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Computational analysis of G-protein-coupled receptor kinase family members as potential targets for colorectal cancer therapy

Adam Hermawan[1,2]* and Herwandhani Putri[2]

Abstract

Background: G-protein-coupled receptor (GPCR) kinases (GRKs) interact with ligand-activated GPCR, causing intracellular phosphorylation and interfering with the intracellular signal transduction associated with the development of cancer. Colorectal cancer (CRC) is a fast-growing disease, and its molecular mechanism involves various regulatory proteins, including kinases. However, the GRK mechanism in CRC has not been explored.

Methods: We used an integrated computational approach to investigate the potential of GRK family members as targeted proteins in CRC. The GRK expression levels in tumor and normal tissues, colon adenocarcinoma samples, and metastatic colon adenocarcinoma were analyzed using ONCOMINE, GEPIA, and UALCAN, as well as TNM plots. Genetic changes in the GRK family genes were investigated using cBioportal. The prognostic value related to the gene expression of the GRK family was examined using GEPIA and UALCAN. Co-expression analysis of the GRK family was conducted using COXPRESdb. Association analysis of the Gene Ontology, KEGG pathway enrichment, and drug-gene analyses were performed using the over-representation analysis (ORA) in WebGestalt.

Results: *GRK2*, *GRK3*, and *GRK5* mRNA levels increased significantly in patients with CRC and metastatic CRC. Genetic changes were detected in patients with CRC, including *GRK7* (1.1%), *GRK2* (1.7%), *GRK4* (2.3%), *GRK5* (2.5%), *GRK6* (2.5%), *GRK3* (2.9%), and *GRK1* (4%). CRC patients with low mRNA of *GRK7* levels had better disease-free and overall survival than those with high *GRK7* levels. Hierarchical clustering analysis revealed significant positive correlations between *GRK5* and *GRK2* and between *GRK2* and *GRK6*. KEGG pathway enrichment analysis showed that the gene network (GN) regulated several cellular pathways, such as the morphine addiction signaling and chemokine signaling pathways in cancer. The drug-gene association analysis indicated that the GN was associated with several drugs, including reboxetine, pindolol, beta-blocking agents, and protein kinase inhibitors.

Conclusion: No research has been conducted on the relation of *GRK1* and *GRK7* to cancer, particularly CRC. In this work, genes *GRK2, GRK3, GRK5,* and *GRK6* were found to be oncogenes in CRC. Although inhibitors against GRK2, GRK5, and GRK6 have previously been developed, further research, particularly preclinical and clinical studies, is needed before these agents may be used to treat CRC.

Keywords: G-protein-coupled receptor kinase, Colorectal cancer, Computational analysis, Targeted therapy

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*Correspondence: adam_apt@ugm.ac.id
[1] Laboratory of Macromolecular Engineering, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Gadjah Mada Sekip Utara II, Yogyakarta 55281, Indonesia
Full list of author information is available at the end of the article

Introduction

G-protein-coupled receptor (GPCR) is a transmembrane receptor that regulates biology and pathology in the human body and is distributed in various organs [1].

GPCRs are involved in the biological processes of cancer development, such as vascular remodeling, invasion, and migration [2], and have various families consisting of many proteins. This protein has been identified as an oncogene or tumor suppressor gene in various types of cancer [3, 4]. GPCR kinases (GRKs) are serine/threonine member kinases that play an important role in GPCR regulation by facilitating arrestin binding and receptor desensitization [5], interacting with ligand-activated GPCRs, and phosphorylating GPCR intracellular domains [6].

GRKs interact with ligand-activated GPCRs, thus phosphorylating intracellular receptor domains and impairing intracellular signaling and desensitization [7]. Seven members of the GRK family have been identified, and the mechanisms of GRK activity are generally classified into subcellular localization, alteration of intrinsic kinase activity, and modification of GRK expression [8]. GRKs regulate the functions of GPCR and growth factor receptor and directly control the components of the cytosol and cell nucleus signaling pathways [9]. The phosphorylation of GPCR intracellular domain desensitizes and triggers the internalization of the receptor, thereby interfering with intracellular signal transduction associated with cancer development [6].

Colorectal cancer (CRC) is a fast-growing global disease, and its molecular mechanism involves various regulatory proteins [10]. The incidence and mortality of early-onset CRC in humans younger than 50 years have increased worldwide [11]. Early detection, biomarkers, and new drugs are necessary to overcome this problem. Nogues mentioned that GRKs are involved in cancer development in a cell-type and tumor-specific way [9]. Furthermore, GRK expression and activity in specific tumor tissues can be changed by modulating various biological processes, including proliferation, survival, and invasion [9]. The review article by Garcia–Aranda [12] discussed the therapeutic targets of CRC on kinases, including tyrosine kinases, serine/threonine kinases, and drug development. GRKs are also targets for developing new drugs belonging to kinase inhibitors [13]. However, the GRK mechanism in CRC has not been deeply explored and needs to be investigated. In this study, we used an integrated computational analysis to explore the potential of GRK family members as target proteins in CRC, especially those related to gene expression, co-expression, alterations, and prognostic value. The integrated bioinformatic approach is carried out using various public databases that validate the potential of GRK members as targets for CRC therapy.

Methods

Analysis of GRK expression levels in tumor and normal tissues

GRK expression levels in tumor and normal tissues were analyzed using the ONCOMINE database (http://oncomine.org) [14] as previously described [15]. The selection criteria were fold change > 2 and p-value < 0.05.

Analysis of GRK expression levels in colon adenocarcinoma samples

GRK expression levels in colon adenocarcinoma samples were analyzed with GEPIA (http://gepia.cancer-pku.cn) [16] and UALCAN (http://ualcan.path.uab.edu) [17]. The gene expression levels were examined from TCGA study samples and compared between normal and colon adenocarcinoma tissues. Student's t-test was used for statistical analysis, and * represents p-value < 0.05.

Analysis of GRK gene expression in normal tissues, tumor tissues, and metastatic colon adenocarcinoma samples

GRK gene expression levels in normal tissues, tumor tissues, and metastatic colon adenocarcinoma samples were analyzed using TNM plots (https://tnmplot.com/analysis/) [18]. Statistical analysis was carried out by ANOVA.

Analysis of genetic alterations

The genetic alterations of GRK family were analyzed using cBioportal (https://www.cbioportal.org) [19, 20]. A study with the highest number of alterations was selected for Oncoprint analysis, mutation profile, and copy number alteration [21].

Prognostic value

Prognostic value related to the gene expression of the GRK family was conducted with GEPIA (http://gepia.cancer-pku.cn) [16] using parameters overall survival (OS) and disease-free survival (DFS) and UALCAN (http://ualcan.path.uab.edu) [17] using the standard settings of the database.

Co-expression analysis

The co-expression of the GRK family was analyzed using COXPRESdb (https://coxpresdb.jp) [22]. Data on the GRK family genes were submitted to coxpresdb.org and further analyzed for hierarchical clustering and network analysis using the standard settings of the database. Query genes (GRK family members) and its co-expressed genes were considered as GRK gene networks (GNs) and further analyzed for gene ontology, KEGG pathway, and drug–gene association analysis.

Gene ontology, KEGG pathway enrichment, and drug–gene association analysis

Gene ontology, KEGG pathway enrichment, and drug–gene association analyses were performed using the over-representation analysis (ORA) of WebGestalt (http://www.webgestalt.org) [23]. GNs were submitted to the ORA of WebGestalt. For gene ontology functional database, gene ontology terms were selected for biological process, cellular component, and molecular function. For KEGG pathway enrichment analysis, functional database pathway and KEGG were selected. For drug–gene association analysis, drug and GLAD4U were selected.

