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Article

In silico evaluation of potential drugs for the treatment of Colorectal Carcinoma

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To evaluate possible new drugs for the treatment of Colorectal Carcinoma (CRC) using in silico tools was the main objective of this study. The method of analysis used was the in silico evaluation of tumor markers and their interaction with selected drugs, through the study of its pharmacokinetic and pharmacodynamic characteristics. A potential therapeutic target pointed out in this study was the Cell Division Cycle 25 B (CDC25B), belonging to the CDC25 phosphatase family. Overexpression of CDC25 phosphatases is often associated with a wide variety of cancers. In addition, CDC25B is an oncogenic protein that induces neoplastic transformation. In CRC, CDC25B is overexpressed to activate the CDC2/cyclin B complex and improve the growth and survival of these tumors. Four drugs were identified for evaluation, with α-amyrin being selected for docking, because it was that had the best characteristics according to the methodology used. The α-amyrin ligand obtained the interaction energy value of -7.6 G (Kcal/mol), while the standard CDC25B ligand obtained -10.0 G (Kcal/mol). TThe results showed that the CDC25B protein was the only structure cocrystallized with α-amyrin and presented favorable outcomes in docking, being a candidate for further studies for its use in the CRC targeted therapy.

Keywords: Molecular Targeted Therapy. Colorectal Neoplasms. Anti Cancer Drug Screens. Antineoplastic Agents.

INTRODUCTION

Colorectal Carcinoma (CRC) incidence and mortality rates diversify distinctly around the world. Differences in eating habits and environmental exposures imposed in a context of genetically determined susceptibility are the most likely features related to these variations. Worldwide, CRC is the third most diagnosed cancer in men and the second in women, with 1.8 million new cases and almost 861,000 deaths in 2018, according to the World Health Organization (WHO) GLOBOCAN database (WHO, 2020). Besides, rates are markedly higher in men than in women (Macrae, 2020).

Despite the improved survival of patients with unresectable metastatic colorectal cancer in recent years, largely due to the introduction of agents targeting the Epidermal Growth Factor Receptor (EGFR) and the Vascular Endothelial Growth Factor (VEGF), these treatments are generally not curative, which adds to the frequent increase in resistance to intrinsic drugs acquired in clinical practice (Arnold, Seufferlein, 2010; Sánchez-Gundín *et al.*, 2010).

The National Cancer Institute (INCA) estimates, for Brazil, for each year of the 2020-2022 triennium, 20,520 cases of colon and rectal cancer in men and 20,470 in women. In the case of mortality, in Brazil, in 2017, there were 9,207 deaths due to CRC (9.12/100 thousand) in men and 9,660 (9.33/100 thousand) in women. CRC comprises tumors that start in the large intestine (called the colon) and in the rectum (end of the intestine, just before the anus) and anus. It is amenable to treatment and, in most cases, it is curable, when detected early and still does not present metastasis (Inca, 2020).

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Risk factors involve poor diet, smoking, polyps, genetic factors, inflammatory bowel disease and aging. Of the diagnoses confirmed, 90% of the patients are over 50 years old and the average is 64 years old; however, the disease is more aggressive in patients who are diagnosed at younger ages (Granados-Romero *et al.*, 2017).

Regarding the treatment of CRC, several strategies have been proposed, including alternative formulations, resistance modulation, antidotes/toxicity modifiers and gene therapy. Recently, the targeted therapy is standing out since it directs its action towards specific cancer cells, which results in less toxicity of the non-target cells (Padma, 2015).

According to Kamble and Khairkar (2017), bioinformatics, which results from the integration between large areas such as biology, information science and computing, stands out as one of the most promising tools currently available for the molecular study of cancer.

