biomolecules

an Open Access Journal by MDPI

Indexed in: PubMed | CITESCORE 5.7 | IMPACT FACTOR 6.064

CERTIFICATE OF PUBLICATION

Certificate of publication for the article titled:

Target-Based Small Molecule Drug Discovery for Colorectal Cancer: A Review of Molecular Pathways and In Silico Studies

Authored by:

Said Moshawih; Ai Fern Lim; Chrismawan Ardianto; Khang Wen Goh; Nurolaini Kifli; Hui Poh Goh; Qais Jarrar; Long Chiau Ming

Published in:

Biomolecules **2022**, Volume 12, Issue 7, 878

MDPI Academic Open Access Publishing since 1996

Basel, April 2023

User Menu

Home (/user/myprofile)
Manage Accounts (/user/manage_accounts)
Change Password (/user/chgpwd)
Edit Profile (/user/edit)
Logout (/user/logout)

Submissions Menu

Submit Manuscript (/user/manuscripts/upload)
Display Submitted Manuscripts (/user/manuscripts/status)
Display Co-Authored Manuscripts (/user/manuscripts/co-authored)
English Editing (/user/pre_english_article/status)
Discount Vouchers (/user/discount_voucher)
Invoices (/user/invoices)
LaTex Word Count (/user/get/latex_word_count)

Reviewers Menu

Reviews (/user/reviewer/status)
Volunteer Preferences (/volunteer_reviewer_info/view)

Journal: Biomolecules (https://www.mdpi.com/journal/biomolecules) (ISSN 2218-273X)

Manuscript ID: biomolecules-1668726

Type: Review

Title: Target-Based Small Molecule Drug Discovery for Colorectal Cancer: A Review of Molecular Pathways and In Silico Studies (https://www.mdpi.com/2218-273X/12/7/878)

Authors: Said Moshawih * , Ai Fern Lim , Chrismawan Ardianto * , Nurolaini Kifli , Hui Poh Goh , Khang Wen Goh , Qais Jarrar , Long Chiau Ming *

Section: Molecular Structure and Dynamics (https://www.mdpi.com/journal/biomolecules/sections/MSD)

Special Issue: Modulating Target Protein Function through the Binding of Small Molecules (https://www.mdpi.com/journal/biomolecules/special_issues/Modulating_Target_Protein)

Abstract: Colorectal cancer is one of the most prevalent cancer types. Although major breakthrough of its treatments, better understanding of the molecular mechanisms and genetic involvement in colorectal cancer have a substantial role in producing novel and targeted treatments with better safety profile. In this review, main molecular pathways and driver genes that are responsible for initiating and propagating the cascade of signaling molecules reaching to carcinoma and the aggressive metastatic stages of colorectal cancer were presented. Protein kinases involved in colorectal cancer, as much as other cancers, have huge fucus and efforts due to their crucial role in subsidizing, inhibiting, or changing the disease course. Moreover, notable improvements of colorectal cancer treatments with in silico studies and better enhanced selectivity on specific macromolecular targets were discussed. Besides, the selective multi-target agents have been made easier by employing in-silico methods in molecular de novo synthesis or target identification and drug repurposing.

The coverletter for this review report has been saved in the database. You can safely close this window.

Authors' Responses to Reviewer's Comments (Reviewer 1)

Author's Notes: We have revised the manuscript according to the reviewer comments. Thank you very much for the highly useful comments. Herein our response to the Reviewers comments

Author's Notes File: Report Notes (/user/review/displayFile/25652763/9XMpISz6?file=author-coverletter&report=18638876)

Review Report Form

Quality of English Language
( ) English very difficult to understand/incomprehensible
( ) Extensive editing of English language and style required
(x) Moderate English changes required
( ) English language and style are fine/minor spell check required
( ) I am not qualified to assess the quality of English in this paper

Is the work a significant contribution to the field? ★ ★ ★ ★ ★

Is the work well organized and comprehensively described? ★ ★ ★ ★ ★

Is the work scientifically sound and not misleading? ★ ★ ★ ★ ★

Are there appropriate and adequate references to related and previous work? ★ ★ ★ ★ ★

Is the English used correct and readable? ★ ★ ★ ★ ★

Comments and Suggestions for Authors

This manuscript reviews our current understanding of molecular pathways and recent reports of in silico small molecule discovery in colorectal cancer. Unfortunately, I think there is a lot of basic knowledge and references that need updating before this can be publishable. While the English is mostly grammatically correct and readable, I think there may be a lot of meaning that is lost in translation/interpretation and would be useful to be carefully read by someone who is both a native English speaker and a cancer expert.

I think I understand the viewpoint the authors are coming from, but I have to disagree with the wording of the first line. "Cancer does not develop from a single gene defect .." There are definitely tumor types that arise from single defects such as the BCR-ABL fusion in CML. Or rhabdoid tumors that are known for having very very few mutations and only SMARCB1 alterations. In fact, that is exactly what oncogenic driver mutations are supposed to be able to do.

"Colorectal cancer has three recognized primary molecular groupings in terms of molecular genetics." This reference is rather old (2004) and there have been more updated consensus molecular subtyping since then. While the old grouping is still valid, it is out of date. Also, the group of MSI should actually be called hypermutated, containing both MSI and POLE-deficiency.

