin for degradation, therefore preventing it from activating Wnt-specific target genes. After Wnt stimulation, which results in full activation of CKI ϵ ^[16], dephosphorylated CKI ϵ disrupts the β -catenin degradation complex and prevents GSK3 β from further phosphorylating β -catenin. Lack of additional GSK3 β -mediated phosphorylation will result in stabilization and accumulation of β -catenin in the cytoplasm, which is required for its subsequent translocation to the nucleus to activate Wnt target genes (Figure 3). Therefore, the reported phosphorylation of β -catenin at S45 seen in TMCH is more consistent with CKI α phosphorylating

β -catenin at S45, but still consistent with the authors’ conclusions that CKI ϵ up-regulation contributes to colonic hyperplasia through up-regulation of the Wnt pathway and subsequent increased transcription of Wnt target genes.

In a separate study based on a clinical family pedigree analysis of colon cancer^[47], one patient was diagnosed at age 46 with several large polyps (5 mm or more), including one > 20 mm. This was approximately 10 years earlier compared to his siblings, suggesting a predisposition to early on-set of colon cancer. Screening identified a point mutation in a highly conserved region of CKI δ (R324H), which was correlated with the more severe condition of the patient through both *in vivo* phenotypic analysis and cell culture transformation potential^[47]. This case further indicates that CKI ϵ/δ contribute to early onset of colon cancer. More specifically, *in vivo* analysis of the R324H mutant in *Xenopus* revealed the axis duplication phenotype indicative of increased canonical Wnt signaling as well as an additional gastrulation phenotype that significantly contributed to the aggressiveness of CKI δ -related polyps^[47]. Taken together, these results suggest that canonical Wnt signaling is not the only pathway that activates CKI ϵ and CKI δ in colon cancer. The non-canonical Wnt/PCP and Wnt/Ca²⁺ pathways, which are involved in gastrulation, are also regulated by CKI ϵ and δ ^[48-50]. Consequently, Tsai *et al*^[47] tested known downstream components of these pathways, namely JNK, RhoA and NF-AT. However, they were not able to detect any changes in either of these pathways when using

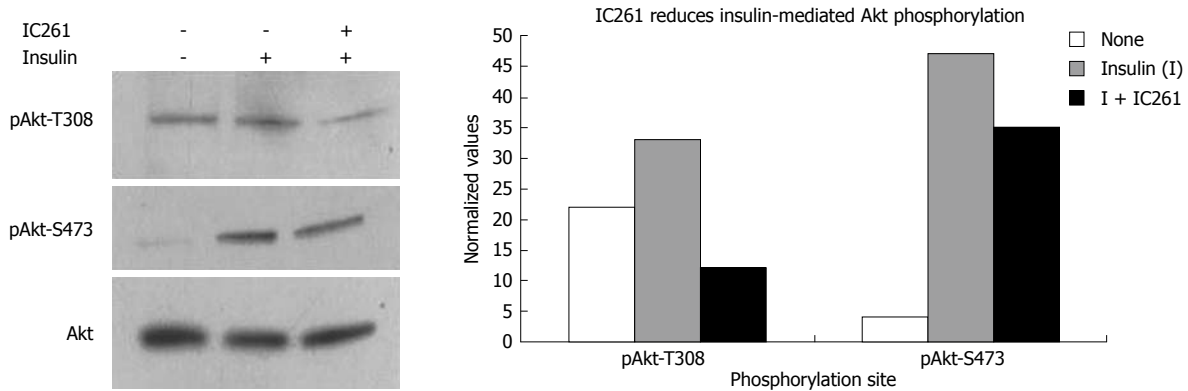


Figure 4 Pharmacological inhibition of CKI ϵ/δ reduces insulin-induced Akt phosphorylation in PDAC cells. Cells were serum starved overnight and then incubated in 10 $\mu\text{g}/\text{mL}$ insulin-containing media for 20 min in the presence or absence of the CKI ϵ/δ inhibitor IC261 (40 $\mu\text{mol}/\text{L}$). Akt activation was measured by phosphorylation at T308 and S473. Bands were quantified using Image J software and values were normalized against total Akt.

the R324H mutant compared to WT, suggesting that the Wnt/PCP and Wnt/ Ca^{2+} pathways are not likely affected by the CKI δ mutation.

