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Integration of genetic variants and gene network for drug repurposing in colorectal cancer

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ABSTRACT

Even though many genetic risk loci for human diseases have been identified and comprehensively cataloged, strategies to guide clinical research by integrating the extensive results of genetic studies and biological resources are still limited. Moreover, integrative analyses that provide novel insights into disease biology are expected to be especially useful for drug discovery. Herein, we used text mining of genetic studies on colorectal cancer (CRC) and assigned biological annotations to identified risk genes in order to discover novel drug targets and potential drugs for repurposing. Risk genes for CRC were obtained from PubMed text mining and for each gene, six functional and bioinformatic annotations were analyzed. The annotations include missense mutations, cis-expression quantitative trait loci (cis-eQTL), molecular pathway analyses, protein-protein interactions (PPIs), a genetic overlap with knockout mouse phenotypes, and primary immunodeficiency (PID). We then prioritized the biological risk candidate genes according to a scoring system of the six functional annotations. Each functional annotation was assigned one point, and those genes with a score ≥2 were designated "biological CRC risk genes". Using this method, we revealed 82 biological CRC risk genes, which were mapped to 128 genes in an expanded PPI network. Further utilizing DrugBank and the Therapeutic Target Database, we found 21 genes in our list that are targeted by 166 candidate drugs. Based on data from ClinicalTrials.gov and literature review, we found four known target genes with six drugs for clinical treatment in CRC, and three target genes with nine drugs supported by previous preclinical results in CRC. Additionally, 12 genes are targeted by 32 drugs approved for other indications, which can possibly be repurposed for CRC treatment. Finally, analysis from Connectivity Map (CMap) showed that 18 drugs have a high potential for CRC.

Abbreviations: BRAF, B-Raf proto-oncogene; CCND2, Cyclin-D2; CRC, Colorectal cancer; CMap, Connectivity map; COX-1, Cyclooxygenase-1; COX-2, Cyclooxygenase-2; CML, Chronic myeloid leukemia; Cis-eQTL, Cis-expressionquantitative trait loci; CYP1A1, Cytochrome P450 Family 1 Subfamily A Member 1; FDA, Food and Drug Administration; FAP, Familial adenomatous polyposis; GWAS, Genome-wide association study; GO, Gene ontology; HER-2, Human epidermal growth factor receptor 2; IUIS, International Union of Immunological Societies; IL17F, Interleukin 17F; KEGG, Kyoto Encyclopedia of Genes and Genomes; KRAS, Kirsten rat sarcoma; LD, Linkage disequilibrium; MLH1, MutL homolog 1; MSI, Microsatellite instability; NHGRI, National Human Genome Research Institute; NOD2, Domain-containing protein 2; NSAIDs, Nonsteroidal anti-inflammatory drugs; NRAS, Neuroblastoma RAS; PARP, Poly (ADP-ribose) polymerase; PMID, PubMed IDentifier; PPI, Protein-protein interaction; PID, Primary immunodeficiency; PI3K, Phosphatidylinositol 3-kinase; PIK3CA, Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; SNPs, Single nucleotide polymorphisms; SSRIs, Selective serotonin-reuptake inhibitors; TGFB1, Transforming growth factor beta-1; TP53, Tumor protein p53; VEGF, Vascular endothelial growth factor; VDR, Vitamin D receptor; WES, Whole exome sequencing.

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1. Introduction

Colorectal cancer (CRC) is the second most common cause of cancer morbidity and mortality, and it occurs as the fourth most frequent in males and the third most frequent in females globally [1]. Transformation of CRC is the result of a progressive accumulation of genetic and epigenetic alterations. Once the CRC is diagnosed, patient outcomes are largely dependent on the disease stage, with early-stage disease generally showing better outcomes after treatment, and advanced disease characterized as clinically aggressive [2]. Thus, more effective anticancer drugs are required, as treatment approaches have been limited [2]. Bringing a new drug through clinical trials to the market is time consuming and expensive, often requiring ~15 years and > US$1 billion [3]. Currently, more than 10,000 clinical trials for cancer are registered at www.clinicaltrials.gov, however, only a few drug candidates continue to subsequent phases [4]. In the end, only around 5 % of new molecules entering phase I clinical trials will eventually be approved by the U.S. Food and Drug Administration (FDA) for disease treatment [5]. Thus, the approach of drug repurposing, which consists of finding new uses for old drugs as therapeutic agents, has become increasingly popular. Advantages of drug repurposing include clear understandings of the drug's safety, pharmacological mechanisms and pharmacokinetic profiles [6]. One example of clinically successful drug repurposing is the use of aspirin for cardiovascular disease and CRC prevention; aspirin was initially used for pain relief [7,8]. Additionally, sildenafil was originally used to treat high blood pressure, but was repurposed for erectile dysfunction [9]. Therefore, drug repurposing based on genomic information may be a promising strategy to improve the efficiency of drug discovery process.

In order to find candidates for drug repurposing, the relationships between diseases and genes, genes and drugs, and diseases and drugs should be identified. One strategy to identify these relationships is literature searching for pathway prioritization [10]. For example, candidate genes can be prioritized using keywords to perform text mining on a database.The database contains a large-scale genomic information that may shed light on germline risk alleles in patients with disease [10,11]. In addition, the PubMed database contains more than 24 million citations from MEDLINE and other biomedical literature data sources [12]. In our study, we queried the PubMed database to identify candidate risk genes. Then, we utilized six bioinformatics repositories to identify the genes that most likely to have biological impact in CRC etiology, and we also identified relationships between those CRC genes and existing drugs. The current study design was adopted from Okada et al., who predicted drugs for repurposing to rheumatoid arthritis by utilizing risk genes of rheumatoid arthritis [13]. Notably, the genomic information from genome-wide association study (GWAS) was utilized by an *in silico* pipeline to study drug repurposing for type 2 diabetes and cancer [14,15].

Germline mutations that alter risks of CRC have been previously identified and used to search for target drugs [16,17]. However, successful translation of this basic research into clinical practice has been limited, and remains as a future challenge. Therefore, in this study, we determined candidate genes associated with CRC and further prioritized the candidate genes using a scoring system based on six *in silico* criteria. As a result, we identified candidate biological risk genes for CRC that provide information to guide the selection of drugs for repurposing to CRC.