Thus, molecular docking is gaining recognition for being an effective method for improving the understanding of the molecular basis of cancer and other pathogenic pathologies not yet fully elucidated (Hoban, Bertorelle, Gaggiotti, 2012; Ritchie, Bush, 2010). but the recent availability of dozens of sophisticated, customizable software packages for simulation now makes simulation an accessible option for researchers in many fields. The in silico genetic data produced by simulations, along with greater availability of population-genomics data, are transforming genetic epidemiology, anthropology, evolutionary and population genetics and conservation. (Hoban, Bertorelle, Gaggiotti, 2011; Ritchie, Bush, 2010

The groupings of data generated in silico provide results from specific hypotheses, which can even be used in the validation and comparison of various methods, such as statistical methods. From this, the research design can be done with factors and hypotheses previously defined by means of in silico simulations under different conditions, using data that best satisfy the empirical data studied (Chen *et al.*, 2015).

Therefore, it is evident that the identification of altered pathways and new therapeutic targets are essential to improve the management of a significant proportion of patients with colorectal cancer. In this sense, Cell Division Cycle 25 B (CDC25B), belonging to the CDC25 phosphatase family, is one of the most cited CRC-related. Overexpression of CDC25 phosphatases is often associated with a wide variety of cancers. In addition, CDC25B is an oncogenic protein that induces neoplastic transformation. In CRC, CDC25B is overexpressed to activate the CDC2/cyclin B complex and improve the growth and survival of these tumors (Takemasa *et al.*, 2000)reported data on smoking behaviors for PLWH by gender.

As a result, in silico studies become a promising alternative in an attempt to speed up the development of new forms of treatment for carcinomas, which currently require long and costly clinical research that often results in ineffective treatments. Therefore, the use of computational tools is beneficial in the evolution of studies related to cancer.

Thus, this paper aimed to evaluate strong targets and possible new drugs for the treatment of CRC using in silico tools.

MATERIAL AND METHODS

Type of analysis

The method of analysis used was the in silico evaluation of tumor markers and their interaction with selected drugs, through the study of the pharmacokinetic and pharmacodynamic characteristics of these selected drugs and the use of computational platforms to verify the interaction between such altered pathways and drug candidates for the CRC cells.

Universe and sample

The research was carried out, a priori, in the scientific base PubMed - NCBI (The United States National Library of Medicine at the National Institutes of Health) with the search for tumor markers involved in the pathogenesis of CRC, using the following DeCS (Descriptors in Health Sciences): “Molecular Targeted Therapy, Intestinal Neoplasms, Antineoplastic Agents and Colorectal Neoplasms”.

Then, the online databases MalaCards: The Human Disease Database, and GeneCards®: The Human Gene

Database were used to follow the identification of the main therapeutic targets for CRC and related drugs, in addition to the characterization of proteins identified as CRC markers in the articles initially raised.

After obtaining the markers in the previous objectives, the PDB Protein Data Bank database (https://www.rcsb.org/) was used to download the .pdb files of the proteins related to the markers. The markers that showed some relationship with the candidate drugs, on the Thomson Reuters Integrity platform, were used to verify the possibility of docking.

The Thomson Reuters Integrity platform brings together biological, chemical and pharmaceutical data on more than 420,000 compounds with proven biological activity, explores their pharmacological and pharmacokinetic parameters, clinical trials, targets and related genes, in addition to more than 235,000 patent family records.

Inclusion and exclusion criteria

The selection of drugs and therapeutic targets in the aforementioned databases was carried out taking into account the drugs in articles published on the PubMed platform and active products considered "elite" in the GeneCards® and MalaCards databases.

From the articles obtained, those that did not show new molecules or that performed the synergism test with more than one molecule were excluded, due to the impossibility of carrying out this type of test via molecular docking methodologies.

After the initial selection, only drugs and targets with greater specificity (pa > 0.6 in PASS Online and having a positive "druglikeness" characteristic on the SwissADME platform) for CRC were analyzed and allocated at the intersection aiming at the interaction between them, in order to discover new drugs with the highest possible specificity for the treatment of CRC.