CKI IN PANCREATIC CANCER

Earlier this year, CKI ϵ and δ were also found in association with another gastrointestinal cancer, pancreatic ductal adenocarcinoma (PDAC), which is the most common type of pancreatic cancer^[24]. Brockschmidt *et al*^[24] showed that CKI ϵ and δ are strongly expressed in both PDAC cell lines and actual tumors tissues. Importantly, inhibition of CKI ϵ/δ by IC261 could effectively re-sensitize cells to apoptosis *in vitro* and reduce tumor growth *in vivo*. More specifically, prolonged treatment of low doses of IC261 in combination with the agonistic anti-Fas antibody CH11 increased cell death by 50% in pancreatic cancer Panc89 cells, which are normally resistant to Fas-mediated apoptosis. This was also correlated with increased Fas-mediated cleavage of caspase-8 and Bid, as well as activation of caspase-3 as measured by cleavage of its substrate PARP^[24]. Moreover, IC261 treatment of SCID mice, which had been previously implanted with PancTu-1 cancer cells to develop pancreatic tumors, lead to a significant reduction in tumor size^[24]. While IC261 was as effective as gemcitabine, a drug currently used to treat pancreatic cancer, combined treatment did not result in any additional benefit^[24]. Real-time PCR analysis of tumor tissues treated with either drug revealed changes in expression of various genes^[24]. Most notably, CKI ϵ and CKI δ were decreased with both drugs, even though the change was greater with IC261 for CKI δ . Moreover, while most genes followed similar patterns between the two treatments, FASLG was strongly increased with IC261 while it was decreased with gemcitabine. Taken together, their data strongly suggests that CKI ϵ/δ promote PDAC through their known anti-apoptotic role in Fas-mediated regulation. However, analysis of their tumor samples revealed that the reduction in tumor size was associated not only with an increase in apoptosis but also with a decrease in cell proliferation. This suggests that CKI ϵ/δ contribute to tumorigenesis in PDAC also by promoting

cell proliferation. Since we recently showed that CKI ϵ can up-regulate the Akt^[30], which is also up-regulated in PDAC^[31,51,52], we tested whether IC261 can inhibit Akt signaling in PDAC cells as well. Our preliminary results show a decrease in Akt phosphorylation at both of its activating sites (Figure 4), indicating that CKI ϵ -mediated Akt up-regulation is not only restricted to breast cancer. However, more studies would be required to determine how much the decrease in Akt signaling is contributing to the decrease in cell proliferation seen by Brockschmidt *et al*^[24] in SCID mice treated with IC261.

CKI IN GASTRIC CANCER

In addition to their role in colon cancer and pancreatic cancer, preliminary data from different research labs strongly suggest that CKI ϵ and CKI δ may also be involved in gastric cancer, the next most common gastrointestinal cancer in the United States after pancreatic cancer^[5]. Recently, von Blume *et al*^[53] showed that gastrin, a major stimulator of gastric acid secretion, increases CKI ϵ/δ activity. This ultimately results in de-repression of HDAC7-regulated genes such as *nur77*. Nur77 has been shown as a potent oncogenic survival factor in several types of cancer, including lung, prostate, breast and colon cancer^[54]. Therefore, while the focus of the study was more at the mechanistic level of signal transduction, these results indicate that CKI ϵ/δ could play a role in gastric cancer as well. Further evidence in support of this comes from two separate studies on H-prune and its binding partner nm23-H1, which are key inducers of cell motility in breast cancer. Oue *et al*^[55] recently showed that increased expression of H-prune and nm23-H1 was strongly correlated with tumor progression and poor survival in gastric cancer, while Garzia *et al*^[56] provided mechanistic evidence on the interaction between H-prune and nm23-H1, implicating CKI ϵ/δ as key mediators of this interaction. Out of the 143 gastric cancer cases analyzed by Oue *et al*^[55], 87% were positive for nm23-H1, which was expressed in 98% of H-prune positive gastric cancer cases. Many of the cases in which H-prune and nm23-H1 were co-expressed showed more advanced T

grade, N grade and tumor stage, and H-prune positive patients had significantly worse survival rate than H-prune negative patients, clearly supporting an important role for H-prune (and nm23-H1) in gastric cancer progression. Since co-expression of H-prune and nm23-H1 resulted in more aggressive tumor stages, the formation of H-prune/nm23-H1 complexes seems to play a critical role in their adverse effects, and disrupting those complexes could be potentially therapeutically advantageous. It is in this context that CKI ϵ/δ could be again promising drug targets, since they phosphorylate a critical region within the H-prune binding region on nm23-H1 and their inhibition with IC261 (or competitive binding with phosphorylated nm23-H1 peptide) disrupted complex formation, resulting in inhibited cell motility^[56].