Similarly, to the first point "Cancerous tumor can be characterized by large volume of somatic mutations such as single nucleotide variants (SNVs), copy number aberrations (CNAs) as well as driver mutations."??? This is not true in many ways

1- doesn't need to be large volume. Many papers have discussed cancers with very few NGS-detectable alterations.

2- missing structural variants and indels.

"Intertumor heterogeneity, where cancer genomes do not share the similar set of somatic mutations and most of the tumor pairs bear different kind of mutation is the most remarkable trait of cancer mutational landscape [7]"

1- I don't think the stratton paper discusses much heterogeneity.

2- Since the authors are talking about tumor pairs, do they mean two different regions of the same tumor in the same patient? This is "intra" tumor heterogeneity. If they mean different tumors from different patients, or different primary tumors in the same patient, this would be "inter"

"SNVs or CNAs mutation" - isn't a standard wording. CNAs are more referred to as alterations.

"top 20 mutated genes in CRC such as APC, TP53, LRP1B, KRAS, BRAF." What is the reference for this ICGC study? LRP1B is a very large gene and very similar to LRP1, thus the presence of many mutations may not be significant (and that's why it often doesn't show up on TCGA and subsequent studies); did this analysis use appropriate control for determining significance?

"Furthermore, amplifications in ERBB2 and IGF2 that might be drug-targeted were also identified in the same project, are two examples of recurrent copy-number alterations as shown in Figure 1(B)" - I don't see these two genes in Fig1B.

"Tumorigenesis is generated in the presence of mutant driver genes such as APC, KRAS, SMAD4, TP53, PIK3A, ARID1A, SOX9, and FAM123B." I don't think FAM123B is an established tumor suppressor gene, and is definitely not at the level of evidence as the other genes in this list.

Fig 2 - "adopted from reference." please include the reference. Also PI3CA typo.

"mutant p53 affects gene expression worldwide via a gain-of-function mechanism" ??? what does worldwide mean.

What is the purpose of focusing on tumor suppressor genes and in silico studies? Why the huge focus on druggable pockets and inhibitors for VEGFR but not EGFR or KRAS? Do these show promising ic50? Are these showing promise in the pre-clinical setting?

I think existing approved and clinical trial drugs should be discussed for these targets, in order to discuss why there is a need to do more in silico discovery for them.

"When compared to the experimental results, in-silico techniques such as docking, pharmacophoric, shape similarity screening, and molecular dynamics were found to be significantly correlated with wet laboratory results." Please include a reference.

There are more, but I think I've gone into more than enough detail already I hope this helps you better understand and improve this manuscript.

Submission Date	21 March 2022
Date of this review	18 Apr 2022 16:51:21

Manuscript ID: biomolecules-1668726

Title: Target-Based Small Molecule Drug Discovery for Colorectal Cancer: A Review of Molecular Pathways and In Silico Studies

We have revised the manuscript according to the reviewer comments. Herein our response to the Reviewers comments:

REVIEWER 1

This manuscript reviews our current understanding of molecular pathways and recent reports of in silico small molecule discovery in colorectal cancer. Unfortunately, I think there is a lot of basic knowledge and references that need updating before this can be publishable. While the English is mostly grammatically correct and readable, I think there may be a lot of meaning that is lost in translation/interpretation and would be useful to be carefully read by someone who is both a native English speaker and a cancer expert.

I think I understand the viewpoint the authors are coming from, but I have to disagree with the wording of the first line. "Cancer does not develop from a single gene defect .." There are definitely tumor types that arise from single defects such as the BCR-ABL fusion in CML. Or rhabdoid tumors that are known for having very very few mutations and only SMARCB1 alterations. In fact, that is exactly what oncogenic driver mutations are supposed to be able to do.

Replies: We totally agree with the given viewpoints. We appreciate your kind suggestions. This idea was quoted from Reference [1] (B. Vogelstein, and K.W. Kinzler, Cancer genes and the pathways they control. Nature medicine 10 (2004) 789-799.https://www.nature.com/articles/nm1087) that was cited more than 5000 times. We appended the original text here: “Unlike diseases such as cystic fibrosis or muscular dystrophy, wherein mutations in one gene can cause disease, no single gene defect ‘causes’ cancer. Mammalian cells have multiple safeguards to protect them against the potentially lethal effects of cancer gene mutations, and only when several genes are defective does an invasive cancer develop”

As for BCR-ABL1 fusion in CML, there are many cases support the idea of that it does not arise from one genetic defect t(9;22), as this defect gives rise to others with the progression of the disease such as:

1. Variant Philadelphia translocations: it is generated in 5-10% of patients by a variant translocation involving 9q34, 22q11.2 and one or more additional genomic regions. (Conventional and molecular cytogenetic studies to characterize 2 complex variant Philadelphia translocations in patients with chronic myeloid leukemia, 2019)
2. Rare cases of translocations involving chromosome 7 were reported in CML patients. (Overview of clinical and genetic features of CML patients with variant Philadelphia translocations involving chromosome 7: A case series. 2021)
3. A unique case of a three-way translocation variant in chronic phase chronic myeloid leukemia (A unique three-way Philadelphia chromosome variant t(4;9;22)(q21;q34;q11.2) in a newly diagnosed patient with chronic phase chronic myeloid leukemia: a case report and review of the literature. 2021)

Blast crisis in CML: Rare cases of chronic myeloid leukaemia blast crisis presented with Philadelphia chromosome-positive acute myeloid leukemia mimicking acute lymphocytic leukemia have been reported in t(9;22) and t(3;14). (Chronic myeloid leukemia blast crisis presented with AML of t(9;22) and t(3;14) mimicking acute lymphocytic leukemia, 2019).