Results

Analysis of GRK expression levels in various tumor tissues

To determine the specific role of the GRK family in various tumor tissues, we compared the expression levels of the GRK family in tumor and normal tissues. The expression of GRK genes showed diverse patterns in various tumor tissues. In general, *GRK1* level is decreased in cervical cancer, lung cancer, and lymphoma (Fig. 1). *GRK2* is increased in melanoma and prostate cancer but decreased in CRC, lymphoma, and pancreatic cancer. *GRK3* is increased in CRC, leukemia, and liver cancer but decreased in brain and CNS cancer, breast cancer, lung cancer, lymphoma, and sarcoma. *GRK4* is decreased in other types of cancer. *GRK5* is generally increased in breast cancer, CRC, kidney cancer, leukemia, myeloma, and pancreatic cancer but decreased in bladder cancer, brain and CNS cancer, breast cancer, gastric cancer, leukemia, lung cancer, melanoma, ovarian cancer, and sarcoma. *GRK6* is increased in gastric cancer but decreased in brain and CNS, leukemia, and pancreatic cancer. *GRK7* did not show any changes in expression in all types of cancer and normal tissues. Gaspar et al. [24] found a significant twofold decrease in *GRK2* in CRC (Table 1). Skrypczak et al. [25] observed a 3–5 times increase in *GRK3* expression in colon cancer cells. Skrypczak et al.
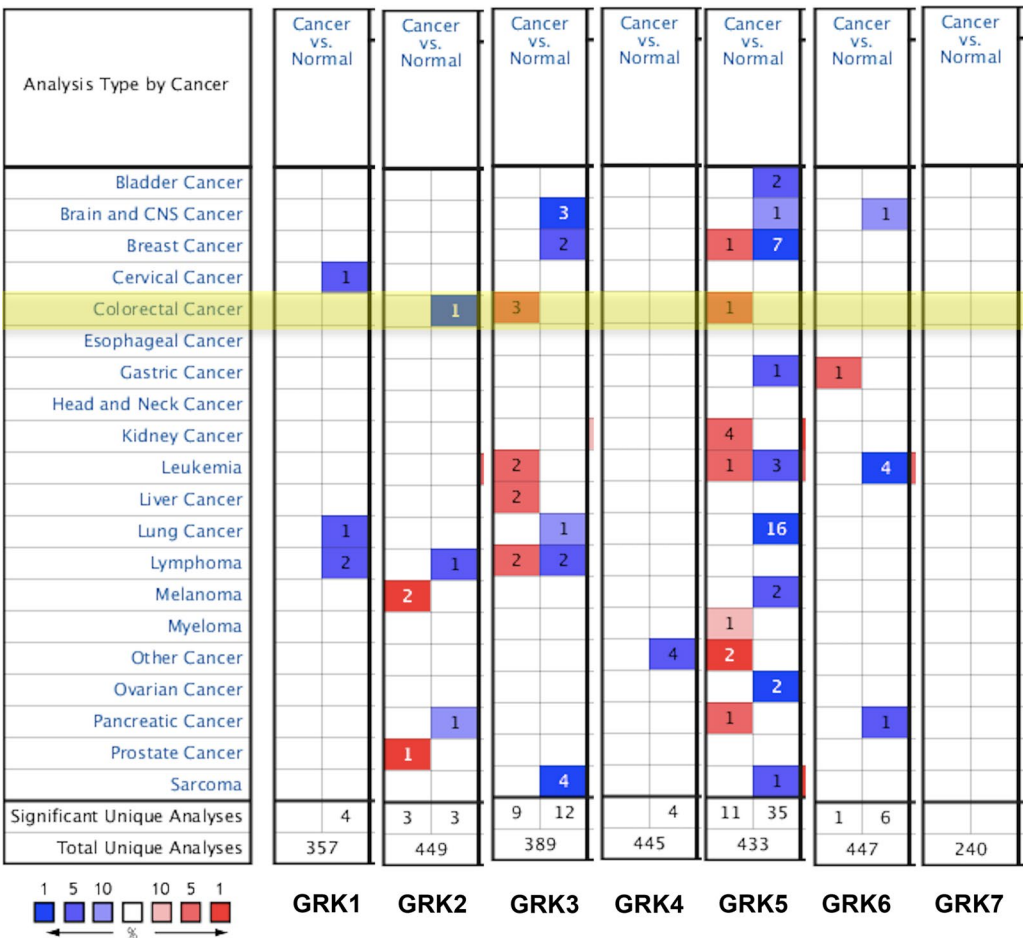
Fig. 1 Expression of GRK family across tumor versus adjacent tissues as analyzed by ONCOMINE. The selection criteria are fold change > 2 and *p*-value < 0.05

Table 1 Expression of GRK family across colorectal cancer studies as analyzed by ONCOMINE

Gene	Tumor versus normal	p	Fold change	References
GRK2	Colon adenoma epithelia versus normal	3.74E−9	− 2.042	Gaspar et al. [24]
GRK3	Colon adenoma epithelia versus normal	6.64E−6	3.049	Skrypczak et al. [25]
	Colon carcinoma epithelia versus normal	3.61E−7	5.023	
	Colon carcinoma versus normal	1.49E−7	3.549	
GRK5	Colon carcinoma epithelia versus normal	4.46E−8	3.042	Skrypczak et al. [25]

[25] found a threefold increase in *GRK5* mRNA expression in colorectal cancer cells.

Analysis of GRK expression levels in colon adenocarcinoma samples

Analysis of GRK expression levels in colon adenocarcinoma samples from TCGA study using GEPIA revealed that only *GRK5* expression levels were significantly downregulated in colorectal cancer cells (Fig. 2A). No significant results were found in the expression levels of GRK family genes in colorectal cancer samples from the TCGA study using the UALCAN database (Fig. 2B). We identified a significant increase in the expression of *GRK1*, *GRK2*, *GRK3*, *GRK4*, *GRK5*, and *GRK6* in metastatic colon cancer cells (Fig. 2C). No data were found on *GRK7*.

Analysis of genetic alterations

We submitted *GRK1-GRK7* as query to cBioportal using samples from TCGA, PanCancer Atlas study. We found genetic alterations in *GRK7* (1.1%), *GRK2* (1.7%), *GRK4* (2.3%), *GRK5* (2.5%), *GRK6* (2.5%), *GRK3* (2.9%), and *GRK1* (4%) in 1.1% to 4% of patients (Fig. 3A). Most of the genetic alterations belong to amplification. Six mutations in *GRK1* occurred in the protein kinase domain, and one of which was named V251M. Mutations in *GRK4*, *GRK5*, and *GRK7* occurred in the RGS and protein kinase domains, and those in *GRK2* and *GRK3* occurred in the RGS, protein kinase, and PH domains (Fig. 3B). Extreme diversity was exhibited by the mutants in the GRK family, namely, *GRK1* (6 mutants), *GRK2* (8 mutants), *GRK3* (14 mutants), *GRK4* (12 mutants), *GRK5* (13 mutants), *GRK6* (15 mutants), and *GRK7* (6 mutants). Significant mutual exclusivity study of GRK family in CRC samples from TCGA study showed only one gene pair *GRK2-GRK3* with co-occurrence tendency (Table 2).

Prognostic value

The prognostic value related to the gene expression of the GRK family and analyzed using GEPIA was classified based on OS and DFS. The patients with CRC and high *GRK6* levels had significantly better OS than their opposite group (Fig. 4A). Meanwhile, the expression levels of *GRK1*, *GRK2*, *GRK3*, *GRK4*, *GRK5*, and *GRK7* had no significant meaning related to the OS of patients with CRC. In terms of DFS, only *GRK7* showed significant results, in which patients with low *GRK7* levels had better DFS in patients with CRC (Fig. 4B). According to UALCAN database, only *GRK7* had significant results, in which patients with low *GRK7* levels had a better OS than their opposite group (Fig. 4C).

Co-expression analysis

Hierarchical clustering analysis with the complete linkage method of the GRK family was conducted to determine the correlation between GRK family members and the functional interaction network. The results showed a significant positive correlation between GRK members, namely, between *GRK5* and *GRK2* and between *GRK2* and *GRK6* (Fig. 5A). We also performed a GN analysis of GRK members based on the 300 neighboring genes most associated with this family (Fig. 5B, Additional file 1: Table 1). The findings revealed that this GN regulated cellular processes in cancer progression.