2. Materials and methods

Integrative analyses of genetics-driven genomic drug repurposing for CRC by utilizing multiple databases are shown in Fig. 1. Single nucleotide polymorphism (SNPs) associated with CRC (CRC-associated SNPs) were obtained from PubMed text mining and expanded using HaploReg (v4.1) with a criterion of $r^2 > 0.8$. The genes corresponding to CRC-associated SNPs are denoted as "CRC-associated genes". Subsequently, genomic data were prioritized based on six functional annotation criteria. Those genes with one functional annotation were awarded one point (score) and those genes with a score ≥2 were classified as "biological CRC risk genes". The biological CRC risk genes were utilized in further analyses, including the use of the STRING database [18] to expand the list of candidate genes as the drug target genes. We mapped the expanded list of genes according to targets of approved drugs which found in the DrugBank and Therapeutic Target Database. The drug targets were examined to determine their clinical status according to ClinicalTrials.gov. Finally, we used the CMap database to prioritize the most promising candidate drugs for repurposing to CRC treatment.

2.1. Literature review by PubMed text mining

CRC-associated SNPs were identified through a systematic search of the PubMed database (articles published 2014–2018). The search terms used were: *"colorectal cancer", "risk gene", "susceptibility", "polymorphism", "SNP", and "genotype"*. Inclusion of data was based on the following criteria: 1) all samples were human samples; 2) the clinical phenotype was "CRC"; and 3) the identified SNPs were associated with CRC.

2.2. CRC–associated genes

CRC-associated SNPs are extended to the SNPs in high LD ($r^2 > 0.8$ in Asian population based on 1000 genome project) with the SNPs

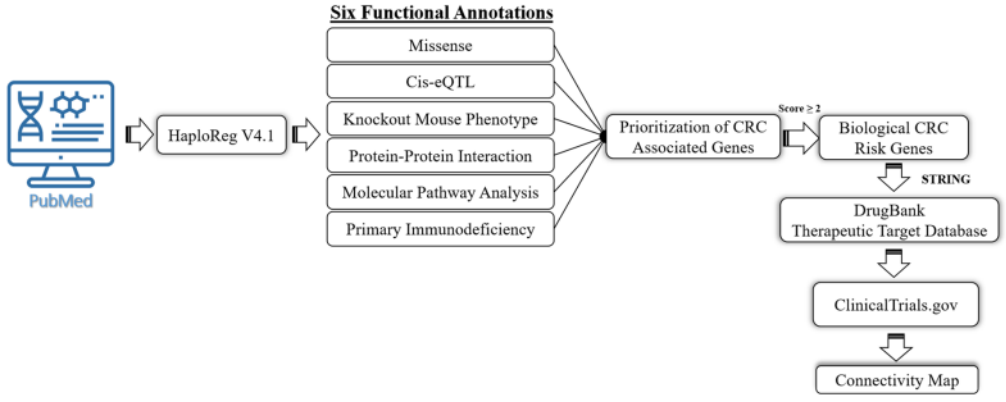

Fig. 1. This schematic model illustrates that genomic-based information cane be integrated for colorectal cancer (CRC) drug repurposing.

identified in the previous step using HaploReg v4.1 [19]. The LD criterion of $r^2 > 0.8$ was adopted from the study of Okada et al [13].

2.3. Functional annotation of CRC-associated genes

In order to prioritize the genes according to their biological function, six annotations were used to construct a scoring system that reflects the most likely candidate genes as CRC targets. The first annotation was missense or nonsense mutation according to HaploReg v4.1, which contains annotations of the functional consequences from a database of (n)SNPs [20]. HaploReg v4.1 also links genetic variants to cis-expression quantitative trait loci (cis-eQTL). Moreover, we examined the cis-eQTL in target tissues of whole blood or colon [20]. After that, in order to understand the connections between mutant genes and phenotypes, we employed WebGestalt 2017 to perform functional enrichment analyses [21]. Mammalian Phenotype (MP) Ontology, which contains information on phenotypes of mice and other mammals, was used [22]. To query the mouse phenotype, we converted genes from the human Ensemble ID to mouse Ensembl ID using BioMart [23]. Genes enriched in mouse phenotypes with a false discovery rate (*FDR*) of < 0.05 were considered significant results. Furthermore, protein-protein interactions (PPIs) were used to identify CRC-associated genes enriched in biological protein networks [24]. WebGestalt 2017 was also used to perform enrichment analyses and investigate if the genes were enriched in any specific functional pathway [25]. Specifically, the biological process Gene Ontology (GO) categories in WebGestalt 2017 were interrogated for this step. Significance of a result was set at *FDR* < 0.05 [25]. To determine what kind of molecular pathways were enriched on the CRC-associated gene list and which gene was involved, enrichment analyses was performed on molecular pathways utilizing the Kyoto Encyclopedia of Genes and Genomes (KEGG), an online pathway database of biochemicals from WebGestalt 2017 [25]. Primary immunodeficiency (PID) genes are the last annotation criterion. PID diseases are innate immune diseases [26] and are often reported to associate with cancer [27]. PID diseases are known as a genetic disorders associated with an increased incidence of malignancies, such as lymphoproliferative disorders, gastric adenocarcinoma, and CRC [28].]. A hypergeometric test was used to perform an enrichment analysis on these data; the criterion for significance was *p*-value < 0.05.

2.4. Prioritization of biological candidate genes

According to these functional annotations, a score was assigned each gene. One point was awarded for each criterion, as follows: 1) genes with missense or nonsense variants; (2) cis-eQTL genes of risk SNPs; (3) genes prioritized by the Knockout Mouse Phenotype; (4) genes prioritized by PPI network; (5) Molecular Pathway; (6) PID. Each gene was scored according to the number of criteria that were satisfied (scores ranged 0–6 for each gene). Genes with a score of ≥2 were categorized as 'biological CRC risk genes'. We set the threshold of biological score ≥ 2 to find much higher number of the biological CRC risk genes.