Kindly, take note that those reports are recent ones, which means a higher percentage of additional chromosomal aberrations in CML could be reported in the future. This following link shows more cases of concurrent mutations in BCR-ABL fusion in CML.
This paper titled: (CBL, CBLB, TET2, ASXL1, and IDH1/2 mutations and additional chromosomal aberrations constitute molecular events in chronic myelogenous leukemia, 2011) states that: "Although translocations resulting in a BCR/ABL1 fusion gene invariably characterize CML, we stipulated that in analogy to other MDS/MPN entities, JAK2V617F, TET2, ASXL1, CBL, and IDH family mutations may also occur in CML, either contributing to phenotypic heterogeneity within BCR/ABL1-associated chronic myeloid disorders or as secondary events leading to their malignant progression to accelerated phase (AP) or blast phase (BP)."

In the second example you mentioned; Rhabdoid tumors are also associated with SMARCB1 alterations in addition to other mutations that are recently discovered (2016 paper): "Three distinct molecular subgroups of ATRTs, associated with differences in demographics, tumor location, and type of SMARCB1 alterations, were identified. Whole-genome DNA and RNA sequencing found no recurrent mutations in addition to SMARCB1 that would explain the differences between subgroups. Whole-genome bisulfite sequencing and H3K27Ac chromatin-immunoprecipitation sequencing of primary tumors, however, revealed clear differences, leading to the identification of subgroup-specific regulatory networks and potential therapeutic targets." From (Atypical Teratoid/Rhabdoid Tumors Are Comprised of Three Epigenetic Subgroups with Distinct Enhancer Landscapes, 2016).

The bottom line here is driver genes initiate the mutation, but alone will not be enough to complete the tumorigenesis. It needs more mutations and molecular pathways involvements to develop a cancer. We summarized this in this paragraph under 2. Driver genes in CRC:
"It begins with the first driver mutation which minimally benefits the cell to survive and turn into a proliferating hyperplastic lesion. This could increase the risk of acquiring the second driver gene mutation and further leads to the third driver gene mutation as the cell gained autonomy and immortality as well as ability to self-renew. In the case when a third driver gene is involved, the tumor cell upgraded to become invasive and metastatic. At this point, the malignant cells disseminate without the assistance of another driver-mutations [12]"

"Colorectal cancer has three recognized primary molecular groupings in terms of molecular genetics." This reference is rather old (2004) and there have been more updated consensus molecular subtyping since then. While the old grouping is still valid, it is out of date.

Replies: This is a valid point. The reference for this paragraph is **2014** not **2004**. Also, we add more recent reference (2020) to support this classification. As our focus on the "**primary** molecular groups", the scope of this article is not the classification and subgroupings of molecular genetics in CRC.

Also, the group of MSI should actually be called hypermutated, containing both MSI and POLE-deficiency.

Replies: Thank you for pointing it out. This sentence was added to the manuscript: High MSI was found in 75% of this group, which is often linked with hypermethylation and MLH1 gene silence, whereas the remaining 25% had mutations in the mis-match-repair gene and polymerase (POLE) gene [2]

Similarly, to the first point "Cancerous tumor can be characterized by large volume of somatic mutations such as single nucleotide variants (SNVs), copy number aberrations (CNAs) as well as driver mutations."??? This is not true in many ways

1- doesn't need to be large volume. Many papers have discussed cancers with very few NGS-detectable alterations.

Replies: Thank you for the suggestion. The sentence was corrected as follows: “A cancerous tumor can be characterized by low frequency of somatic mutations such as single nucleotide variants (SNVs), copy number aberrations (CNAs), structural variations, and indels. As indicated by the name, SNVs are aroused by a single nucleotide variant that occurred in one particular genetic position while CNAs are the amplifications or deletions of copies of a DNA region at a larger scale.”

2- missing structural variants and indels.

Replies: A small paragraph was added: However, structural variation is used to describe an area of DNA that is 1 kb or bigger in size and can include inversions, balanced translocations, and genomic imbalances, which are also known as copy number variations. Insertions and deletions called indels, are changes to the DNA sequence that result in the addition or deletion of one or more nucleotides [7].

"Intertumor heterogeneity, where cancer genomes do not share the similar set of somatic mutations and most of the tumor pairs bear different kind of mutation is the most remarkable trait of cancer mutational landscape [7]"

1- I don't think the stratton paper discusses much heterogeneity.

Replies: The reference was confused with the previous one. We corrected this.

2- Since the authors are talking about tumor pairs, do they mean two different regions of the same tumor in the same patient? This is "intra" tumor heterogeneity. If they mean different tumors from different patients, or different primary tumors in the same patient, this would be "inter"

Replies: We meant: the presence of different genetic alterations in different metastatic tumors from a single patient that called inter-heterogeneity.

We further clarify the sentence in the manuscript.

"SNVs or CNAs mutation" - isn't a standard wording. CNAs are more referred to as alterations.