Gene ontology, KEGG pathway enrichment, and drug–gene association analysis

Gene ontology analysis (Fig. 5C) showed that the GN was involved in several biological processes, including cell communication and stimulus response, was located in the cell membrane and cytoplasm, and played a role in the molecular function of protein binding and ion binding. KEGG pathway enrichment analysis (Fig. 5D) revealed that GN regulated several cellular pathways such as morphine addiction signaling pathway and chemokine signaling pathway in cancer. Drug–gene association analysis (Fig. 5E) showed that the GN was associated with several drugs including reboxetine, pindolol, beta-blocking agents, and protein kinase inhibitors.

Discussion

On the basis of previous studies, the regulation of GRKs in cancer progression is highly dependent on specific cells and tissues. Hence, the role of GRKs in the progression of CRC is crucial for the development of GRKs as a target for colon cancer therapy. This study aims to

explore the potential of GRK family members including GRK1, GRK2, GRK3, GRK4, GRK5, GRK6, and GRK7 as therapeutic targets in CRC using an integrated computational approach. Results showed that the mRNA expression levels of *GRK2*, *GRK3*, and *GRK5* significantly differed in CRC tissues compared with adjacent tissues. This result is supported by Matthees et al. [3], who showed the upregulations of protein levels GRK2, GRK3, GRK5, and GRK6 in patients with CRC. Moreover, mutual exclusivity analysis revealed one co-occurring gene pair, namely, *GRK2–GRK3*. OS analysis showed that patients with low *GRK7* levels and high *GRK6* levels had better OS than their opposite groups. Co-expression analysis showed two significantly co-expressed significant gene pairs, namely, *GRK5–GRK2* and *GRK2–GRK6*.

Gene ontology enrichment analysis of the GN showed the GN pathway regulation of chemokine signaling pathway in cancer. Drug–gene association analysis showed that kinase inhibitors were associated with the GN.

GRK1 and GRK7 were found to regulate several cellular processes. The phosphorylation of GRK1 and GRK7 by cAMP-dependent protein kinase diminishes their enzymatic activities [26]. GRK1B and GRK7A are involved in the recovery of the photoresponse of zebrafish larva [27]. GRK1 regulates the normal kinetics of dark adaption [28]. The role of GRK1 and GRK7 in cancer development, especially CRC, remains elusive and thus deserves further investigation.