Data analysis

The selected molecules were then drawn in the ChemAxon Marvin ChemSketch 18.24 software. Its 2D structures were transformed into a better state of 3D compliance through algorithms of the software itself. Then, the drugs designed (using their SMILES code) were submitted to the SwissADME online platforms (http://www.swissadme.ch/) for verification of pharmacokinetic characteristics. SwissADME data include molecular weight, TPSA, LogP consumption, LogS solubility classification, GI absorption, BBB permeability, P-gp substrate, CYP1A2 inhibition, Log Kp, lipinski pharmacokinetic ratio and lead likeness ratio. On the PASS Online platform (http://www.pharmaexpert.ru/passonline/), a screening was carried out to check possible locations and types of action. Only indexes greater than 0.6 were used.

Pa (probability of "being active") estimates a chance that the studied compound belongs to the subclass of compounds (similar to molecule structures, which are the most common in a subset of "assets" in the set of molecules in the PASS database Online). Whereas the pharmacodynamic variable qualitatively assesses the chance of a molecule becoming a drug orally in relation to its bioavailability, according to Lipinski's rule of five.

The targets raised in the first objective were verified from the possible targets listed in the PASS Online results. Those present in both were taken to the next phase of the project, which carried out molecular docking studies using UCSF Chimera 1.13-1 software for assembling the molecular target structure and ligands and AutoDock Vina 1.1.2 to assess the interaction of the target and binder. Both were run on the Linux Ubuntu 18.10 operating system.

Hardware and software

Drownings of molecules and docking studies were done out in the Intel® Core i3-2100 CPU, processor 3.10 GHz x 4, memory (RAM) 4.00 GB, 64-bit with Ubuntu Linux (version 14.04.3 LTS) as the operational system. Ligand preparation was performed and analyses were performed with UCSF Chimera version 1.13.1, University of California. The combinations of ligand and receptor in a single file were performed using the software PyMOLversion 0.99rc6 and the visualization of the ligand-target interaction was performed in Maestro version 12.1.013 in an Intel® Core™ i3-6100T CPU, processor 3.10 GHz x 8.00 GB 64-bit with Windows 7 Professional.

Ligand preparation

All the selected molecules were drawn using 2D and 3D option of Marvin ChemSketch version 18.20.0 and saved in mol2 format. Energy minimization, conformational analysis, and ligand preparation were performed and exported in the SDF format. They were later imported into the chimera for the docking study by the AutoDock Vina tool.

Docking using Autodock/Vina

Intermediary steps, such as pdbqt files for protein and ligands preparation and grid box creation were completed using Graphical User Interface program UCSF Chimera through AutoDock Tools (ADT). ADT, internally used at UCSF Chimera, had assigned polar hydrogens, charges using Gasteiger, deleted solvent and incomplete side chains replaced using Dunbrack 2010 library. During H addition was considered H-bonds method. Protonation states for histidine was residue-named-based. In the AutoDock Vina tool, internally used at UCSF Chimera, the grid size was set to $60 \times 60 \times 60$ xyz points with grid spacing of 0.375 Å and grid center was designated at dimensions (x, y, and z): -1.095, -1.554 and 3.894. A scoring grid was calculated from the ligand structure to minimize the computation time. During the docking procedure, both the protein and ligands are considered as semi-rigid. The pose with lowest energy of binding or binding affinity was extracted and aligned with receptor structure for further analysis.

Graphical analysis of the result of molecular docking

The preparation of the file for the responses of the interaction levels of the receiver and ligand was performed using the software PyMOL. The file created with the interaction of both was submitted to the interaction analysis tool of the receptor-ligand to the Maestro software, generating interaction images with a 5Aº cut-off from the amino acids' residues. The final images were generated at .tif format.

RESULTS

Tumor markers

Initially, after searching for articles with tumor markers related to the pathogenesis of colorectal carcinoma, performed on the PubMed platform, 65 articles were found that contained one or more markers linked to tumorigenesis characteristics of the CRC or another segment of the gastrointestinal tract, as shown in Table I.