2.5. STRING database

The purpose of the STRING database (http://string-db.org/) is to identify functional interactions related to protein expression by integrating predicted protein-protein association data [18]. A large number of disease-protein networks may provide targets for diseases [29]. The proteins in direct PPI with these encoded by biological CRC risk genes were identified using STRING database version 10.5 (https://string-db.org/cgi/network,). The genes encoding these protein were defined as "drug target gene". Protein products from the identified biological CRC risk genes, or several genes from a direct PPI network, were explored as potential targets of approved drugs, either for CRC or other indications.

2.6. Drug validation and discovery

The DrugBank database (https://www.drugbank.ca/) and Therapeutic Targets Database (http://bidd.nus.edu.sg/BIDD-Databases/TTTD/TTD.asp) were utilized to evaluate potential drug targets. Drug target genes were used to interrogate the databases according to several criteria, such as drugs with pharmacological activities, effectiveness in humans, and annotations of 'approved', 'clinical trial' or 'experimental drugs'. The identified drugs were reviewed on ClinicalTrials.gov (https://clinicaltrials.gov) to identify any clinical investigations for CRC or other diseases.

2.7. Connectivity map (CMap) analyses

To prioritize the list of drugs for CRC repurposing, we used the CMap touchstone database platform to rank drugs according to a connectivity score (-100 to 100) [30]. The CMap scores were obtained from the 'Touchstone' tool in the CLUE website (https://clue.io/). The CMap database can be used to perform profile similarity analyses, which is based on a collection of genome-wide transcriptional expression profiles from cultured human cancer cells treated with different drug compounds [31]. In this study, drugs were compared with capecitabine as standard treatment for CRC according to the National Comprehensive Cancer Network (NCCN) guideline [32].

2.8. Statistical analyses

All analytic workflows were performed using R studio v3.4.3 and the haploR package (https://www.r-project.org/ and https://cran.r-project.org/web/packages/haploR/index.html). Over-representation analysis (ORA) was used to prioritize the genes in Knockout Mouse Phenotype, PPI network and Molecular Pathway. The *FDR* of <0.05 was determined to indicate the statistical significance. A hypergeometric test was used to perform an enrichment analysis for PID; the criterion for significance was *p*-value < 0.05.

3. Results

We retrieved SNPs associated with CRC from PubMed text mining. Forty CRC-associated studies were collected from genotyping studies and GWAS (Table 1). In total of 170 unique SNPs were found to associate with CRC risk (**Table S1**). The number of SNPs was expanded based on LD, using HaploReg v4.1 with criterion of $r^2 > 0.8$, and this expansion yielded 210 CRC-associated genes **(Table S2)**.

3.1. Functional annotations of CRC risk SNPs

In order to prioritize genes according to known biological processes, we devised six biological functional annotations based on 210 CRC-associated genes and assigned one point for each functional annotation. The results of six biological functional annotations as shown in **Table S2** and Fig. 2; we determined that 29 out of 210 genes (13.80 %) were missense or nonsense variants. We then assessed the cis-eQTL effects with HaploReg v4.1, using this strategy, we found 101 genes (48.09 %) risk SNPs have cis-eQTL effects on 210 genes, in either whole blood or colon tissues. Phenotype data were then retrieved from MP Ontology, which contains information on phenotypes of mice and other mammals. WebGestalt 2017 was used to perform an ORA, and 24 genes (11.43 %) were found to overlap with CRC risk genes (*FDR* < 0.05). Gene Ontology (GO) annotations obtained from WebGestalt 2017 were then used to evaluate PPIs. According to the PPIs, 95 genes (45.24 %) that overlapped with other CRC risk genes (*FDR* < 0.05) were found. The Kyoto Encyclopedia of Genes and Genomes (KEGG) was used to perform an ORA on molecular pathways. A total of 49 CRC-associated genes (23.33 %) in KEGG pathways were successfully identified by the enrichment analysis. We also analyzed PID data from the IUIS and subsequently confirmed

Table 1
Profiles of colorectal cancer (CRC) reports in this study.

No	Type of the Study	Population	Source of Study (PMID)
1	WES	Chinese	30018674
2	GWAS	Italian	28915899
3	GWAS	European	24737748
4	Genotype	Saudi Arabian	25811207
5	GWAS	East Asian, Chinese, Japanese Korean	26965516
6	GWAS	East Asian	24836286
7.	Genotyping	Saudi Arabian	30233234
8.	GWAS	Chinese	26515597
9	GWAS	Japanese	25105248
10	Genotyping	Chinese	27698911
11	GWAS	Chinese	27145994
12	GWAS	Chinese	28218435
13	ChIP-Seq, DNase-Seq	Chinese	29267898
14	GWAS	East Asian	24448986
15	Genotyping	Korean	23875689
16	GWAS	European-Asian	23946381
17	GWAS	European	25990418
18	Genotyping	Chinese	29428571
19	Genotyping	Taiwanese	24968322
20	Genotyping	Tunisians	29689450
21	Genotyping	Iran	29953646
22	Genotyping	European	29372689
23	Genotyping	Chinese	29419695
24	GWAS	NHGRI	27146020
25	Genotyping	Chinese	29766219
26	Genotyping	German	29119627
27	Genotyping	Chinese	28915636
28	Genotyping	Chinese	28039327
29	Genotyping	Saudi Arabia	27746584
30	Genotyping	Chinese	27665685
31	Genotyping	Korea	27354594
32	Genotyping	Chinese	26547791
33	GWAS	Chinese	26148620
34	Genotyping	Tunisian	26083022
35	Genotyping	Chinese	25834816
36	Genotyping	Iran	25680555
37	GWAS	Iran	25640388
38	Genotyping	Chinese	25222241
39	Genotyping	Chinese	24777809
40	Genotyping	African Americans	24562971

Abbreviations: ChIP-Seq: Chromatin immunoprecipitation-sequencing, CRC: Colorectal cancer, DNase-seq: DNase I hypersensitive sites sequencing, GWAS: Genome-wide association study, WES: Whole exome sequencing, NHGRI: National Human Genome Research Institute.

overlapping genes with our findings. Five genes (2.38 %) with statistically significant overlap in PID ($p < 0.05$) were identified. After compiling the scores from 0-6, we found that genes with a score of 0 and 1 have the same numbers of genes, 64 genes, respectively. Genes with a score of 2 were 32 while those with a score of 3 were 30. There were 16 genes with a score of 4, three genes with a score of 5 and one gene with a score of 6 (Fig. 3). In total, we obtained eighty-two genes with a score of ≥2, which were categorized as 'biological CRC risk genes' (Table 2). Here are the top five of biological CRC risk genes which were domain-containing protein 2 (NOD2), followed by MutL homolog 1 (MLH1), transforming growth factor beta-1 (TGFB1), tumor protein p53 (TP53) and interleukin 17 F (IL17 F).