GRK2 interacts with caveolin, in which caveolin 1 inhibits GRK-mediated phosphorylation of GPCR [29].
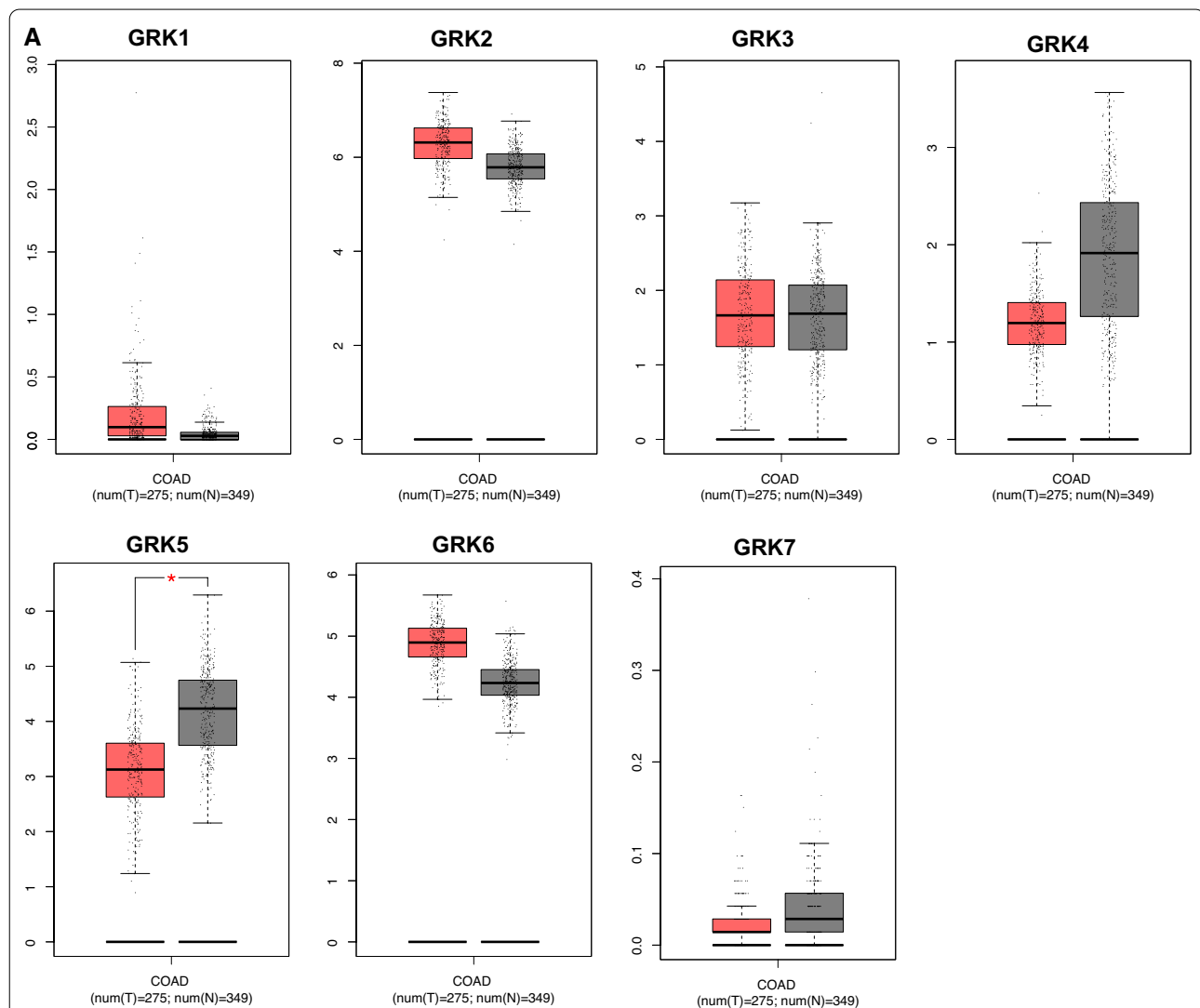
Fig. 2 **A** mRNA expression profile of GRK family across colon adenocarcinoma samples from TCGA study as analyzed by GEPIA. **B** mRNA expression profile of GRK family across colon adenocarcinoma samples from TCGA study as analyzed by UALCAN. **C** Differential gene expression analysis of GRKs in tumor, normal, and metastatic tissues as analyzed by TNMPlot
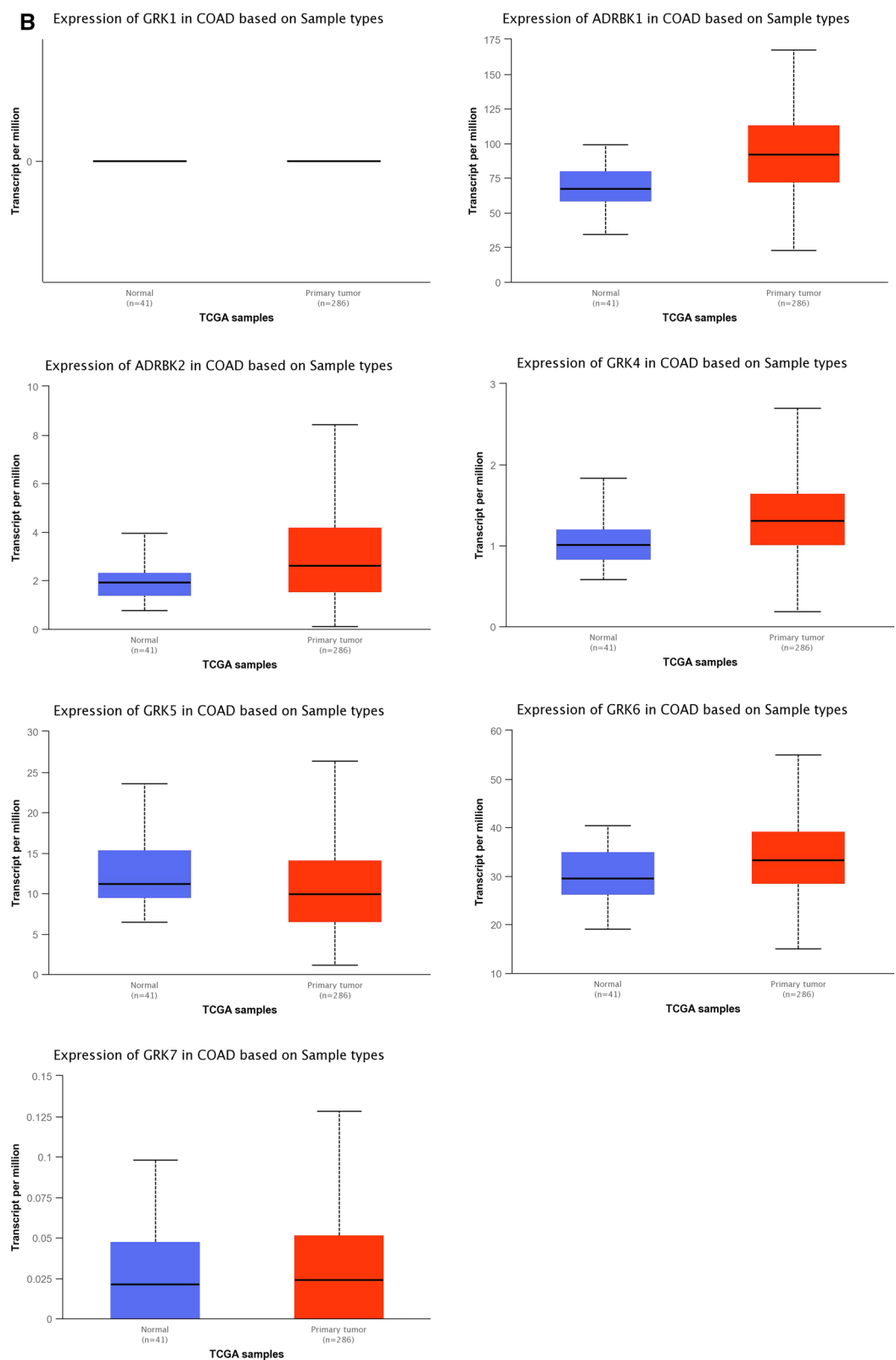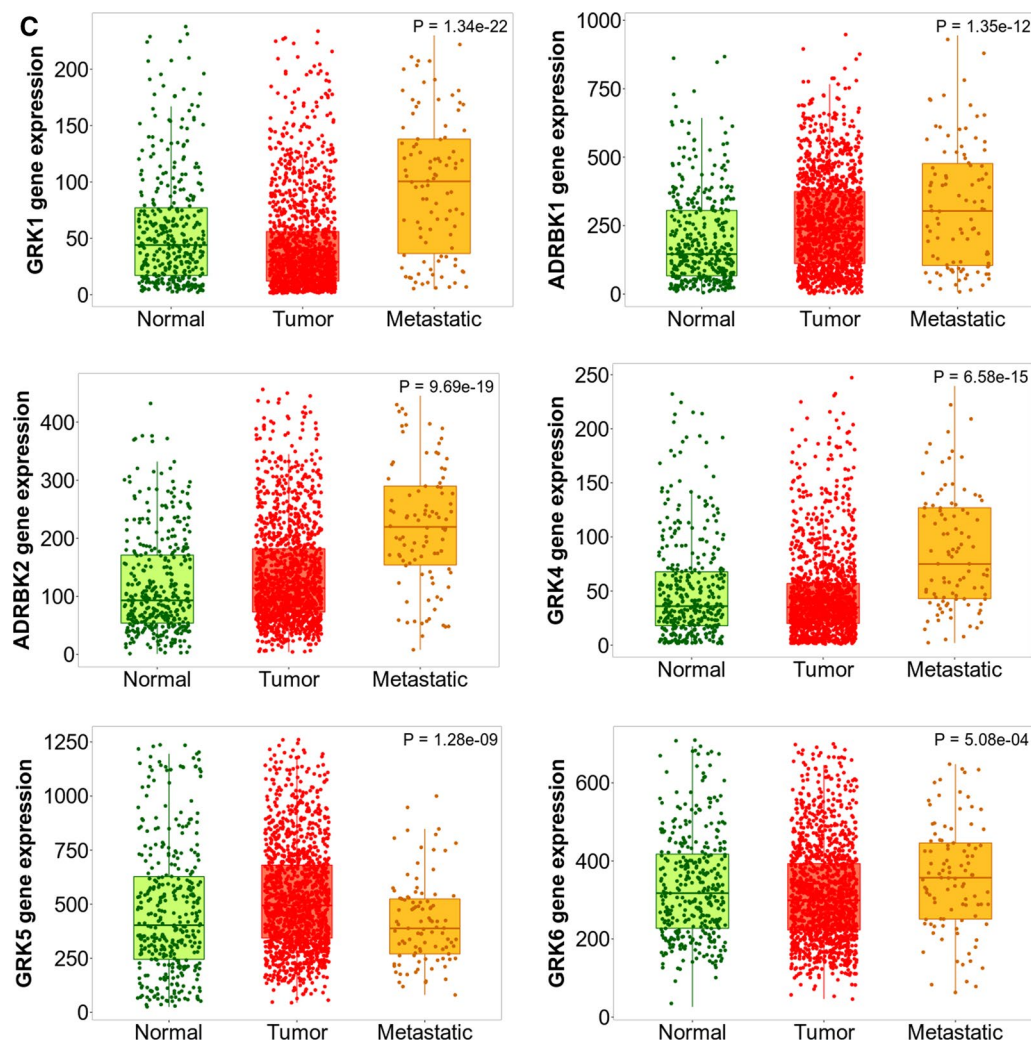
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Fig. 2 continued

GRK2 is a key player of TNF-a-induced wound healing in colon epithelial cells [30]. GRK2 can play a role as an oncogene or tumor suppressor gene depending on certain tissue types. GRK2 promotes carcinogenesis in breast cancer [9]. The stabilization of GRK2 by EIF3D promotes the progression of gallbladder cancer by activating the PI3K/Akt pathway [31]. Moreover, an increase in *GRK2* expression is involved in the progression of laryngeal carcinoma [32]. Downstream GPCR signaling involves crosstalk with other kinase pathways, including insulin-like growth factor-1 receptor (IGF1R) and beta-arrestin [33]. Inhibition of GRK2 blocks IGF1R signaling, thereby suppressing sarcoma progression. GRK2 increases the sensitivity of breast cancer cells to cisplatin by interacting with NADPH oxidase 4 [34].

