BIOINFORMATICS ANALYSIS OF MICRORNAS ASSOCIATED WITH KRAS AND EGFR MUTATION IN COLORECTAL CANCER (MICRORNAS PREDICTION IN COLON CANCER)

Namphon Klaewkla[1] | *Surasak Wanram*[2]

Abstract: Colorectal cancer (CRC) is the third most common cancer in the world. Epigenetic regulation by non-coding RNAs or microRNAs (miRNAs) plays a role in cancer development and progression. Mutated genes such as KRAS and EGFR are associated with CRC carcinogenesis and play an important role in controlling the cellular process. The specific expressions of miRNAs serving as biomarkers for early diagnosis or prognosis of CRC are still unclear. The purpose of this study[3] was to identify potential miRNAs specific to KRAS and EGFR mutation using bioinformatics tools for prediction. The candidate miRNAs associated with interested target genes can be predicted. Selection of the candidate miRNAs should be validated and required for further development as biomarkers for colon cancer diagnosis and treatment.
Keywords: MicroRNAs (miRNAs); Prediction; KRAS mutation; EGFR mutation; Colorectal Cancer.

Previsão De Micrornas No Cancro Do Cólon

Resumo: O cancro colorretal (CCR) é o terceiro tipo de cancro mais comum no mundo. A regulação epigenética por RNAs não codificantes ou microRNAs (miRNAs) desempenha um papel no desenvolvimento e na progressão do cancro. Os genes mutantes, como o KRAS e o EGFR, estão associados à carcinogénese do CCR e desempenham um papel importante no controlo do processo celular. As expressões específicas dos miRNAs que servem como biomarcadores para o diagnóstico precoce ou prognóstico do CCR ainda não são claras. O objetivo deste estudo foi identificar potenciais miRNAs específicos da mutação KRAS e EGFR utilizando ferramentas de bioinformática para previsão. Os miRNAs candidatos associados aos genes-alvo interessados podem ser previstos. A seleção dos miRNAs candidatos deve ser validada e necessária para um maior desenvolvimento como biomarcadores para o diagnóstico e tratamento do cancro do cólon.
Palavras-chave: MicroRNAs (miRNAs); Previsão; Mutação KRAS; Mutação EGFR; Cancro colorrectal.

[1] Biomedical Sciences Research Unit, College of Medicine and Public Health, Ubon Ratchathani University, Ubon Ratchathani, Thailand. https://orcid.org/0000-0002-1448-2966.

[2] Biomedical Sciences Research Unit, College of Medicine and Public Health.
Ubon Ratchathani University, Ubon Ratchathani, Thailand. Department of Pathology, College of Medicine and Public Health, Ubon Ratchathani University, Ubon Ratchathani, Thailand. Associate Professor. Dr. Surasak Wanram, Ph.D. Department of Pathology, College of Medicine and Public Health, Ubon Ratchathani University, Ubon Ratchathani, Thailand 85, Sathollmark Rd., Mueang Si Khai, Warin Chamrap, Ubon Ratchathani 34190 Thailand. Tel: +66-815-457-311. E-mail: Surasak.w@ubu.ac.th. https://orcid.org/0000-0002-1866-0723.

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Introduction

Globally, colorectal cancers (CRCs) affect over a quarter of a million people each year. The risk of developing CRC in industrialized nations is approximately 5% while the risk of developing an adenoma, a noncancerous colon tumor, is approximately 20%. When the disease is localized or confined, effective treatment success rates range from 70 - 90%; however, advanced CRC has a high mortality rate, consistently ranking in the top three causes of cancer-related death worldwide [1, 2]. The 5-year survival rate of patients with localized stage colorectal cancer is 90%. About 39% of patients are diagnosed at an early stage. If cancer has spread to surrounding tissues or organs and/or the regional lymph nodes, the 5-year survival rate is 71%. If cancer has spread to distant parts of the body, the 5-year survival rate is 14% [3]. Delayed diagnosis and different prognosis factors can affect the survival rate of CRC patients, such as tumour stage, age, gender, histology type, tumour grade, tumour size, lymph node level, as well as pathological metastases, and tumour location [4]. Therefore, early detection is important to reduce cancer mortality and effective monitoring assists in tracking the relapse of cancer and guides decisions for cancer treatment, as well as it is an effective way of decreasing cancer death [5]. The molecular carcinogenesis of CRC is very complex and multifarious because of the genetic and epigenetic instability during CRC development [6]. Accumulation of various genetic mutations and/or epigenetic changes is important to drive the progression of carcinogens through disruption to the progression of tumour suppressor genes and oncogenes. The progression of CRC has been associated with a dysregulation expression in genes such as KRAS, DCC, APC, and TP53 [7]. The oncogenic mutations of CRC such as the EGFR and KRAS are responsible for the activation of the RAS/MAPK kinase pathway [8].