3.2. Expanding the list of biological CRC risk genes

We used the STRING database of PPIs to expand the number of biological CRC risk genes. After expansion with the STRING database, 128 genes were included in the list (**Table S3**). These genes comprised the final list of candidate genes, which were used for futher analysis.

3.3. Discovery of CRC drug targets

We obtained 1108 gene pairs from curated PPI networking (**Table S4**) and found21 target genes in DrugBank and the Therapeutic Target Database (**Table S5**). The 166 drugs are either approved, in clinical trials, or experimental drugs for human diseases (**Table S6**). Four target genes with six drugs available used for CRCwere identified: genistein, marimastat and bevacizumab, dalteparin, aflibercept and tegafur uracil (Fig. 4). Importantly, three drugs (bevacizumab, aflibercept and tegafur uracil) are the most frequently used as chemotheraputic drugs for the treatment of CRC. Further, correlations of drugs approved for other diseases with biological CRC risk genes were also assessed. We found 12 known targets with 32 drugs approved for other diseases, but areunder clincal investigation for CRC now.These drugs may have potential to be repositioned for treatment of CRC (Fig. 5). An example is dasatinib, which is approved for treating chronic myeloid leukemia (CML), but has been repurposed for CRC treatment in clinical trials. Another drug, genistein, cytochrome P450 family 1 subfamily a member 1 (CYP1A1) targeting agent, is also a promising drug for treatment of CRC. Genistein may inhibit cancer cell growth by blocking Wnt signaling required for CRC cell growth **(Table S7**). In addition, we also identified five non-steroidal anti-inflammatory drugs (NSAIDs) and four selective serotonin reuptake inhibitors (SSRIs), which have been widely reported in the preclinical *in vitro* or *in vivo* (CRC model) investigations for repurposing to CRC (Fig. 6). Especially, NSAIDs include balsalazide, tolfenamic acid, adapalene, sulindac, and parecoxib

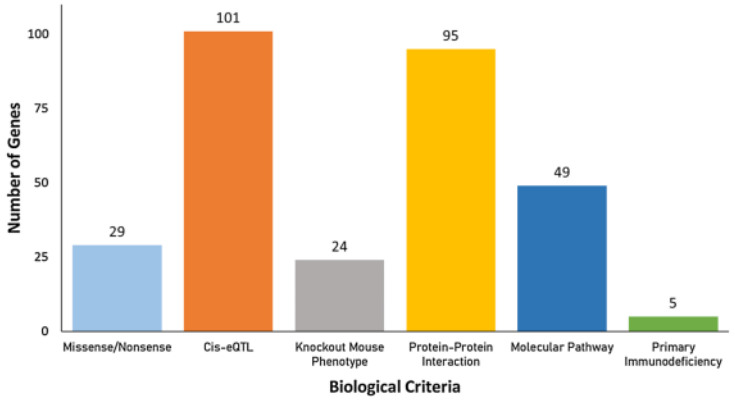

Fig. 2. Bar chart shows the number of genes (*y*-axis) satisfying each of the six biological criteria (*x*-axis) for drug prioritization.

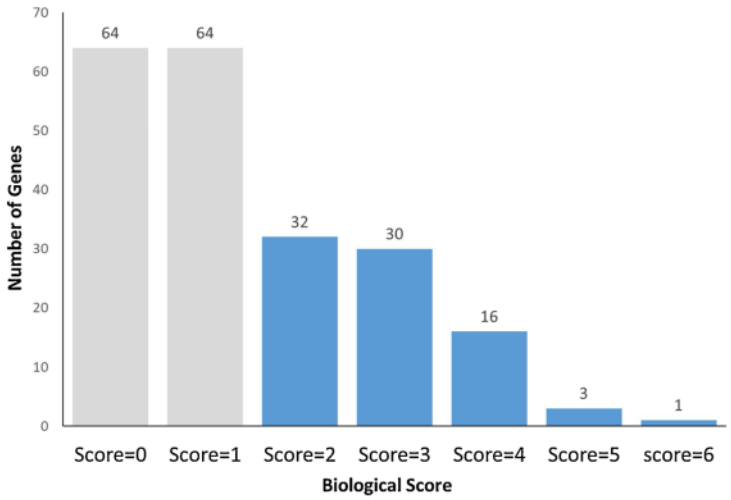

Fig. 3. Distribution plot shows the number of genes (*y*-axis) with different biological (gene) scores (*x*-axis). After compiling the scores, 82(=32+30+16+3+1) genes with a score of ≥2 were categorized as 'biological CRC risk genes' (blue).

Table 2
Prioritized biological annotations for colorectal cancer (CRC) genes with a score ≥ 2.