TABLE I - Tumor targets of CRC identified in articles searched in PubMed, with their respective journals and year of publication highlighted

Title	Markers	Journal	Year of Publication
Subsequent anti-VEGF therapy after first-line anti-EGFR therapy improved overall survival of patients with metastatic colorectal cancer	**EGFR e VEGF**	OncoTargets and Therapy	2018
A novel pretherapeutic gene expression-based risk score for treatment guidance in gastric cancer	**CCL5, CTNNB1, EXOSC3 e LZTR1**	Annals of Oncology	2018
STAT3 inhibition by STA21 increases cell surface expression of MICB and the release of soluble MICB by gastric adenocarcinoma cells)	**STAT3**	Immunobiology	2017
Application of unique sequence index (USI) barcode to gene expression profiling in gastric adenocarcinoma	**FAT4 e CDX1**	The Journal of Cell Communication and Signaling	2017

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Title	Markers	Journal	Year of Publication
Growth hormone-releasing hormone receptor antagonists inhibit human gastric cancer through downregulation of PAK1-STAT3/NF-κB signaling	**PAK1-STAT3/NF-κB**	Proc Natl Acad Sci U S A	2016
Expression of the anaphylatoxin C5a receptor in gastric cancer: implications for vascular invasion and patient outcomes	**C5AR**	Med Oncol	2016
Co-expressed miRNAs in gastric adenocarcinoma	**miRNAs 100, let-7c, 125b, 99a, 181 miRNA family e miRNA 21**	Genomics	2016
Interleukin-13 receptor α2 is associated with poor prognosis in patients with gastric cancer after gastrectomy	**IL-13Rα2**	Oncotarget	2016
(A nCounter CNV Assay to Detect HER2 Amplification: A Correlation Study with Immunohistochemistry and In Situ Hybridization in Advanced Gastric Cancer	**HER2**	Mol Diagn Ther	2016
Tumor-infiltrating macrophages express interleukin-25 and predict a favorable prognosis in patients with gastric cancer after radical resection	**IL-25**	Oncotarget	2016
Overexpression of CD39 and high tumoral CD39+/CD8+ ratio are associated with adverse prognosis in resectable gastric cancer	**CD39 e CD8in**	Int J Clin Exp Pathol	2015
Low Expression of the bcl2 Gene in Gastric Adenocarcinomas in Mazandaran Province of Iran	**BCL2**	Asian Pac J Cancer Prev	2015
Spinophilin loss correlates with poor patient prognosis in advanced stages of colon carcinoma	**Brca1, NM23, prohibitin, e spinophilin**	Clin Cancer Res	2013
MSI1 overexpression in diffuse type of gastric cancer	**MSI1**	Pathol Res Pract	2013
Targeted Casp8AP2 methylation increases drug resistance in mesenchymal stem cells and cancer cells	**Casp8AP2**	Biochem Biophys Res Commun	2012
CHIP functions as a novel suppressor of tumour angiogenesis with prognostic significance in human gastric cancer	**CHIP**	Gut	2013
H2S donor, S-propargyl-cysteine, increases CSE in SGC-7901 and cancer-induced mice: evidence for a novel anti-cancer effect of endogenous H2S?	**SGC-7901**	PLoS One	2011