The epidermal growth factor receptor (EGFR) is one of the most important genes intervening in CRC carcinogenesis pathways. It belongs to the transmembrane receptor HER/ErbB family receptor tyrosine kinase (RTKs) and controls the intracellular signal transduction involving several pathways, including the RAS-mitogen-activated protein kinase

(MAPK), phosphoinositide 3-kinase (PI3K)/Akt, signal transducer and activator of transcription (STAT) and SRC/FAK pathways. When activated, it promotes cell division and migration, proliferation, apoptosis, angiogenesis, adhesion, and motility [9-12]. The Kirsten RAS (KRAS) is a component of the epidermal growth factor receptor (EGFR) signal transduction pathway and affects proliferation, and angiogenesis. KRAS mutations are present in about 40% of CRC [13]. KRAS mutations have reduced GTPase activity that results in increased cell proliferation potentially related to early tumorigenesis affecting more persistent growth--promoting signals [14]. The most frequent alterations detected involve three codons [12-13] that appear to play a major role in the progression of colorectal cancer [15].

MicroRNAs (miRNAs) are small non-protein-coding RNA molecules that are key regulators in several biological processes genes and regulate expression post-transcriptionally, including proliferation, differentiation, and survival of normal cells, and tumorigenesis and the development of various types of cancers (brain, lung, breast, liver, prostate, and colorectal cancer). Circulating miRNAs have also emerged as promising diagnostic biomarkers for CRC screening [16-19]. By the 3′UTR of target genes, miRNAs may function as tumour suppressors or oncogenes by regulating different targets [20]. For example, miR-18a [21], miR155 [22], and miR--106b-5p [23] inhibit proliferation, migration, invasion, and metastasis of CRC cells, whereas miR-433 [24], miR-885-5p [25] and miR-181a [26] promote cell proliferation, migration, and invasion. Moreover, no single miRNA alone has been identified as an ideal CRC biomarker up to now, a panel of miRNAs can be used to distinguish CRC patients from healthy controls with relatively high sensitivity and specificity, in the testing of a large population of subjects [27]. The purpose of this study was to identify potential microRNAs specific to KRAS and EGFR using bioinformatics tools for prediction. Five different prediction tools were employed to identify microRNAs using 3'UTR of KRAS and 3'UTR of EGFR for target genes.

Methods

MicroRNA Target Prediction Databases

The ensemble database retrieved comprises the 3′UTR sequence of KRAS (human KRAS 3′UTR based on transcript NM_033360 (chr12:25205246...25250929)) and EGFR (human EGFR 3′UTR based on transcript NM_005228 (chr7:55019017...55211628)) from NCBI database. The bioinformatic tools for miRNA prediction that relate to KRAS and EGFR were conducted from TargetScanHuman, miRTar2GO, miRDB, MiRanda, and DIANA-microT-CDS. Workflow was established considering all miRNA target site predictions.