GENCODE_ID	GENCODE_name	Missense	Cis-eQTL	KO mice	PPI	KEGG	PID	Total Score
ENSG00000167207	NOD2	1	1	1	1	1	1	6
ENSG00000076242	MLH1	1	1	1	1	1	0	5
ENSG00000105329	TGFB1	1	1	1	1	1	0	5
ENSG00000141510	TP53	1	1	1	1	1	0	5
ENSG00000112116	IL17F	1	0	0	1	1	1	4
ENSG00000113318	MSH3	1	1	0	1	1	0	4
ENSG00000135862	LAMC1	1	1	0	1	1	0	4
ENSG00000073756	PTGS2	0	1	1	1	1	0	4
ENSG00000088305	DNMT3B	0	1	0	1	1	1	4
ENSG00000095303	PTGS1	0	1	1	1	1	0	4
ENSG00000112115	IL17A	0	1	1	1	1	0	4
ENSG00000132781	MUTYH	1	0	1	1	1	0	4
ENSG00000134899	ERCC5	1	1	0	1	1	0	4
ENSG00000143799	PARP1	0	1	1	1	1	0	4
ENSG00000149485	FADS1	0	1	1	1	1	0	4
ENSG00000150093	ITGB1	0	1	1	1	1	0	4
ENSG00000164362	TERT	0	0	1	1	1	1	4
ENSG00000183765	CHEK2	1	0	1	1	1	0	4

Abbreviations: Missense: Missense mutation; Cis-eQTL: Cis expression quantitative trait loci, KO mice: Knockout mouse phenotype; PPI: Protein-protein interaction; KEGG: Kyoto Encyclopedia of Genes and Genomes; PID: Primary immunodeficiency.

[33–36], and SSRI drugs include amitriptyline, fluoxetine, paroxetine and sertaline [37–40] (**Table S8**). Therefore, our results suggest that this combinational analysis of PubMed text-mining and six functional annotations can efficiently identify potential candidate drugs for CRC drug repurposing.

3.4. Connectivity Map (CMap) analyses prioritize the most promising drugs for CRC

To prioritize the most potential candidate drugs for CRC, we analyzed 41 drugs (32 drugs under clinical and 9 drugs preclinical investigation for CRC) using the CMap database. If two drugs have a strongly positive relationship, they may have similar effects in treating CRC. We found that 18 of 41 drugs had positive correlations with capecitabine, the standard treatment for CRC, according to the high CMap scores (Table 3). Among the 18 drugs, nine drugs (celecoxib, ibuprofen, naproxen, balsalazide, aspirin, sulindac, rofecoxib, diclofenac and parecoxib) target cyclooxygenase (COX)-1 and COX-2, three drugs (sertraline, fluoxetine and amitriptyline) target SLC6A4, two drugs (rucaparib and olaparib) target poly (ADP-ribose) polymerase (PARP), and one drug (dasatinib) targets cyclin-D2 (CCND2). As depicted in Table 3, the top five drugs that exhibited strong relationships with capecitabine were celecoxib, rucaparib, ibuprofen, naproxen and balsalazide, with respective CMap scores of 99.09, 94.14, 92.25, 86.40, and 81.55. This finding suggests the biological plausibility of repurposing cancer drugs from non-cancer drugs or other drugs for CRC

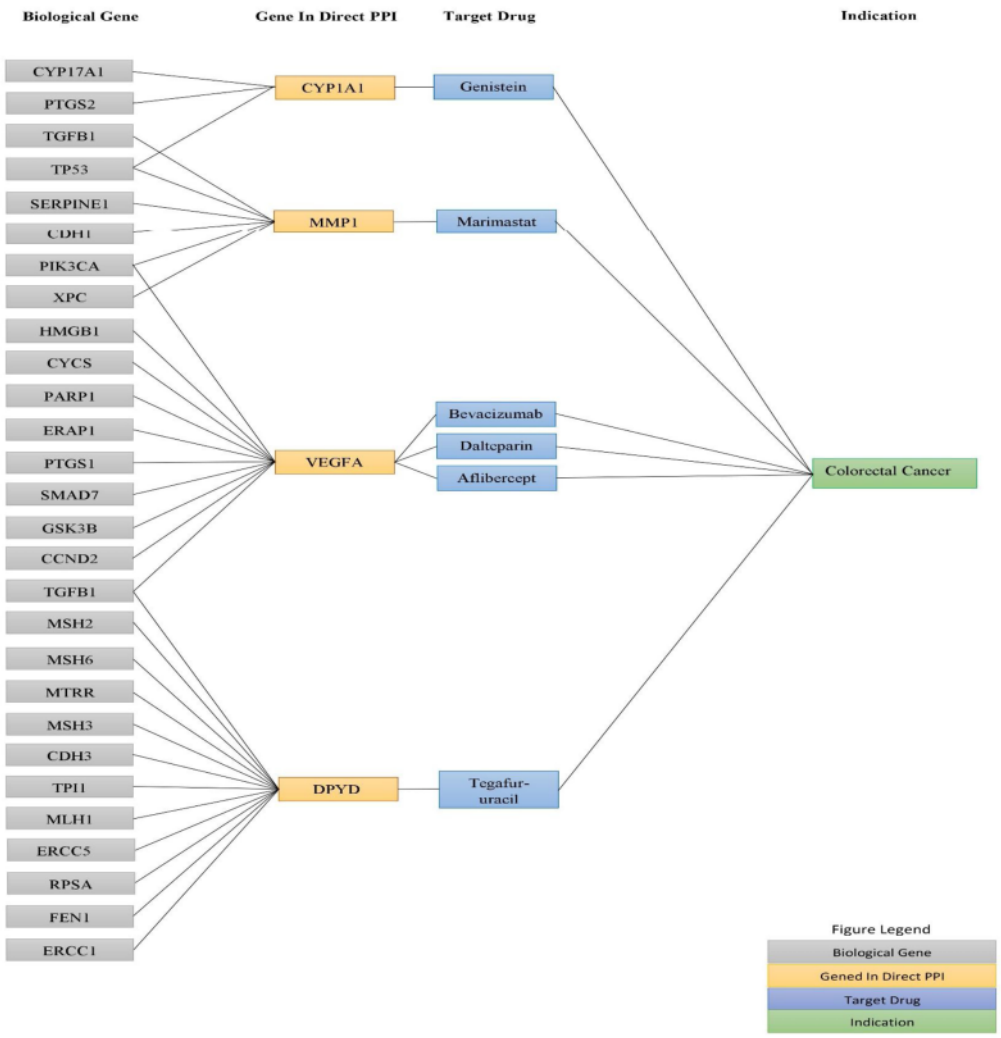

Fig. 4. Connections between biological CRC genes, genes from protein-protein ineteractions (PPIs, and drugs available for CRC.

treatment.