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microRNA-451 regulates macrophage migration inhibitory factor production and proliferation of gastrointestinal cancer cells	**miR-451**	Clin Cancer Res	2009
Tie2: a journey from normal angiogenesis to cancer and beyond	**Tie2**	Histol Histopathol	2008
Signal transduction proteins in tumors from Puerto Rican and Caucasian gastric adenocarcinoma patients: expression differences with potential for specific targeted therapies	**HER2/NEU**	Dig Dis Sci	2008
Adenovirus-mediated FasL gene transfer into human gastric carcinoma.)	**FasL**	World J Gastroenterol	2005
Chemotherapy targeted to cancers through tumoral hormone receptors	**LH-RH, AN-152 e AN-207**	Trends Endocrinol Metab	2004
Target Gene Mutation Profile Differs between Gastrointestinal and Endometrial Tumors with Mismatch Repair Deficiency	**TGFβRII, Bax, IGFIIR, hMSH3, hMSH6, and GRB-14, Bat-25 e Bat-26, hMLH1**	Cancer Res	2002
No loss of sst receptors gene expression in advanced stages of colorectal cancer	**hsst5 e hsst2 mRNA**	Eur J Endocrinol	1999
Critical factors for optimizing the 5-fluorouracil-folinic acid association in cancer chemotherapy	**thymidylate synthase**	Ann Oncol	1996
Comparative study of the content of 1,25-dihydroxyvitamin D3 receptors in digestive mucosa and adenocarcinoma.	**1,25-dihydroxyvitamin D3 receptor**	Bull Cancer	1990
Blockade of LAG3 enhances responses of tumor-infiltrating T cells in mismatch repair-proficient liver metastases of colorectal cancer	**LAG3**	Oncoimmunology	2018
Modulation of the colon cancer cell phenotype by pro-inflammatory macrophages: A preclinical model of surgery-associated inflammation and tumor recurrence	**FN1 e VIM, TCF4**	PLoS One	2018
A Deregulated PI3K-AKT Signaling Pathway in Patients with Colorectal Cancer	**PI3K-AKT**	J Gastrointest Cancer	2019
Protein drug target activation homogeneity in the face of intra-tumor heterogeneity: implications for precision medicine	**MAPK e AKT-mTOR**	Oncotarget	2017
Advances in immunotherapeutic strategies for colorectal cancer commentary on: tumoral immune cell exploitation in colorectal cancer metastases can be targeted effectively by anti-CCR5 therapy in cancer patients	**CCL5/CCR5**	Journal for ImmunoTherapy of Cancer	2016

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Title	Markers	Journal	Year of Publication
Salinomycin inhibits metastatic colorectal cancer growth and interferes with Wnt/β-catenin signaling in CD133+ human colorectal cancer cells	**CD133+**	BMC Cancer	2016
Normalizing Microbiota-Induced Retinoic Acid Deficiency Stimulates Protective CD8(+) T Cell-Mediated Immunity in Colorectal Cancer	**atRA**	Immunity	2016
The profile of melatonin receptors gene expression and genes associated with their activity in colorectal cancer: a preliminary report	**GNA11, OXTR, TPH1**	J Biol Regul Homeost Agents	2015
Sphingosine kinase 2 promotes colorectal cancer cell proliferation and invasion by enhancing MYC expression.	**SphK2**	Tumour Biol	2016
KRAS discordance between primary and recurrent tumors after radical resection of colorectal cancers	**KRAS**	J Surg Oncol	2015
Overexpression of the Promigratory and Prometastatic PTK7 Receptor Is Associated with an Adverse Clinical Outcome in Colorectal Cancer	**PTK7**	PLoS ONE	2015
Cathepsin B promotes colorectal tumorigenesis, cell invasion, and metastasis	**Cathepsin B**	Mol Carcinog	2016
Predictors of Tumor Response to Cetuximab and Panitumumab in 116 Patients and a Review of Approaches to Managing Skin Toxicity	**EGFR**	Actas Dermosifiliogr	2015
The cholesterol biosynthesis enzyme oxidosqualene cyclase is a new target to impair tumour angiogenesis and metastasis dissemination	**oxidosqualene cyclase**	Sci Rep	2015
The role of VEGFR-2 expression in outcomes and survival of patients with peritoneal carcinomatosis from appendiceal cancer	**VEGF**	Eur J Surg Oncol	2013
Dietary restriction-resistant human tumors harboring the PIK3CA-activating mutation H1047R are sensitive to metformin.	**H1047R**	Oncotarget	2013
The proto-oncogene KRAS and BRAF profiles and some clinical characteristics in colorectal cancer in the Turkish population	**G12D**	Genet Test Mol Biomarkers	2013
Chemokine CXCL14 is associated with prognosis in patients with colorectal carcinoma after curative resection	**CXCL14**	J Transl Med	2013