MicroRNA Target Prediction

Five different prediction programs were performed as shown in Figure 1. TargetScan software program is one of the most common user-friendly programs based on the two classic features for miRNA target prediction. First is the seed match sequence between miRNA and 3'UTR of a target gene using the base-pairing rule and other features of this algorithm [28]. In mammals, predictions are ranked based on the predicted efficacy of targeting as calculated using cumulative weighted context and scores of the sites that are free energy [29]. MiRTar2GO is designed to predict miRNA target sites using more relaxed miRNA–target binding characteristics, ranks the candidate interactions based on MFE of hybridization as a primary parameter miRTar2GO against other widely used miRNA target prediction algorithms and demonstrates that miRTar2GO produced significantly higher F1 and G scores [30]. MiRDB is the online database for miRNA target prediction and functional annotation, predicted by a bioinformatics tool, MirTarget, which was developed by analyzing thousands of miRNA--target interactions from high-throughput sequencing experiments [31-32]. MiRanda is a resource for microRNA target predictions and microRNA expression, primarily in the 3′-UTR regions. The lower mirSVR score is a method for ranking microRNA target sites by a down-regulation score. The algorithm trains a regression model on the sequence and contextual features extracted from MiRanda-predicted target sites. In a large-scale

evaluation, MiRanda-mirSVR is competitive with other target prediction methods in identifying target genes and predicting the extent of their downregulation at the mRNA or protein levels [33-34]. DIANA-microT-CDS is the 5th version of the micro-T algorithm. It is specifically trained on a positive and a negative set of miRNA Recognition Elements (MREs) located in both the 3'-UTR and CDS regions. The prediction of candidate miRNA was applied according to the following selection criteria including or exhibiting greater than or equal to 4 in 5 prediction tools and ranks of high context^{++} score, maximum MFE value, high miTG score, and high mirSVR score.

Results

Prediction Of KRAS-Specific miRNAs

The prediction results of KRAS-specific miRNAs using five prediction tools including TargetScanHuman, miRTar2GO, miRDB, MiRanda, and Diana tools were performed. The results showed a total of 913 miRNAs, 63 miRNAs, 147 miRNAs, 330 miRNAs, 47 miRNAs, and 326 miRNAs, respectively. The application of candidate miRNAs was decided and selected according to our established criteria. When using 4 of 5 prediction tools, we found 8 miRNAs including miR-143-3p, miR-155-5p, miR-193a-3p, miR--30a-5p, miR-30d-5p, miR-30e-5p, miR-543 and miR-877-5p, respectively. The prediction of miRNAs from all prediction tools showed similar candidate miRNAs for miR-206.

Prediction of EGFR-specific miRNAs

The prediction results of EGFR-specific miRNAs using five prediction tools including TargetScanHuman, miRTar2GO, miRDB, MiRanda, and Diana tools were performed. The results showed a total of 635 miRNAs, 36 miRNAs, 146 miRNAs, 166 miRNAs, 34 miRNAs, and 253 miRNAs, respectively. The application of candidate miRNAs was decided and selected according to our established criteria. When using 4 of 5 prediction tools, we found 9 miRNAs including miR-302a-3p, miR-302b-3p, miR-302d-3p, miR-302e, miR-520a-3p, miR-520b, miR-520c-3p, miR-7-5p and miR-

-875-5p, respectively. The prediction of miRNAs from all prediction tools showed similar candidate miRNAs for miR-133b.

Discussion

Currently, there are many challenges and excitement in the field of miRNA research. MiRNAs have been suggested to play a key regulatory role in numerous processes of cancer. More than 50 per cent of miRNA genes are located in cancer-associated, genomic regions or fragile sites, which suggests that miRNAs may play a more important role in the pathogenesis of human cancers [35]. MiRNA regulates the progression of the tumour by regulating target genes. Therefore, it is of great significance to study the expression profile of miRNAs and predict the target genes in colon cancer [36]. Our result showed the possibility of miRNA associated with the target gene. Further research on circulating microRNA should be performed. The prediction miRNAs associated with KRAS gene result found that miR-206, miR-143--3p, miR-155-5p, miR-193a-3p, miR-30a-5p, miR-30d-5p, miR-30e-5p, miR-543 and miR-877-5p. Prediction miRNAs associated EGFR gene result found that miR-133b, miR-302a-3p, miR-302b-3p, miR-302d-3p, miR-302e, miR-520a-3p, miR-520b, miR-520c-3p, miR-7-5p and miR-875-5p. The selected miRNAs were reported to be associated with the prognosis of CRC. Previous studies have shown that a unique role of miR-206 in 5-FU resistance in CRC is associated with reduced survival of colon cancer patients and supports the development of miR-206 mimic as a potential target for reversing drug resistance [37].