4. Discussion

In the current study, we mined PubMed texts to extract potential candidate genes that may guide drug repurposing to CRC. Six functional annotations were applied to build a scoring system and to prioritize CRC risk genes which are likely to be useful drug targets. We found 128 genes from the expanded PPI network, including four known targets of six clinical CRC drugs, 12 known targets for 32 drugs under clinical investigation for CRC. In addition, three known target genes assocaited with CRC can be modulated by nine drugs. Finally, by using the CMap database, we observed that18 drugs might have high potential to be repurposed for CRC. We also found six drugs, including genistein, marimastat, bevacizumab, dalteparin, aflibercept, and tegafur-uracil, that are currently in clinical trials for CRC. Genistein [NCT01985763] and Aflibercept [NCT03264274] are currently in a phase 2 clinical trial for treatment of metastatic CRC. Xeloda or UFT (tegafur-uracil) with folinic acid is in a phase 3 investigation for metastatic CRC [NCT00905047]. Bevacizumab is in a phase 2 clinical trial for advanced CRC in combination with irinotecan, lederfolin, and 5FU (FOLFIRI) [NCT01853813]. Dalteparin was assessed by a double-blinded phase 3 randomized control trial for treating advanced cancers, including breast, lung and colorectal [NCT00003674].

Although adjuvant therapy is available for patients with high-risk stage II and stage III CRC, around 40–50 % of patients experience recurrence with the development of metastatic disease [41]. For advanced CRC, targeting the vascular endothelial growth factor (VEGF) pathway has a pivotal role in increasing overall survival time in patients receiving chemotherapy [42]. Herein, VEGF is one of the drug targets in this study; VEGF can be targeted by bevacizumab, dalteparin, and aflibercept. Two of these drugs (bevacizumab and aflibercept) have been approved for use in metastatic CRC [43]. Indeed, most drugs that we found have indications for other cancer types. 32 drugs identified in this category are depicted in Fig. 5 (*e.g.*, ovarian cancer [rucaparib, niraparib, olaparib], non-small cell lung cancer [pemetrexed], prostate cancer [abiraterone], osteosarcoma [mifamurtide], CML [dasatinib], multiple myeloma [pomalidomide]). Another set of drugs identified in our study are currently used for autoimmune disease (*e.g.*, ankylosing spondylitis [etanercept, adalimumab, infliximab, golimumab,

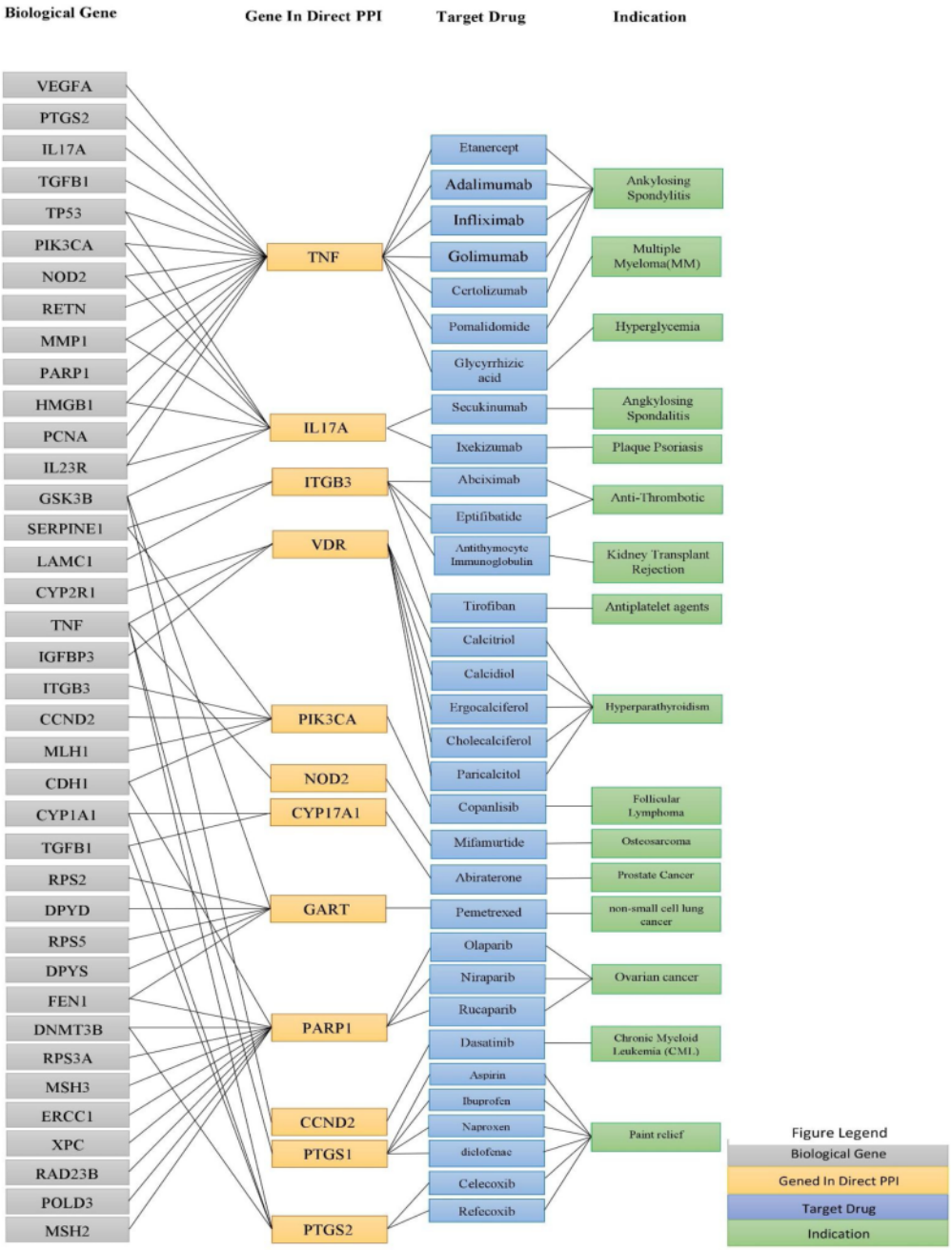

Fig. 5. Connections between biological CRC genes with the drugs that approved for other indications and are under clinical investigation for CRC.