GRK2 mRNA and protein levels show no difference between normal and thyroid cancer tissues [35]. GRK2 exhibits tumor suppressor activity in prostate cancer, in which loss of *GRK2* function speeds up cancer progression to the deadliest stage [36]. Downregulation of *GRK2* in head and neck squamous cell carcinoma promotes epithelial-to-mesenchymal transition [37]. Selective GRK2 inhibitors are available as treatment for several diseases, such as heart failure and cancer [38]. Kang [39] studied the structure and function of the GRK2 protein and developed GRK2 substrates and inhibitors to examine the molecular function of GRK2 and develop drugs for targeted therapy. GRK2 prevents metastasis and invasion in hepatocellular carcinoma by regulating the Akt pathway [40] and inhibits the progression of lymphoma by inhibiting MALT1 [41].
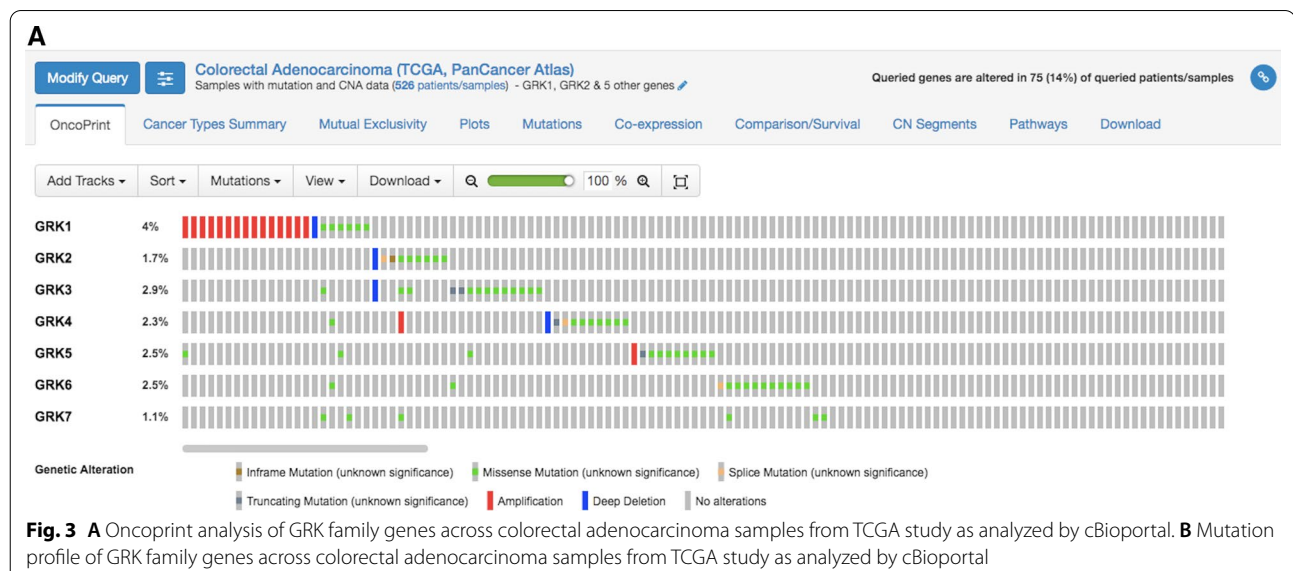
GRK3 was found to regulate several biological processes, including cancer. The expression of *GRK3* and *GRK4* is increased in hyperfunctioning thyroid nodules [42]. Woerner [43] found that GRK3 is a negative regulator of cell proliferation, and its levels are reduced in patients with glioblastoma multiforme. GRK3 is also an oncogene and negative regulator of the chemokine receptor CXCR4, and its GRK3 is positively correlated with progression and metastasis in triple-negative breast cancer [44]. Increased levels of *GRK3* are a negative predictive marker for malignant CRC [45]. *GRK3* is downregulated in patients with hepatocellular carcinoma and thus can be used as a good prognosis for this disease [46]. In addition, GRK2 and GRK3 are involved in the internalization of μ-opioid receptor and β-arrestin2 recruitment [47]. Further investigations on GRK3 expression in CRC must be conducted.

KGN malignant human ovarian granulose-like tumor cells have lower levels of GKR4 alpha and beta and higher levels of GRK2 and GRK4 gamma/delta than non-malignant human granulosa cells [48]. *GRK4* expression is increased in hyperfunctioning thyroid nodules [42]. High *GRK4* levels can trigger beta-arrestin-mediated MAPK signaling in breast cancer cells [49]. GRK4 induces cellular senescence via the p53-independent pathway [50]. GRK4 is a regulatory gene in hypertension and promotes breast cancer cell proliferation [51]. GRK4 expression in CRC must be explored in detail.

GRK5 mRNA and protein levels are decreased in differentiated thyroid carcinoma compared with those in normal thyroid tissues [35]. *GRK5* is overexpressed in aggressive prostate cancer cells, and its knockdown leads to cell growth inhibition, G2/M cell cycle arrest,

and decreased cyclin D1 levels [52]. *GRK5* is downregulated and increases CRC cell proliferation [53]. *GRK5* overexpression is observed in glioblastoma stem cells and is correlated with increased cell proliferation [54]. GRK5 regulates metastasis in prostate cancer by targeting moesin, a cytoskeletal membrane attachment protein, as its substrate [55]. GRK5 exhibits oncogenic activity in non-small-cell lung cancer, and its knockdown increases G2/M cell cycle arrest and induces apoptosis [56]. Low *GRK5* levels promote the resistance of HeLa cervical cancer cells and MDA-MB 231 breast cancer cells to paclitaxel by increasing tubulin acetylation [57]. GRK5 increases proliferation, invasion, and migration in renal cell carcinoma [58] and is a key regulator of the migration and invasion of breast cancer [59]. The downregulation of *GRK5* inhibits the signaling pathway of migration in triple-negative breast cancer. A review article by Marzano [60] discussed the potential of GRK5 for cancer chemoprevention. GRK5 is localized in the nucleus and overexpressed in MDA-MB 231 triple-negative breast cancer cells [61]. *GRK5* knockdown increases the sensitivity of HeLa cervical and MDA-MB triple-negative breast cancer cells toward actinomycin D [61]. Rowlands et al. [62] developed selective and potent GRK5 inhibitors. However, the regulating role of GRK5 in CRC needs further investigation.