Replies: We agree with you. We removed “mutations”

"top 20 mutated genes in CRC such as APC, TP53, LRP1B, KRAS, BRAF." What is the reference for this ICGC study? LRP1B is a very large gene and very similar to LRP1, thus the presence of many mutations may not be significant (and that's why it often doesn't show up on TCGA and subsequent studies); did this analysis use appropriate control for determining significance?

Replies: We appreciate your kind comment. The title of this bar graph is “Top 20 Mutated Cancer Genes with High Functional Impact SSMs (866 Unique SSM-Tested Donors)”. Therefore, those mutated genes are the top 20 with high functional impact, that is why we consider them significant. This graph is downloaded from this link: https://dcc.icgc.org/ = projects. with no interference from our side.

The three projects with donors number and detailed conditions are available on the website with interactive properties to show all required details.

"Furthermore, amplifications in ERBB2 and IGF2 that might be drug-targeted were also identified in the same project, are two examples of recurrent copy-number alterations as shown in Figure 1(B)" - I don't see these two genes in Fig1B.

Replies: Thank you for the comment. The sentence has been further clarified

"Tumorigenesis is generated in the presence of mutant driver genes such as APC, KRAS, SMAD4, TP53, PIK3A, ARID1A, SOX9, and FAM123B." I don't think FAM123B is an established tumor suppressor gene, and is definitely not at the level of evidence as the other genes in this list.

Replies: It was modified. Thank you

Fig 2 - "adopted from reference." please include the reference. Also PI3CA typo.

Replies: thank you for your suggestion. It was corrected

"mutant p53 affects gene expression worldwide via a gain-of-function mechanism" ??? what does worldwide mean.

Replies: It was corrected to: globally. Thank you

What is the purpose of focusing on tumor suppressor genes and in silico studies? Why the huge focus on druggable pockets and inhibitors for VEGFR but not EGFR or KRAS? Do these show promising ic50? Are these showing promise in the pre-clinical setting?

Replies: We focused on VEGFR as a representative of the growth factor pathway, others such as egfr and kras were discussed briefly. If we discussed them exactly as we did with VEGFR, we will write thousands of pages.

Yes, this is right, our main focus for this paper is in silico studies and the druggable pockets of CRC macromolecular targets. The main idea we tried to present here is to find a collective reference for in silico studies to find inhibitors against targets in CRC.

I think existing approved and clinical trial drugs should be discussed for these targets, in order to discuss why there is a need to do more in silico discovery for them.

Replies: Clinical studies are out of this paper's scope. This could be a different paper and to be done by more specialized researchers in this field. Our research group is specialized in cancer in silico studies.

"When compared to the experimental results, in-silico techniques such as docking, pharmacophoric, shape similarity screening, and molecular dynamics were found to be significantly correlated with wet laboratory results." Please include a reference.

Replies: Thank you. The examples cited in the tables of the in-silico studies in this manuscript have many references.

There are more, but I think I've gone into more than enough detail already I hope this helps you better understand and improve this manuscript.

Replies: Our sincere appreciation for these very useful comments. Thank you.

User Menu

Home (/user/myprofile)
Manage Accounts (/user/manage_accounts)
Change Password (/user/chgpwd)
Edit Profile (/user/edit)
Logout (/user/logout)

Submissions Menu

Submit Manuscript (/user/manuscripts/upload)
Display Submitted Manuscripts (/user/manuscripts/status)
Display Co-Authored Manuscripts (/user/manuscripts/co-authored)
English Editing (/user/pre_english_article/status)
Discount Vouchers (/user/discount_voucher)
Invoices (/user/invoices)
LaTex Word Count (/user/get/latex_word_count)

Reviewers Menu

Reviews (/user/reviewer/status)
Volunteer Preferences (/volunteer_reviewer_info/view)

Journal: Biomolecules (https://www.mdpi.com/journal/biomolecules) (ISSN 2218-273X)
Manuscript ID: biomolecules-1668726
Type: Review
Title: Target-Based Small Molecule Drug Discovery for Colorectal Cancer: A Review of Molecular Pathways and In Silico Studies (https://www.mdpi.com/2218-273X/12/7/878)
Authors: Said Moshawih * , Ai Fern Lim , Chrismawan Ardianto * , Nurolaini Kifli , Hui Poh Goh , Khang Wen Goh , Qais Jarrar , Long Chiau Ming *
Section: Molecular Structure and Dynamics (https://www.mdpi.com/journal/biomolecules/sections/MSD)
Special Issue: Modulating Target Protein Function through the Binding of Small Molecules (https://www.mdpi.com/journal/biomolecules/special_issues/Modulating_Target_Protein)
Abstract: Colorectal cancer is one of the most prevalent cancer types. Although major breakthrough of its treatments, better understanding of the molecular mechanisms and genetic involvement in colorectal cancer have a substantial role in producing novel and targeted treatments with better safety profile. In this review, main molecular pathways and driver genes that are responsible for initiating and propagating the cascade of signaling molecules reaching to carcinoma and the aggressive metastatic stages of colorectal cancer were presented. Protein kinases involved in colorectal cancer, as much as other cancers, have huge fucus and efforts due to their crucial role in subsidizing, inhibiting, or changing the disease course. Moreover, notable improvements of colorectal cancer treatments with in silico studies and better enhanced selectivity on specific macromolecular targets were discussed. Besides, the selective multi-target agents have been made easier by employing in-silico methods in molecular de novo synthesis or target identification and drug repurposing.