secuximab]). Surprisingly, one drug we identified here as potentially useful for CRC is vitamin D. Vitamin D deficiency may affect various human cancers, and the Vitamin D receptor (VDR), which plays a role in regulating active vitamin D metabolites, is highly expressed in the small intestine and colon [44]. Vitamin D has been shown to protect from CRC tumorigenesis by binding long-chain fatty acids and bile acids in the small intestine and protecting colonic epithelial cells from mutagens [45]. Notably, Vitamin D-related drugs are currently being evaluated in CRC: cholecalciferol is in a phase 2 trial [NCT01074216]; cholecalciferol [NCT02172651] and paricalcitol [NCT01197664] are in phase 1 trials. Furthermore, we identified calcitriol as a promising drug for CRC repurposing based on the CMap analysis (Table 3). However, molecular evidence for the role of vitamin D in CRC is limited, so future functional studies are needed to investigate the mechanisms of how vitamin D affect CRC.

Another category of drugs identified in our study is NSAIDs, which

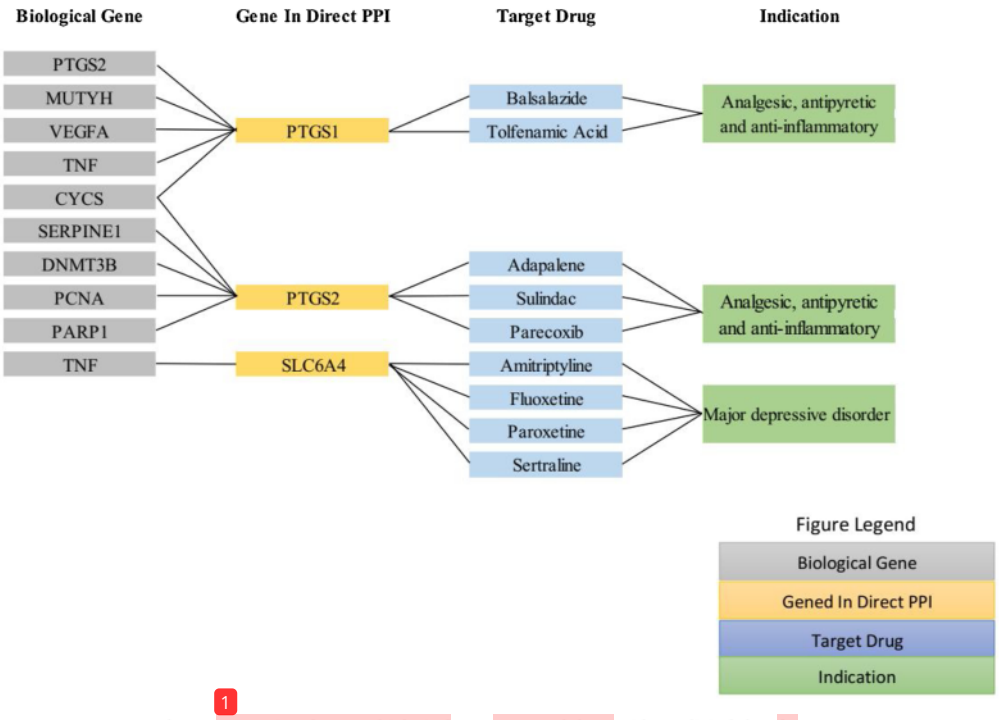

Fig. 6. Connections between biological CRC genes and drugs with preclinical data for CRC.

generally hold indications as anti-inflammatory agents [46]. From the results of a nationwide population-based case–control study, Kuo et al., indicated that NSAIDs use was associated with lower incidence of colorectal cancer [47]. According to the CMap analysis we performed in this study, nine NSAIDs (celecoxib, ibuprofen, naproxen, balsalazide, aspirin, sulindac, rofecoxib, diclofenac and parecoxib) were among the most promising for CRC repurposing. Furthermore, NSAIDs were also reported to reduce the growth of cancer cells through inhibition of two enzymes, COX-1 and COX-2 [48]. Aspirin is commonly used in cardiovascular disease treatment and to prevent myocardial infarction. Previous clinical studies indicated that post-diagnosis use of aspirin is correlated with favorable survival in CRC patients [49,50]. Ibuprofen was also reported to effectively inhibit the growth of prostate cancer [51]. In an *in vitro* study on adenocarcinoma gastric cells, the antitumor activity of ibuprofen was attributed to the induction of apoptosis and the reduction of cell proliferation, which attenuated angiogenesis [52]. Naproxen is a propionic-acid derivative and non-selective COX-inhibitor that was shown to inhibit cell proliferation, trigger apoptosis and suppress metastasis. *In vitro* and *in vivo* studies revealed that naproxen also had antineoplastic properties in leukemia, breast, colon, bladder, and osteosarcoma cell lines [53]. Furthermore, according to an *in vivo* study on a colon cancer animal model, the combination of naproxen and atorvastatin significantly inhibited growth of colonic adenocarcinomas [54]. Diclofenac is a derivative of acetic acid that has a moderate selectivity for COX-2 inhibition. Diclofenac was reported to have antineoplastic effects in various tumor types, including fibrosarcomas, hepatomas, and colon, ovarian and pancreatic cancer [55]. Diclofenac also was reported to have anti-proliferative effects in human colon cancer cell lines [56]. In addition, other NSAIDs that we identified are celecoxib and rofecoxib for CRC. Celecoxib and rofecoxib are two COX-2 inhibitors approved by the U.S. FDA for FAP patients, and these drugs are also being studied in patients with sporadic adenomas and other cancer types [57]. A study of celecoxib as an adjuvant chemotherapy is ongoing in patients with metastatic CRC (phase 4) [NCT03645187]. Yang et al., reported that it may be effective for ovarian cancer [12]. Kim et al. suggested that the combination of celecoxib and paclitaxel could be an effective treatment for ovarian cancer [58]. Moreover, NSAIDs are being evaluated in preclinical models for repurposing to CRC (Fig. 6); such drugs including balsalazide, tolfenamic acid, adapalene and sulindac, which showned anticancer effects in human colon cancer cells [33–36].