GRK6 is overexpressed in multiple myeloma cells with increased chemoresistance [63]. GRK6 possesses oncogenic activity, and its overexpression is correlated with progression and poor prognosis of papillary thyroid carcinoma [64]. Tao [65] showed that *GRK6* overexpression is strongly correlated with the poor prognosis and progression of CRC. GRK6 exhibits tumor suppressor activity,
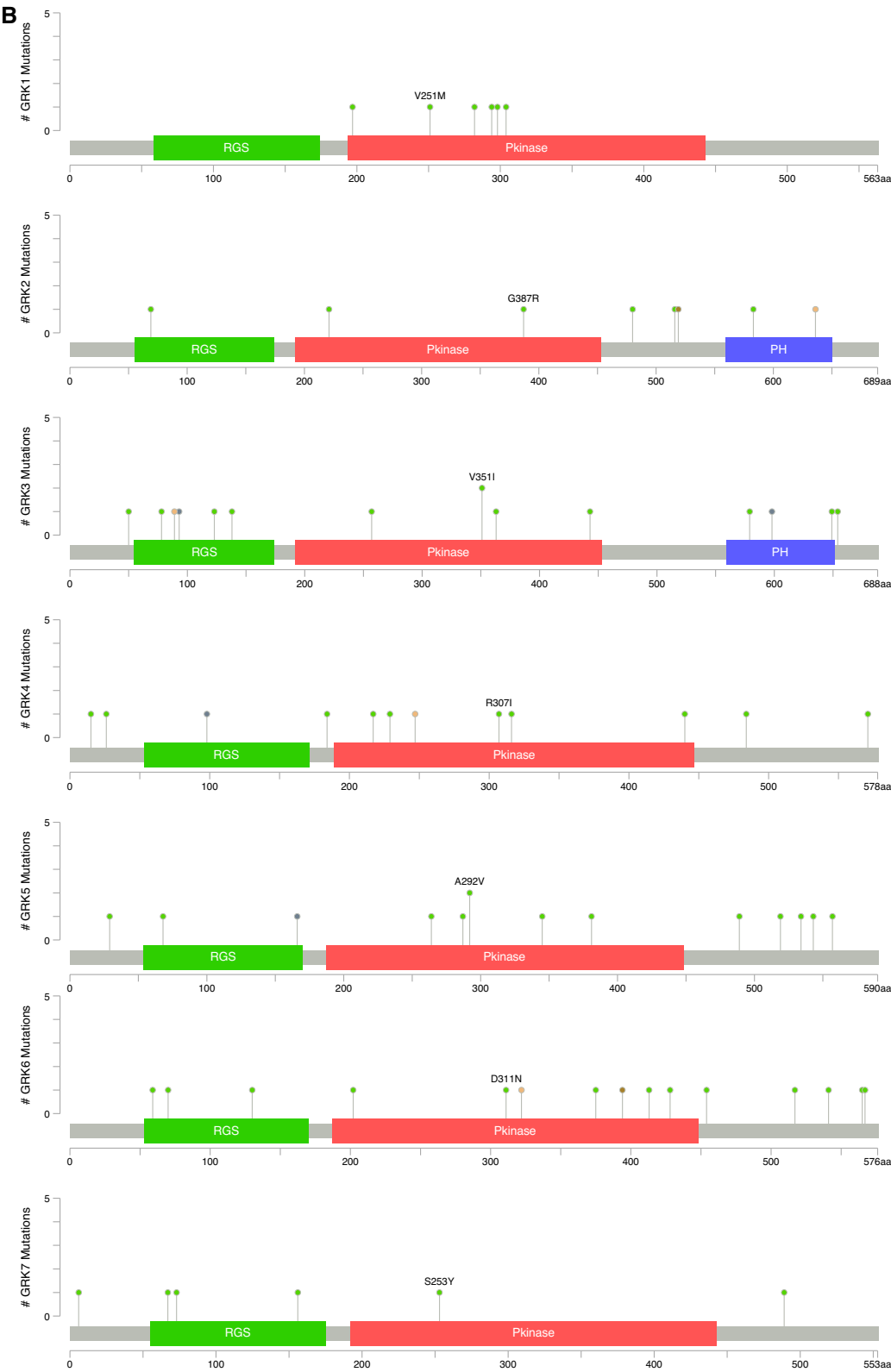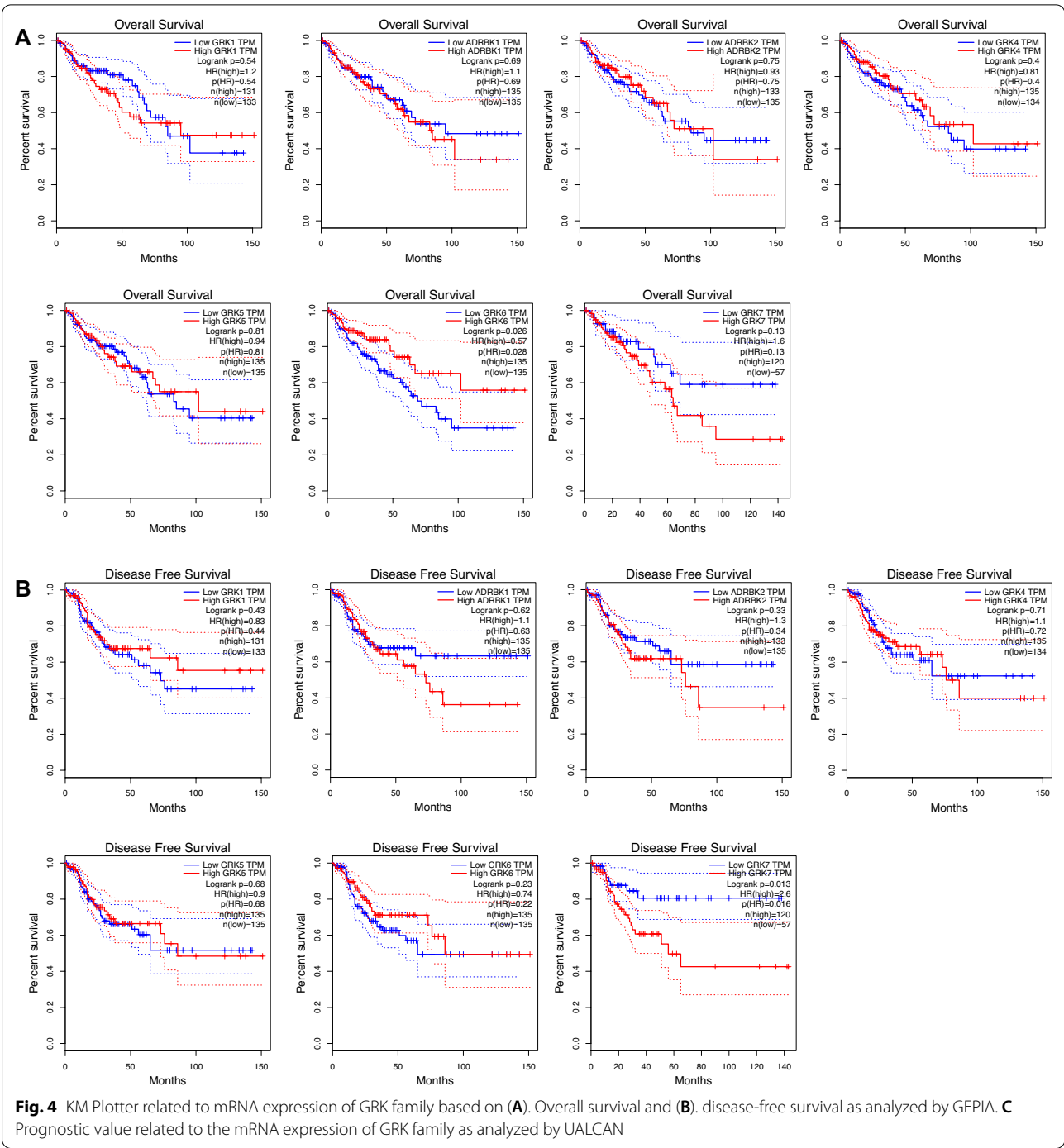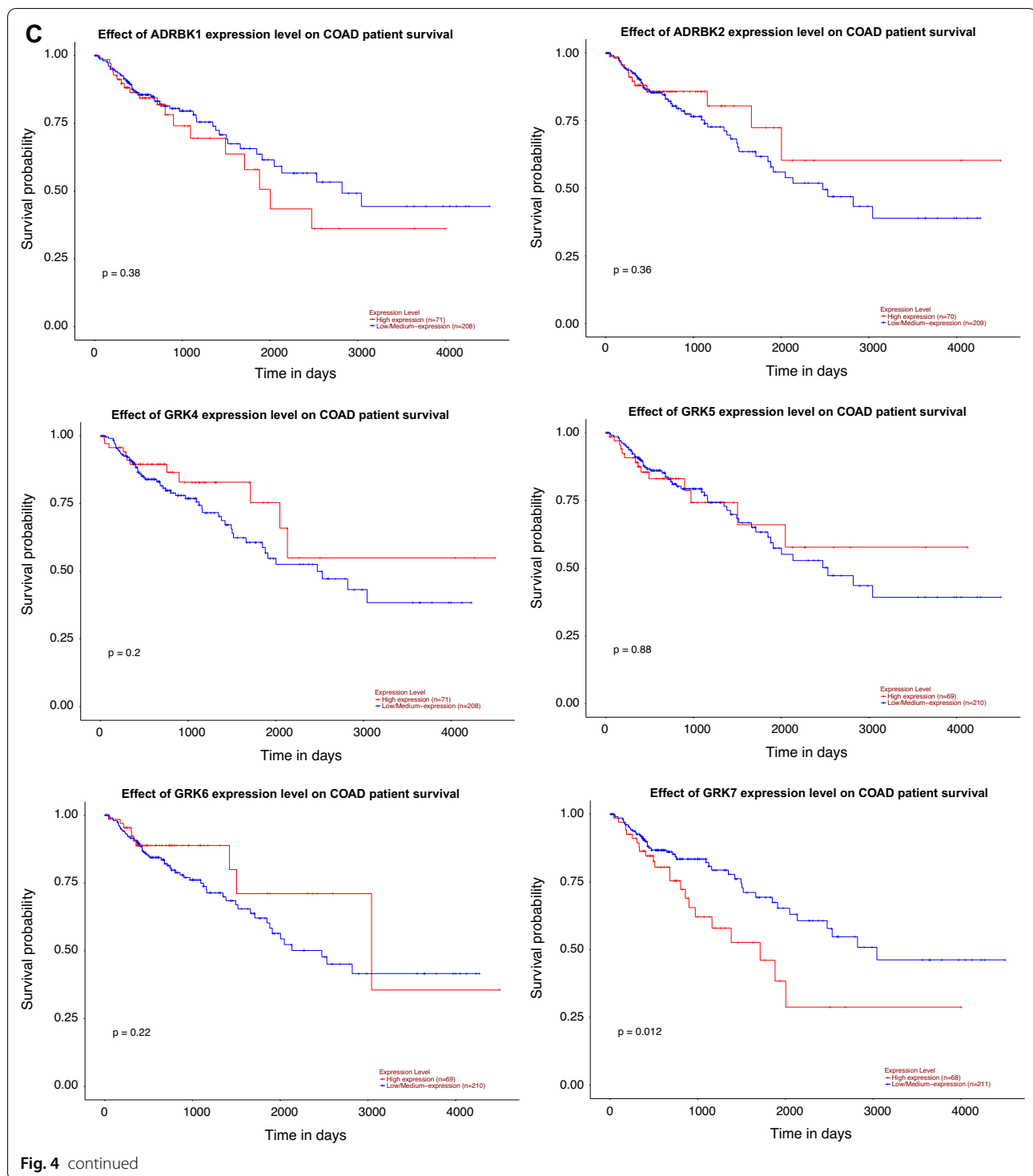