The coverletter for this review report has been saved in the database. You can safely close this window.

Authors' Responses to Reviewer's Comments (Reviewer 2)

Author's Notes: We have revised the manuscript according to the reviewer comments. Thank you very much for the highly useful comments. Herein our response to the Reviewers comments

Author's Notes File: Report Notes (/user/review/displayFile/25692482/NA8MWEp9?file=author-coverletter&report=18673968)

Review Report Form

Quality of English Language
( ) English very difficult to understand/incomprehensible
( ) Extensive editing of English language and style required
( ) Moderate English changes required
(x) English language and style are fine/minor spell check required
( ) I am not qualified to assess the quality of English in this paper

Is the work a significant contribution to the field? ★ ★ ★ ★ ★
Is the work well organized and comprehensively described? ★ ★ ★ ★ ★
Is the work scientifically sound and not misleading? ★ ★ ★ ★ ★
Are there appropriate and adequate references to related and previous work? ★ ★ ★ ★ ★
Is the English used correct and readable? ★ ★ ★ ★ ★

Comments and Suggestions for Authors

The paper by Moshawi et al. depicts exhaustively the state of the art of the current target-based small molecule in colorectal cancer discovered through specific in silico studies, with a particular focus on their most involved molecular pathways.

The manuscript owns the main structure of the review article, however in my opinion, I would suggest a thorough check of the entire manuscript before final publication and to address the following issues.

1. The last part of the Introduction should include also a brief overview outline about in silico methods applied to molecular pathways in CRC in general. The authors mention only virtual screening studies, and they should spend further few words about the other in silico approaches available from literature that were mentioned in the other sections. Moreover, line 340-342 describing virtual screening could be moved here.
2. Please, if possible, provide the Figure 1, 2, 3, 4, 5, and 6 in high quality resolution.
3. Please revise the misprints, typos and minor issues throughout the text:

Keywords:

Kinanes -> Kinases

Introduction:

line 31: it's better -> it is better;

line 49: CRC has not been specified extensively before having mentioned the acronym;

line 52-53: colorectal is split erroneously when starting a new line;

line 69: two full-stop are present;

Driver Genes in CRC

line 121: Figure2 -> Figure 2;

line 146: seratodenoma -> maybe the authors mean adenomatous or some other cancer type;

line 148: please specify from which reference the authors retrieved the information.

Figure 2:

in the part of "Driver Genes in Colorectal Carcinoma" please revise PI3CA with PIK3CA;

please substitute "Signaling pathways in the case of mutation" with "Signaling pathways targeted by mutations";

please edit "shifts from a tumor suppressor to a tumor promoter" with "shifts from a tumor suppressor gene to tumor promoter gene";

in the part of "Somatic/Germline mutation" please modify Germ-line with Germline in all blocks;

Inactivation of Tumor-Suppressor Genes

line 151: Inactivation OF Tumor-Suppressor Genes -> Inactivation of Tumor-Suppressor Genes;

line 160: please specify in which kind of cells phosphorylated β-catenin is attached to the destruction complex without being stimulated by extracellular Wnt signal;

line 182: leading the cell phenotypic modification -> leading to the cell phenotypic modification;

line 183: Dotted arrow illustrates induction -> The dotted arrow illustrates induction mechanisms;

line 184: the specification for LRP is missing;

line 186: relative to -> related to; moreover, the authors should explain to which studies they are referring to;

line 196: encodes BAX -> encoding BAX;

line 201: degrade -> degrades;

line 207: Bax -> BAX; are downregulated -> is downregulated;

lines 208-210: please, revise the entire sentence that it is a bit hard to read "*Due to the resulting permeabilization of its outer membrane, mitochondria releases cytochrome c which then binds to Apaf-1 forming a complex to activate initiator caspase-9 followed by executioner capase-3, -6 and -7",*

line 213: assist -> assists;

line 215: attach -> binds;

line 244: MSH6 -> MutS homolog 6 (MSH6);

line 263: please insert a comma after "In Table 1";

line 267: and the summaries of the findings. . -> and the summaries of the findings.

Table 1

Virtual screening was revealed 4 lead compounds -> Virtual screening revealed 4 lead compounds;

Please delete the full stop from the columns "Ligands" in Table 1.

Growth Factor Pathways

line 282: please insert a comma between "switch" and "where;

lines 284-285: therefore, It is believed that -> therefore, it is believed that;

line 286: as it act as a medium -> as it acts as a medium; exerts -> exert;

line 289-290: there exists an active site which is surrounded by a flexible activation loop on its circumference. -> an active site which is surrounded by a flexible activation loop on its circumference exists;

line 297: Adapted from Schrodinger tutorials. -> (Adapted from Schrodinger tutorials); please also insert the proper reference for Schrodinger software;

line 299-301: Please revise the following sentence: "*The competitive Type I 299 and II enzyme inhibitors which interact with ATP-binding pocket and Mg2+ ion in the 300 active site of the domain between N-terminal and C-terminal lobes in the presence of ATP".* It seems that a verb is missing;

line 312: "out' -> "out";

line 335: showing the DFG -> showing that the DFG;

line 340: Virtual screening use computer models -> Virtual screening uses computer models;

line 426: In Table 4. we summarized -> In Table 4, we summarized;

Submission Date	21 March 2022
Date of this review	04 Apr 2022 22:07:30

Dear reviewer

Manuscript ID: biomolecules-1668726

Title: Target-Based Small Molecule Drug Discovery for Colorectal Cancer: A Review of Molecular Pathways and In Silico Studies

We have revised the manuscript according to the reviewer comments. Herein our response to the Reviewers comments:

The paper by Moshawi et al. depicts exhaustively the state of the art of the current target-based small molecule in colorectal cancer discovered through specific in silico studies, with a particular focus on their most involved molecular pathways.