TABLE I - Tumor targets of CRC identified in articles searched in PubMed, with their respective journals and year of publication highlighted

Title	Markers	Journal	Year of Publication
Production and characterization of a colon cancer-specific immunotoxin based on the fungal ribotoxin α-sarcin	**GPA33**	Protein Eng Des Sel	2012
β-catenin confers resistance to PI3K and AKT inhibitors and subverts FOXO3a to promote metastasis in colon cancer	**FOXO3a**	Nat Med	2012
AEZS-108: a targeted cytotoxic analog of LHRH for the treatment of cancers positive for LHRH receptors	**AEZS-108**	Expert Opin Investig Drugs	2012
Upregulation of trefoil factor 3 (TFF3) after rectal cancer chemoradiotherapy is an adverse prognostic factor and a potential therapeutic target	**TFF3**	Int J Radiat Oncol Biol Phys	2012
Analysis of select members of the E26 (ETS) transcription factors family in colorectal cancer	**ETS**	Virchows Arch	2011
Prognostic significance of AMP-activated protein kinase expression and modifying effect of MAPK3/1 in colorectal cancer	**AMPK e MAPK**	Br J Cancer	2010
Down-regulation of the phosphoenolpyruvate carboxykinase gene in human colon tumors and induction by omega-3 fatty acids	**PEPCK**-C	Biochimie	2010
Endogenous expression of proteases in colon cancer cells facilitate influenza A viruses mediated oncolysis	**Caco-2, HT-29 e SW-620**	Cancer Biol Ther.	2010
PTGER2 overexpression in colorectal cancer is associated with microsatellite instability, independent of CpG island methylator phenotype	**PTGER2**	Cancer Epidemiol Biomarkers Prev.	2010
Pregnane X Receptor (PXR) expression in colorectal cancer cells restricts irinotecan chemosensitivity through enhanced SN-38 glucuronidation	**PXR**	Mol Cancer	2010
Increased proteasome subunit protein expression and proteasome activity in colon cancer relate to an enhanced activation of nuclear factor E2-related factor 2 (Nrf2)	**Nrf2**	Oncogene	2009
Identification of tumor-associated autoantigens for the diagnosis of colorectal cancer in serum using high density protein microarrays	**PIM1, MAPKAPK3, e ACVR2B**	Mol Cell Proteomics	2009
EpCAM, a human tumor-associated antigen promotes Th2 development and tumor immune evasion	**EpCAM**	Blood	2009
In vivo tumor targeting by the B-subunit of shiga toxin	**glycosphingolipid Gb3**	Mol Imaging	2008

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Title	Markers	Journal	Year of Publication
A novel DNA vaccine encoding PDGFRbeta suppresses growth and dissemination of murine colon, lung and breast carcinoma	**mPDGFRbeta**	Vaccine	2006
Proteomics-based validation of genomic data: applications in colorectal cancer diagnosis	**ANXA3, BMP4, LCN2, SPARC, MMP7, e MMP11**	Mol Cell Proteomics	2006
Combination of thymidine phosphorylase gene transfer and deoxyinosine treatment greatly enhances 5-fluorouracil antitumor activity in vitro and in vivo	**LS174T**	Mol Cancer Ther.	2001
Indomethacin, a cox inhibitor, enhances 15-PGDH and decreases human tumoral C cells proliferation	**15-PGDH**	Prostaglandins Other Lipid Mediat	2001
Evolution of instability at coding and non-coding repeat sequences in human MSI-H colorectal cancers	**GRB-14, RHAMM, RAD50**	Human Molecular Genetics	2001
Overexpression of matrix metalloproteinase-2 and tissue inhibitor of matrix metalloproteinase-2 in liver from patients with gastrointestinal adenocarcinoma and no detectable metastasis	**6TIMP2**	Int J Cancer	1997
Decreased folylpolyglutamate synthetase activity in tumors resistant to fluorouracil-folinic acid treatment: clinical data	**Thymidylate synthase**	Clin Cancer Res	1997

Source: Findings of Research.