MicroRNA-143-3p was reported to be suppressed in colorectal cancer metastases and tumorigenesis [38-39]. In addition, it has been reported that miR-143-3p may be suppressed in pancreatic ductal adenocarcinoma by targeting KRAS [40], and miR-143 has grown inhibitory anti-metastatic effect on breast cancer [41]. Moreover, miRNA-143 was reported to be aberrantly downregulated in gastric cancer cell lines. Ectopic expression of miRNA-143 resulted in a significant inhibition of AGS gastric cancer cell proliferation suggestive of the tumor-suppressive role of miRNA-143 [42].

MicroRNA-193a-3p was decreased in CRC cell lines, and upregulation of miR-193a-3p inhibited tumour development and progression in vitro through regulating cell growth, migration, and angiogenesis partly through targeting the PLAU pathway [43], miR-193a-3p could suppress proliferation and promote apoptosis by targeting CCND1 in HCC cells [44]. In addition, it has been reported that miR-193a-3p was reported in the potential tumour suppressor function of miR-193a-3p in PDAC progress by targeting CCND1 [45]. MicroRNA-543 was reported that act as an oncogene to promote tumor cell invasion, proliferation, and migration of CRC [46], hepatocellular carcinoma [47], gastric cancer [48], oral squamous cell carcinoma [49], and nasopharyngeal carcinoma [50]. Moreover, miRNA-543 revealed promotes proliferative ability in AGS and MKN45 cells. Interestingly, miRNA-543 participated in cell proliferation and metastasis by targeting PIAS3 in CRC [51-52]. In addition, it has been reported that miR-543 enhances the resistance of CRC cells to 5-FU and that the downregulation of miR-543 increases the sensitivity of CRC cells to 5-FU by suppressing the PTEN/PI3K/AKT signalling pathway [53]. MicroRNA-302a/b/c and microRNA-133b were reported to act as tumour suppressors to inhibit metastasis and cetuximab resistance of CRC [54], tumour cell angiogenesis, proliferation, and promote apoptosis of hepatocellular carcinoma [55-56], chronic myeloid leukaemia [57], bladder cancer [58], and oesophagal squamous cell carcinoma [59]. Furthermore, miR-133b was reported to up-regulate miR-133b reduce cell proliferation and colony formation, induce cell apoptosis and G0/G1 phase arrest, and decrease cell invasion by inhibiting SOX9/b-catenin signaling [60].

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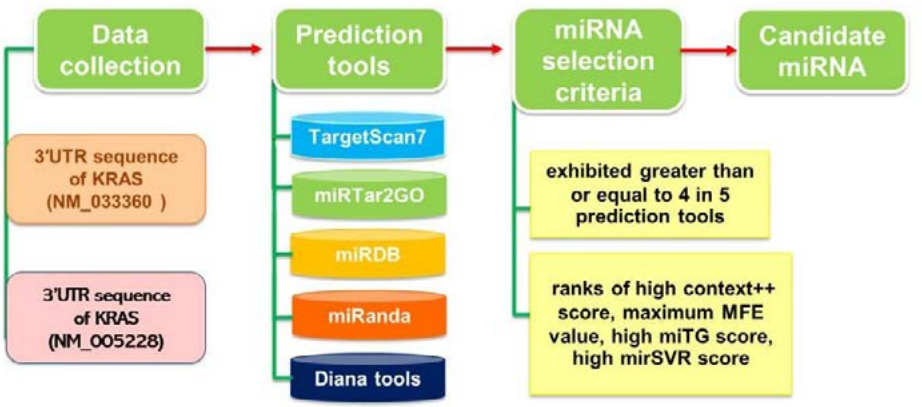

Figure 1. Conceptual framework of the computational bioinformatic prediction tools on interested target genes in colon cancer. A couple of interested EGFR and KRAS genes show significant candidate miRNA by using five prediction tools.