The manuscript owns the main structure of the review article, however in my opinion, I would suggest a thorough check of the entire manuscript before final publication and to address the following issues.

1. The last part of the Introduction should include also a brief overview outline about in silico methods applied to molecular pathways in CRC in general. The authors mention only virtual screening studies, and they should spend further few words about the other in silico approaches available from literature that were mentioned in the other sections. Moreover, line 340-342 describing virtual screening could be moved here.

Replies: This is a very valid point. Thank you. We added this paragraph:
"In silico techniques have long been considered crucial in the efforts of predicting inhibitors, new targets, and diagnostic tools for CRC treatment plans. Exploring binding pockets, residue interactions, and different virtual screening methods are approaches among others, that were utilized to target CRC [13]. Gene-mutated CRC was targeted by topological in silico simulations to predict the best treatment combinations that can be successful in clinically advanced conditioned [14]. Furthermore, other tac-tics such as the simulations that predict the interplay between tumor microenvironment components could enhance or reduce immunotherapy success or failure [15], and the gut-on-chip model that delineates the molecular mechanism of symbiotic effects on CRC genes' expression [16] are examples of significant accomplishments in this field. The use of computational methods has also proved a distinguished efficacy by analysing cell surface proteins overexpression in predicting disease progression, diagnosis, and drug resistance in CRC [17]. MicroRNA was employed as a biomarker for CRC through its attachment to the predicted target gene. The molecular pathways and functional analysis of this non-coding RNA with its target macromolecules can predict CRC pathogenesis [18]."

2. Please, if possible, provide the Figure 1, 2, 3, 4, 5, and 6 in high quality resolution.

Replies: Thanks for the suggestions. We have redrawn those Figures. The quality of figures was improved now.

3. Please revise the misprints, typos and minor issues throughout the text:
   Replies: The whole manuscript was reviewed.

- **Keywords:**

Kinanes -> Kinases

- **Introduction:**

line 31: it's better -> it is better;

Replies: Thank you for your suggestions. We have corrected the text now.

line 49: CRC has not been specified extensively before having mentioned the acronym;

Replies: Thank you for your suggestions. We have corrected the text now.

line 52-53: colorectal is split erroneously when starting a new line;

Replies: Thank you for pointing it out. This is corrected now.

line 69: two full-stop are present;

Replies: Thank you for your suggestions. We have corrected the text now.

- **Driver Genes in CRC**

line 121: Figure2 -> Figure 2;

Replies: Thank you for your suggestions. We have corrected the text now.

line 146: seratodenoma -> maybe the authors mean adenomatous or some other cancer type;
Replies: Thanks for pointing it out. We have changed it to adenoma-carcinoma sequence instead

line 148: please specify from which reference the authors retrieved the information.
Replies: The reference was added. Thank you.

- **Figure 2:**

in the part of "Driver Genes in Colorectal Carcinoma" please revise PI3CA with PIK3CA;

please substitute "Signaling pathways in the case of mutation" with "Signaling pathways targeted by mutations";

please edit "shifts from a tumor suppressor to a tumor promoter" with "shifts from a tumor suppressor gene to tumor promoter gene";

in the part of "Somatic/Germline mutation" please modify Germ-line with Germline in all blocks;

Replies: Thank you for your suggestions. All corrections were applied

- **Inactivation of Tumor-Suppressor Genes**

line 151: Inactivation OF Tumor-Suppressor Genes -> Inactivation of Tumor-Suppressor Genes;

Replies: It was corrected now. Thank you.

line 160: please specify in which kind of cells phosphorylated β-catenin is attached to the destruction complex without being stimulated by extracellular Wnt signal;

Replies: Thank you for your suggestions. We have corrected the text now.

line 182: leading the cell phenotypic modification -> leading to the cell phenotypic modification;

line 183: Dotted arrow illustrates induction -> The dotted arrow illustrates induction mechanisms;

line 184: the specification for LRP is missing;

line 186: relative to -> related to; moreover, the authors should explain to which studies they are referring to;

Replies: Thank you. These studies were cited now.

line 196: encodes BAX -> encoding BAX;

line 201: degrade -> degrades;

line 207: Bax -> BAX; are downregulated -> is downregulated;

lines 208-210: please, revise the entire sentence that it is a bit hard to read "*Due to the resulting permeabilization of its outer membrane, mitochondria releases cytochrome c which then binds to Apaf-1 forming a complex to activate initiator caspase-9 followed by executioner capase-3, -6 and -7*",

line 213: assist -> assists;

line 215: attach -> binds;

line 244: MSH6 -> MutS homolog 6 (MSH6);

line 263: please insert a comma after "In Table 1";

line 267: and the summaries of the findings. . -> and the summaries of the findings.