As a result, after identifying the articles and their respective target molecules, it was possible to list the following markers: CTNNB1, BAX, HMSH6, HMLH1, PTGS2, CCND1, KRAS, EGFR, PIK3CA and BRAF.

Drugs

Secondly, 55 articles were identified on PubMed, these containing active molecules with therapeutic potential for colorectal carcinoma (Table II). Then, the drugs were screened for the specificity criterion and 31 articles were reached. Of these, 16 were selected for evaluation, since 15 articles did not have the molecular structures in the scope of the text available for evaluation. It is noteworthy that some articles have more than one drug.

TABLE II – Potential CRC-related drugs identified in PubMed articles, followed by related tumor targets and their respective journals and year of publication

Title	Compounds/Targets	Journal	Year of Publication
Cepharanthine exhibits a potent anticancer activity in p53-mutated colorectal cancer cells through upregulation of p21Waf1/Cip1.	**Cepharanthine/p53**	Oncol Rep.	2018
Quercetin: A functional dietary flavonoid with potential chemo-preventive properties in colorectal cancer	**Quercetin/Caco-2**	J Cell Physiol.	2018
Enhanced Therapeutic Activity of Non-Internalizing Small-Molecule-Drug Conjugates Targeting Carbonic Anhydrase IX in Combination with Targeted Interleukin-2	**L19-IL2/CT26**	Clin Cancer Res.	2018
Dihydroartemisinin potentiates antitumor activity of 5-fluorouracil against a resistant colorectal cancer cell line	**DHA/BCL-2/BAX**	Biochem Biophys Res Commun.	2018
Evaluation of Anticancer Activity of Camellia Sinensis in the Caco-2 Colorectal Cancer Cell Line	**Camellia Sinensis/ Caco-2**	Asian Pac J Cancer Prev.	2018
Novel ruthenium methylcyclopentadienyl complex bearing a bipyridine perfluorinated ligand shows strong activity towards colorectal cancer cells.	**Ruthenium methylcyclopentadienyl/ Bipyridine perfluorinated**	Eur J Med Chem.	2018
Improved Anticancer Effect of Magnetite Nanocomposite Formulation of GALLIC Acid (Fe_3O_4-PEG-GA) Against Lung, Breast and Colon Cancer Cells	**Gallic Acid/HT-29**	Nanomaterials (Basel)	2018
Hemidesmus indicus induces immunogenic death in human colorectal cancer cells	**Hemidesmus indicus/DLD1**	Oncotarget	2018
Hispidulin suppresses cell growth and metastasis by targeting PIM1 through JAK2/ STAT3 signaling in colorectal cancer	**Hispidulin/PIM1**	Cancer Sci.	2018
A novel resveratrol derivative selectively inhibits the proliferation of colorectal cancer cells with KRAS mutation	**Resveratrol/HCT116**	Mol Cell Biochem.	2018
MicroRNA-195 desensitizes HCT116 human colon cancer cells to 5-fluorouracil	**5-fluorouracil/HCT116**	Cancer Lett.	2018
Anticancer activities of a benzimidazole compound through sirtuin inhibition in colorectal cancer	**BZD9L1/HCT 116 and HT-29**	Future Med Chem.	2018
The Deceptively Similar Ruthenium(III) Drug Candidates KP1019 and NAMI-A Have Different Actions. What Did We Learn in the Past 30 Years?)	**KP1019/SW480 and HT29**	Met Ions Life Sci.	2018