CONCLUSIONS AND FUTURE

DIRECTIONS

Results from recent studies in gastrointestinal cancers provide strong evidence of an association between CKI and carcinogenesis. However, they also raise important questions as to whether these kinases could be successfully targeted for cancer screening or treatment. For colon cancer, while activation of the canonical Wnt pathway was evidenced, results by Tsai *et al.*^[57] also indicate the possibility of some undefined pathway (related to cell migration and morphogenesis during gastrulation) that may be affected by CKI. Identifying this additional pathway is certainly a daunting task due to the complexity of gastrulation^[58,59] and the wide number of known (and unknown) targets of CKI. However, at least two new targets of CKI ϵ could be possible alternative candidates for the R324H mutant, given the great similarity between CKI ϵ and δ . The first one is Rap1, an alternative GTPase that was later shown by the same group to promote gastrulation through CKI ϵ ^[57,60]. The second is Akt, which we recently identified as a new target of CKI ϵ ^[30]. Akt is also involved in cell migration, even though its role in gastrulation is still unclear^[61]. Regardless which pathways are involved, it may be even more interesting to see whether the CKI ϵ/δ -specific inhibitor IC261 could reduce hyperplasia and/or polyp formation effectively.

Several lines of evidence show that the CKI ϵ/δ -specific inhibitor IC261 has potential therapeutic effects for cancer. On the one hand, CKI ϵ/δ inhibition could be successfully achieved within non-toxic levels both *in vitro* and *in vivo*^[24,27]. Moreover, IC261 was also shown to be more specific, resulting in fewer side-effects compared to some currently used alternatives^[27,62,63]. For instance, while it has been shown to cause transient mitotic arrest, its effect is limited to mitosis, unlike other mitotic spindle drugs like nocodazole, which affect microtubule stability in general^[62]. This feature could actually be advantageous to cancer therapy by targeting primarily actively dividing cancer cells, as was also shown *in vitro* by another group^[63]. In addition, Behrend *et al.*^[62] also provided a more detailed analysis of the potential effects of IC261 in PDAC and showed that it is as effective as gemcitabine,

the chemotherapeutic drug most commonly used for pancreatic cancer patients^[27]. However, combinatorial treatment did not have any additive advantages^[27], possibly because the two drugs for the most part affect the same pathways. This is tentatively supported by their gene expression data showing mostly similar trends in up and down-regulation of genes such as CKI ϵ and CKI δ . These results highlight the importance of understanding at the molecular level which pathways contribute to the disease, how these pathways are regulated and connected, and which components are affected by specific drug targets. In this case, Brockschmidt *et al.*^[24] convincingly showed that IC261 promotes apoptosis in PDAC by blocking CKI ϵ/δ -mediated inhibition of Fas-mediated signaling. However, their data also raises questions about which CKI ϵ/δ -mediated cell proliferative signal is also inhibited by IC261. Answering this question would help determine what other drug could be more effective in combinatorial treatment. For instance, Morgan-Lappe *et al.*^[64] mentioned that the Akt specific inhibitor A443654 was successful in combination with specific inhibition by siRNA of the CKI γ 3 isoform, which is another positive regulator of Wnt signaling^[65]. However, this may not be the best candidate in combination with IC261, which is specific to only CKI ϵ/δ , if IC261 is already blocking Akt signaling.

Taken together, results presented by Tsai, Umar, Brockschmidt, von Blume, Oue and Garzia further support a role of CKI ϵ/δ in carcinogenesis, particularly of the gastrointestinal tract, and make them very promising markers for both early detection/risk assessment and cancer therapy. Understanding the mode of regulation of these kinases can provide insight into how naturally occurring CKI ϵ/δ mutations work, and also help identify which portions of the gene are more likely mutated. This is particularly important for conditions like colon cancer, as well as many other cancers including pancreatic cancer, where asymptomatic early detection (i.e. through screening of mutations such as R324H) could be critical for better prognosis. More studies are required to further explore this area and also determine whether CKI ϵ/δ can be pharmacological targets for treatment as well, particularly in light of the promising results with the CKI ϵ/δ inhibitor IC261 against pancreatic cancer^[24] and AD^[27], both of which currently do not offer many successful options.

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