Replies: Thank you for your suggestions. We have corrected the text now.

Table 1

Virtual screening was revealed 4 lead compounds -> Virtual screening revealed 4 lead compounds;

Please delete the full stop from the columns “Ligands” in Table 1.

Replies: Thank you for your suggestions. We have corrected the text now.

- **Growth Factor Pathways**

line 282: please insert a comma between “switch” and “where;

lines 284-285: therefore, It is believed that -> therefore, it is believed that;

line 286: as it act as a medium -> as it acts as a medium; exerts -> exert;

line 289-290: there exists an active site which is surrounded by a flexible activation loop on its circumference. -> an active site which is surrounded by a flexible activation loop on its circumference exists;

Thank you for your suggestions. We have corrected the text now.

line 297: Adapted from Schrodinger tutorials. -> (Adapted from Schrodinger tutorials); please also insert the proper reference for Schrodinger software;

Replies: Thank you. We added a reference for Schrodinger web page.

line 299-301: Please revise the following sentence: “*The competitive Type I 299 and II enzyme inhibitors which interact with ATP-binding pocket and Mg2+ ion in the 300 active site of the domain between N-terminal and C-terminal lobes in the presence of ATP”*. It seems that a verb is missing;

line 312: “out’ -> “out”;

line 335: showing the DFG -> showing that the DFG;

line 340: Virtual screening use computer models -> Virtual screening uses computer models;

line 426: In Table 4. we summarized -> In Table 4, we summarized;

Replies: Thank you for your suggestions. We have corrected the text now.

User Menu

Home (/user/myprofile)
Manage Accounts (/user/manage_accounts)
Change Password (/user/chgpwd)
Edit Profile (/user/edit)
Logout (/user/logout)

Submissions Menu

Submit Manuscript (/user/manuscripts/upload)
Display Submitted Manuscripts (/user/manuscripts/status)
Display Co-Authored Manuscripts (/user/manuscripts/co-authored)
English Editing (/user/pre_english_article/status)
Discount Vouchers (/user/discount_voucher)
Invoices (/user/invoices)
LaTex Word Count (/user/get/latex_word_count)

Reviewers Menu

Reviews (/user/reviewer/status)
Volunteer Preferences (/volunteer_reviewer_info/view)

Journal: Biomolecules (https://www.mdpi.com/journal/biomolecules) (ISSN 2218-273X)
Manuscript ID: biomolecules-1668726
Type: Review

Title: Target-Based Small Molecule Drug Discovery for Colorectal Cancer: A Review of Molecular Pathways and In Silico Studies (https://www.mdpi.com/2218-273X/12/7/878)

Authors: Said Moshawih * , Ai Fern Lim , Chrismawan Ardianto * , Nurolaini Kifli , Hui Poh Goh , Khang Wen Goh , Qais Jarrar , Long Chiau Ming *

Section: Molecular Structure and Dynamics (https://www.mdpi.com/journal/biomolecules/sections/MSD)
Special Issue: Modulating Target Protein Function through the Binding of Small Molecules (https://www.mdpi.com/journal/biomolecules/special_issues/Modulating_Target_Protein)

Abstract: Colorectal cancer is one of the most prevalent cancer types. Although major breakthrough of its treatments, better understanding of the molecular mechanisms and genetic involvement in colorectal cancer have a substantial role in producing novel and targeted treatments with better safety profile. In this review, main molecular pathways and driver genes that are responsible for initiating and propagating the cascade of signaling molecules reaching to carcinoma and the aggressive metastatic stages of colorectal cancer were presented. Protein kinases involved in colorectal cancer, as much as other cancers, have huge fucus and efforts due to their crucial role in subsidizing, inhibiting, or changing the disease course. Moreover, notable improvements of colorectal cancer treatments with in silico studies and better enhanced selectivity on specific macromolecular targets were discussed. Besides, the selective multi-target agents have been made easier by employing in-silico methods in molecular de novo synthesis or target identification and drug repurposing.

The coverletter for this review report has been saved in the database. You can safely close this window.

Authors' Responses to Reviewer's Comments (Reviewer 3)

Author's Notes: We have revised the manuscript according to the reviewer comments. Thank you very much for the highly useful comments. Herein our response to the Reviewers comments

Author's Notes File: Report Notes (/user/review/displayFile/26036151/ET5J1cWj?file=author-coverletter&report=18966168)

Review Report Form

Quality of English Language
( ) English very difficult to understand/incomprehensible
( ) Extensive editing of English language and style required
( ) Moderate English changes required
(x) English language and style are fine/minor spell check required
( ) I am not qualified to assess the quality of English in this paper

Is the work a significant contribution to the field? ★ ★ ★ ★ ★
Is the work well organized and comprehensively described? ★ ★ ★ ★ ★
Is the work scientifically sound and not misleading? ★ ★ ★ ★ ★
Are there appropriate and adequate references to related and previous work? ★ ★ ★ ★ ★
Is the English used correct and readable? ★ ★ ★ ★ ★

Comments and Suggestions for Authors

Said Moshawih and colleagues submitted a well-written review manuscript describing target-based small molecule drug discovery for colorectal cancer with focus on molecular pathways and in silico studies.