Fig. 3 **A** Oncoprint analysis of GRK family genes across colorectal adenocarcinoma samples from TCGA study as analyzed by cBioportal. **B** Mutation profile of GRK family genes across colorectal adenocarcinoma samples from TCGA study as analyzed by cBioportal

Fig. 3 continued

Table 2 Significant mutual exclusivity study of GRK family in colorectal cancer TCGA as analyzed by cBioportal

A	B	*p*-value	Tendency
GRK2	GRK3	0.001	Co-occurrence

and its knockdown increases lung adenocarcinoma cell migration and invasion [66]. Olson [67] found that *GRK6*

levels are overexpressed in multiple myeloma compared with that in epithelial cells; hence, they modified and expressed GRK6 protein to support the structure-based drug development of supportive therapy for multiple myeloma. Uehling [68] synthesized 4-aminoquinazolines as GRK6 inhibitors and found that compound no. 18 is a novel, potent, and selective GRK6 inhibitor that can be used for treating multiple myeloma. Further studies on

Fig. 4 KM Plotter related to mRNA expression of GRK family based on (**A**). Overall survival and (**B**). disease-free survival as analyzed by GEPIA. **C** Prognostic value related to the mRNA expression of GRK family as analyzed by UALCAN

Fig. 4 continued

the molecular mechanism and development of GRK6 inhibitors for CRC therapy are required.