Additionally, CMap analysis also showed that the SSRIs drugs, sertraline, fluoxetine and amitriptyline, may be promising candidates for repurposing to CRC. SSRI drugs have been widely used for the treatment of depression and anxiety [59]. Recently, several reports indicated that SSRI drugs have anticancer activity based on the inhibition of proliferation and induction of apoptosis in human colon cancer cells [37–40]. Stopper et al., reported that fluoxetine reduced tumor growth of colon cancer in animals and the incidence of colon cancer in humans through a blockage in tumor metabolism [38]. A following investigation also supported the anticancer activity of sertraline in the two human colon cancer cell lines (HT29 and LS1034) [40]. Currently, biomarkers for CRC are still limited to kirsten rat sarcoma (KRAS), neuroblastoma RAS (NRAS) and B-Raf proto-oncogene (BRAF), with the upcoming standard of care including phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), human epidermal growth factor receptor 2 (HER-2) and microsatellite instability (MSI) [43]. PIK3CA is one of the therapeutic targets that we found in this study. PIK3CA is very likely to be targeted by copanlisib, which has an original indication for follicular lymphoma. Copanlisib, a phosphatidylinositol 3-kinase (PI3K) inhibitor, combined with nivolumab, an anti-PD-1 agent, is under evaluation in patients with mismatch-repair proficient (MSS) colorectal cancer [NCT03711058]. Furthermore, the safety and efficacy of copanlisib in combination with nivolumab are being assessed in an on-going phase 1b/2 study [NCT03735628].

The advantage of drug repurposing is that the safety data of identified drugs are already available [60]. Thus, *in silico* approach might be useful to narrow down candidate drugs for CRC disease, resulting in cost

Table 3
Prioritization of drug repositioning candidates for the treatment of colorectal cancer (CRC) based on CMap comparison to capecitabine.

Drugs	Original Use	Mode of Action	Target Drugs	CMap (Score)	ID
Celecoxib	Pain	Antagonist	PTGS1/ PTGS2	99.09	7541
Rucaparib	Advanced Ovarian Cancer	Antagonist	PARP	94.14	0311
Ibuprofen	Pain	Antagonist	PTGS1/ PTGS2	92.25	5518
Naproxen	Pain	Antagonist	PTGS1/ PTGS2	86.40	9232
Balsalazide	Pain	Antagonist	PTGS1/ PTGS2	81.55	0256
Aspirin	Pain	Antagonist	PTGS1/ PTGS2	80.06	3652
Sulindac	Pain	Antagonist	PTGS1/ PTGS2	77.92	6108
Sertraline	Antidepresant	Antagonist	SLC6A4	74.63	6761
Abiraterone	Prostate Cancer	Antagonist	CYP17A1	72.01	1415
Calcitriol	Hypocalcemia	Agonist	VDR	65.92	7315
Fluoxetine	Antidepresant	Antagonist	SLC6A4	61.71	9102
Olaparib	Metastatic Breast Cancer (MBC)	Antagonist	PAPR	60.14	3016
Rofecoxib	Pain	Antagonist	PTGS2	61.11	3600
Diclofenac	Pain	Antagonist	PTGS1/ PTGS2	61.38	2256
Amitriptyline	Antidepresant	Antagonist	SLC6A4	56.96	7926
Parecoxib	Pain	Antagonist	PTGS2	50.75	0121
Paroxetine	Antidepresant	Antagonist	SLC6A4	45.72	1163
Dasatinib	Chronic Myelogenous leukemia (CML)	Antagonist	CCND2	45.33	8571

Abbreviations: Connectivity Map (CMap), the results were ranked by CMap score (ranging -100 to 100); higher scores denote higher similarities with capecitabine (a standard drug for CRC). CMap scores were obtained from the 'CMap Touchstone' tool in the CLUE website (https://clue.io/).

and time savings. In particular, we would like to emphasize that CRC gene network -driven drug repurposing is a new model to contribute to CRC treatment.. However, our approaches have some limitations. The target genes that we identified are not all in pharmacological activities, therefore these genes might potentially miss the target of the drugs (undruggable). Our analysis showed that only 21 targets of the 1108 CRC target genes are druggable (genetic driven druggable). The comprehensive analysis on the druggability of genes performed by Finan et al. also revealed that only 4479 (22 %) among 20,300 protein coding genes are druggable [61]. Moreover, functional studies and clinical are still needed to confirm the safety and efficacy.

5. Conclusion

By utilizing genomic data to map disease-gene-protein-drug relationships, we found 82 biological CRC risk genes. Then, 128 genes were further expanded byPPI network. Among the identified targets, six drugs are clinically used for CRC, nine drugs were widely supported by preclinical data, and 32 drugs already approved for other indications might be good candidates for CRC treatment. Moreover, CMap results indicated that 18 drugs have potential to be repurposed for CRC. In conclusion, our study demonstrates the feasibility of utilizing genomic data and gene network as a potential method for drug discovery.

Author contributions

L.M.I., H.S.C.W., W.H.C and W.C.C conceived and designed the study. L.M.I performed the computational analysis. L.M.I wrote the manuscript. W.C.C provided the funding. H.S.C.W., W.H.C., W.C.H., W. A., E.M., and W.C.C revised the manuscript. W.C.H and W.C.C supervised and coordinated this study. All authors have read and approved of the manuscript and have made significant contributions to this study.

Data availability

The dataset used and/or analyzed in the study are obtained from ClinicalTrials.gov (https://clinicaltrials.gov/); CMap 'Touchstone' tool in the CLUE website (https://clue.io/); Drug bank databases (https://www.drugbank.ca/); HaploReg (v4.1) (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php); STRING database (http://string-db.org/); Therapeutic Targets Database (http://bidd.nus.edu.sg/BIDD-Databases/TTD/TTD.asp); WebGestalt 2017 (http://www.webgestalt.org/2017/option.php); PubMed database (https://pubmed.ncbi.nlm.nih.gov/).

Declaration of Competing Interest

The authors disclose no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:10.1016/j.phrs.2020.105203.

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