Authors systematically review advances in main molecular pathways and driver genes that are responsible for initiating and propagating the cascade of signaling molecules reaching to carcinoma and the aggressive metastatic stages of colorectal cancer. Authors also cover protein kinases involved in colorectal cancer that have huge fucus and efforts due to their crucial role in subsidizing, inhibiting, or changing the disease course. They also discuss improvements of colorectal cancer treatments with in silico studies and better enhanced selectivity on specific macromolecular targets.

Authors summarized the molecular pathways involved in colorectal cancer and the main driver genes that have the greatest triggering impacts. They also discussed the main tumor suppressor genes that are inactivated such as APC, TP53, and TGF-β, the growth factor pathways mainly, VEGFR and EGFR, and the microsatellite instability pathway involving genes.

Particularly important are TP53 mutations since the most frequent type of gene alterations occur in human cancers is the TP53 gene mutations.

Finally, authors conclude that the advances that are being made on virtual drug discovery models and algorithms are time, effort, and cost-saving in discovering new selective inhibitors for allosteric cancer targets and complicated pathways.

Overall, authors present a quality and well-written bioinformatics manuscript valuable for the scientific community and should be accepted for publication after minor edits are made.

==========

Other comments:

1) Please check for typos and punctuation.

2) With regards to p53 (and TP53) authors are kindly encouraged to cite the following article that describes various aspects of p53 signaling regulation. DOI: 10.3389/fonc.2020.01460

Submission Date 21 March 2022

Date of this review 15 Apr 2022 17:17:15

Dear reviewer

Manuscript ID: biomolecules-1668726

Title: Target-Based Small Molecule Drug Discovery for Colorectal Cancer: A Review of Molecular Pathways and In Silico Studies

We have revised the manuscript according to the reviewer comments. Herein our response to the Reviewers comments:

Said Moshawih and colleagues submitted a well-written review manuscript describing target-based small molecule drug discovery for colorectal cancer with focus on molecular pathways and in silico studies.

Authors systematically review advances in main molecular pathways and driver genes that are responsible for initiating and propagating the cascade of signaling molecules reaching to carcinoma and the aggressive metastatic stages of colorectal cancer. Authors also cover protein kinases involved in colorectal cancer that have huge fucus and efforts due to their crucial role in subsidizing, inhibiting, or changing the disease course. They also discuss improvements of colorectal cancer treatments with in silico studies and better enhanced selectivity on specific macromolecular targets.

Authors summarized the molecular pathways involved in colorectal cancer and the main driver genes that have the greatest triggering impacts. They also discussed the main tumor suppressor genes that are inactivated such as APC, TP53, and TGF-β, the growth factor pathways mainly, VEGFR and EGFR, and the microsatellite instability pathway involving genes.

Particularly important are TP53 mutations since the most frequent type of gene alterations occur in human cancers is the TP53 gene mutations.

Finally, authors conclude that the advances that are being made on virtual drug discovery models and algorithms are time, effort, and cost-saving in discovering new selective inhibitors for allosteric cancer targets and complicated pathways.

Overall, authors present a quality and well-written bioinformatics manuscript valuable for the scientific community and should be accepted for publication after minor edits are made.

Replies: Thank you so much for your generous comments.

Other comments:

1) Please check for typos and punctuation.

Replies: The manuscript has been carefully proofread now by a native speaker. Thanks for the comment

2) With regards to p53 (and TP53) authors are kindly encouraged to cite the following article that describes various aspects of p53 signaling regulation. DOI: 10.3389/fonc.2020.01460

Replies: thank you for your suggestions. This paragraph was added:

Replies: A variety of small compounds have been created to target and stabilize certain mutant versions of p53, restoring wild-type (WT)-like transcriptional activity and causing mutant tumor cells to undergo cell cycle arrest or apoptosis. The nine most common mutations of p53 protein (R175H, R248Q, R273H, R248W, R273C, R282W, G245S, R249S, Y220C) account for around 30% of all its

cancer-driving mutations [44]. PRIMA-1 and its methyl analog APR-246 are potential small molecules that interact with the DNA binding domain of mutant p53, encouraging correct folding/function and, as a result, increasing the production of pro-apoptotic genes Puma, Noxa, and Bax in p53 mutant cells [45]. The Y220C mutation is the ninth most common p53 missense mutation, and it is linked to more than 100,000 new cancer cases each year. The Y220C pocket's hydrophobic and "druggable" characteristics make it a good candidate for targeting with small-molecule stabilizers. The mutation-induced crevice is also far away from the p53 surfaces involved in DNA recognition or protein-protein interactions, permitting the creation of tailored chemical agents that stabilize the DNA binding domain without interfering with its natural substrate binding [44]. Several powerful lead compound families that bind Y220C pocket have been identified in recent years using fragment-based and in silico screening approaches. PK9328 is a carbazole derivative that was identified by computational screening techniques that fit in p53-Y220C binding pocket with a low micromolar affinity significantly decreased cell viability in various Y220C cancer cell lines [46]. Moreover, the pyrazole derivative PK7088 restored p53-Y220C transactivation and downstream upregulation of p21 and Noxa expression, correlated with cell cycle arrest and apoptosis [47].