The results of our study demonstrated that not all GRK family members were correlated with or played an important role in the progression of CRC, as only GRK2, GRK5, and GRK6 played essential roles in CRC progression. Moreover, inhibitors of GRK2, GRK5, and GRK5 have been widely studied as anticancer inhibitors but not in CRC; therefore, further studies of those inhibitors on CRC cells will provide comprehensive data for
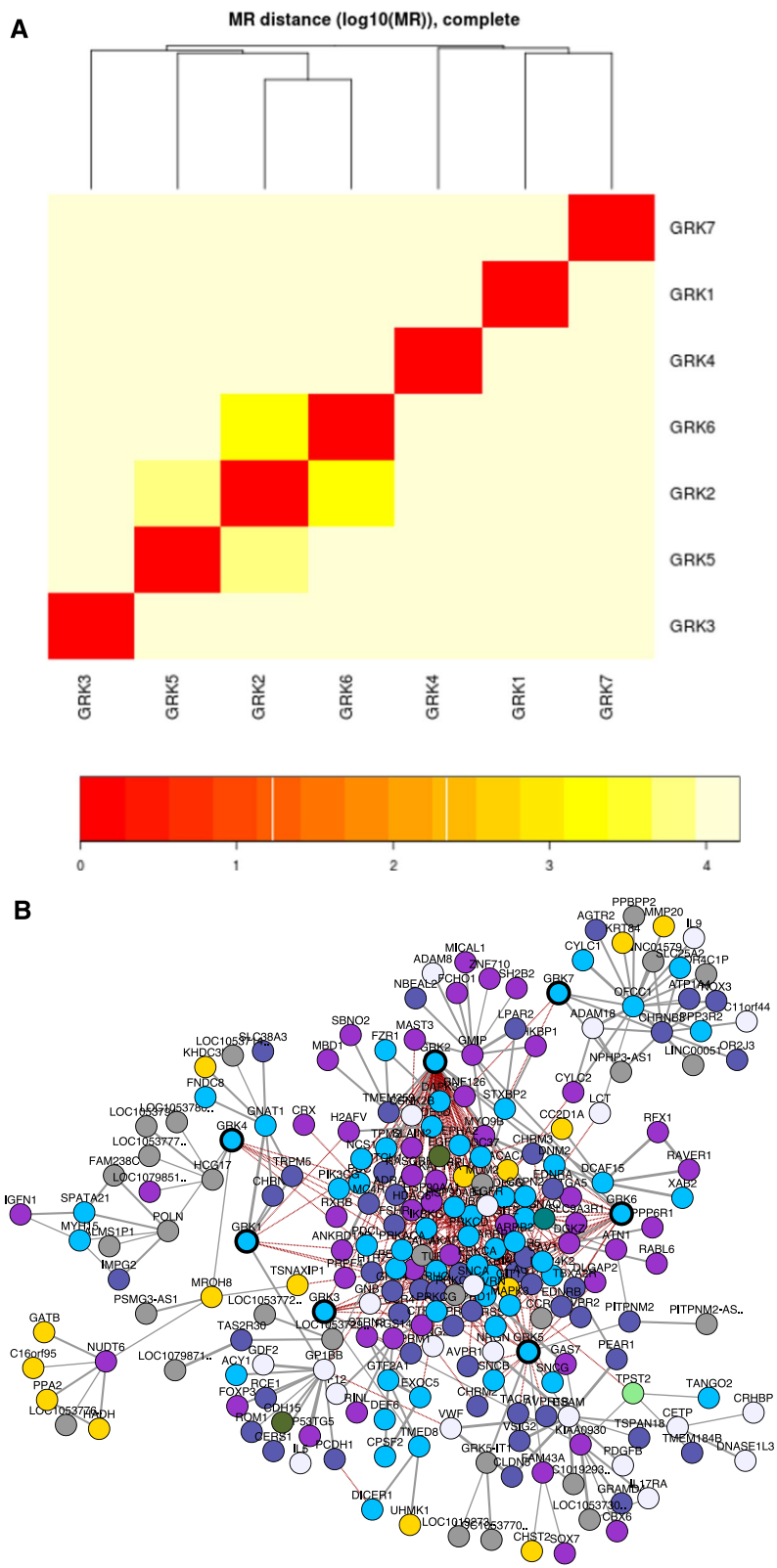
Fig. 5 **A** Hierarchical clustering of GRK family genes constructed by mutual rank distance as analyzed by COXPRESdb. **B** Network analysis of co-expressed genes related to GRKs as analyzed by COXPRESdb. **C** Gene ontology enrichment analysis of the co-expressed GRK genes as analyzed by WebGestalt. **D** KEGG pathway enrichment analysis of co-expressed GRK genes as analyzed by WebGestalt. **E** Drug–gene association analysis of co-expressed GRK genes as analyzed by WebGestalt
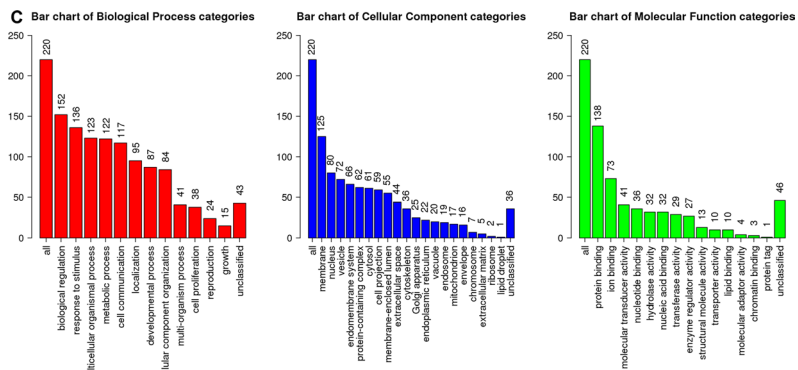
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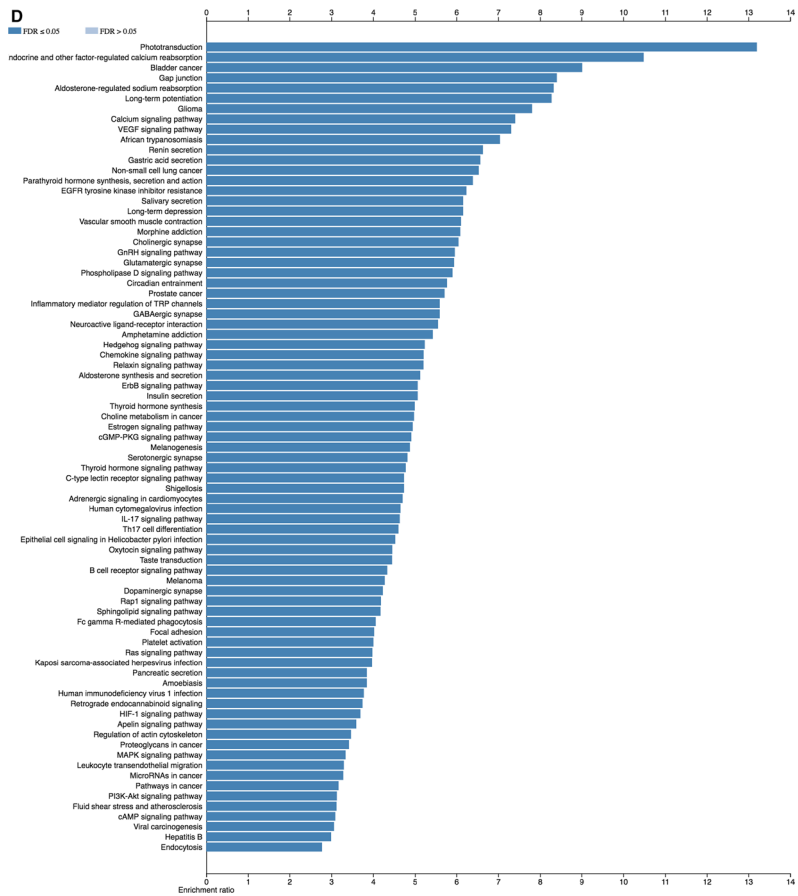
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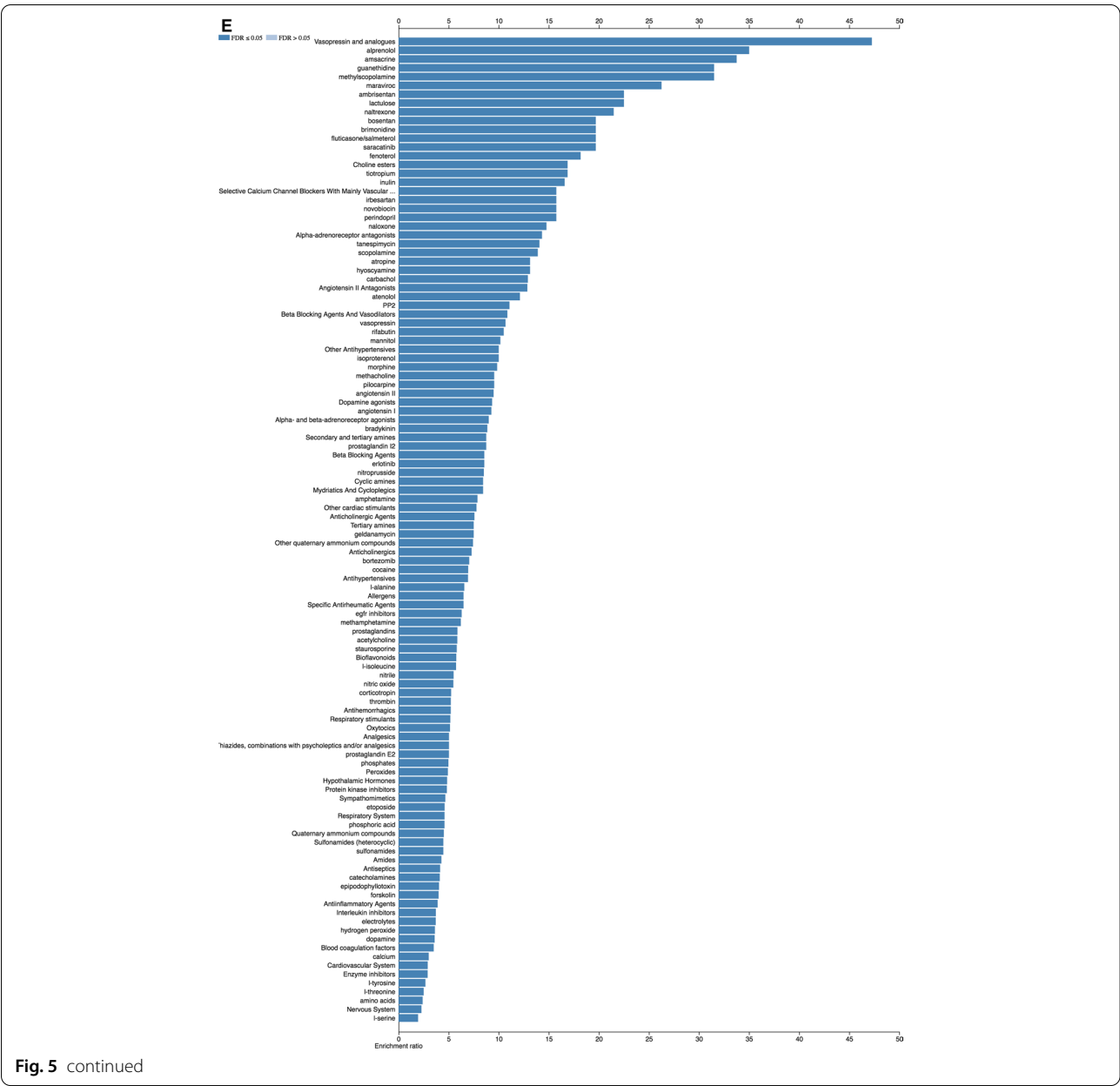
their development as anti-CRC agents. This study used a bioinformatics approach and was in the early stages of the drug discovery process of exploring potential GRK families for CRC therapy; therefore, the results must be validated by other studies such as in vitro, in vivo, and clinical trials. Despite these limitations, this is the first study to apply bioinformatics to explore the role and potential of GRKs as therapeutic targets in CRC.

Conclusion

This study highlights the potential of GRK family members, especially GRK2, GRK3, GRK5, GRK6, and GRK7, as targets for the treatment of CRC. To date, no research has focused on the relation of GRK1 and GRK7 to cancer, especially CRC. *GRK2*, *GRK3*, *GRK5*, and *GRK6* were found to be oncogenes in CRC. Although inhibitors against GRK2, GRK5, and GRK6 have been developed, further investigations, especially preclinical and clinical studies, are required for their potential use against CRC.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors indicate that they have no competing interests.

Author details
[1]Laboratory of Macromolecular Engineering, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Gadjah Mada Sekip Utara II, Yogyakarta 55281, Indonesia. [2]Cancer Chemoprevention Research Center, Faculty of Pharmacy, Universitas Gadjah Mada Sekip Utara II, Yogyakarta 55281, Indonesia.

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