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Title	Compounds/Targets	Journal	Year of Publication
A452, an HDAC6-selective inhibitor, synergistically enhances the anticancer activity of chemotherapeutic agents in colorectal cancer cells	**A452/HDAC6**	Mol Carcinog.	2018
Trichostatin A preferentially reverses the upregulation of gene-expression levels induced by gain of chromosome 7 in colorectal cancer cell lines	**Trichostatin A/SW480**	Genes Chromosomes Cancer	2018
Bacterial ghosts as adjuvant to oxaliplatin chemotherapy in colorectal carcinomatosis	**Oxaliplatin/CT26**	Oncoimmunology	2018
A review: Therapeutics potentials of phytochemical drugs and their loading in pH specific degradable Nano-drug carrier targeting colorectal cancer	**Thymoquinone/ HCT116**	J Pak Med Assoc.	2018
Wee1 inhibition can suppress tumor proliferation and sensitize p53 mutant colonic cancer cells to the anticancer effect of irinotecan	**MK1775/Wee1**	Mol Med Rep.	2018
Antimalarial drug mefloquine inhibits nuclear factor kappa B signaling and induces apoptosis in colorectal cancer cells	**Mefloquine/Kappa B**	Cancer Sci.	2018
Simvastatin induces G1 arrest by up-regulating GSK3β and down-regulating CDK4/cyclin D1 and CDK2/cyclin E1 in human primary colorectal cancer cells	**SIM/CPs**	J Cell Physiol.	2018
Proteomics Study Reveals That Docosahexaenoic and Arachidonic Acids Exert Different In Vitro Anticancer Activities in Colorectal Cancer Cells	**Docosahexaenoic and Arachidonic Acids/HT-29**	J Agric Food Chem.	2018
Targeting the SET and RING-associated (SRA) domain of ubiquitin-like, PHD and ring finger-containing 1 (UHRF1) for anti-cancer drug development	**Luteolin/UHRF1**	Oncotarget	2018
Drug-like dietary vanilloids induce anticancer activity through proliferation inhibition and regulation of bcl-related apoptotic proteins	**Vanilloids/HT-29 and HCT116**	Phytother Res.	2018
Hormetic dose response to L-ascorbic acid as an anti-cancer drug in colorectal cancer cell lines according to SVCT-2 expression	**L-Ascorbic acid/p300**	Scientific Reports	2018
Discovery of new A- and B-type laxaphycins with synergistic anticancer activity	**Laxaphycins A e B4/HCT116**	Bioorg Med Chem.	2018

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Title	Compounds/Targets	Journal	Year of Publication
A Low-Toxicity DNA-Alkylating N-Mustard-Quinoline Conjugate with Preferential Sequence Specificity Exerts Potent Antitumor Activity Against Colorectal Cancer	**SL-1/SW620**	Neoplasia	2018
Anticancer activity study of A3 adenosine receptor agonists	**A3AR/Caco-2**	Life Sciences	2018
Anticancer effect of Polyphyllin I in colorectal cancer cells through ROS-dependent autophagy and G2/M arrest mechanisms	**Polyphyllin I/HCT116**	Nat Prod Res.	2018
Synthesis, Anti-Proliferative Activity Evaluation and 3D-QSAR Study of Naphthoquinone Derivatives as Potential Anti-Colorectal Cancer Agents	**Naphthoquinone/HT-29**	Molecules	2018
Magnolin promotes autophagy and cell cycle arrest via blocking LIF/Stat3/Mcl-1 axis in human colorectal cancers	**Magnolin/Stat3**	Cell Death & Disease	2018
1-Aroylindoline-hydroxamic acids as anticancer agents, inhibitors of HSP90 and HDAC	**1-Aroylindoline-hydroxamic acids/ HSP90 and HDAC**	Eur J Med Chem.	2018
Astragaloside IV inhibits cell proliferation of colorectal cancer cell lines through down-regulation of B7-H3	**Astragaloside IV/B7-H3**	Biomed Pharmacother.	2018
Identification of a fluorescent small-molecule enhancer for therapeutic autophagy in colorectal cancer by targeting mitochondrial protein translocase TIM44	**IR-58/TIM44**	Gut	2018
Antiproliferative efficacy of curcumin mimics through microtubule destabilization	**Curcumin mimic 6a/DLD1**	Eur J Med Chem.	2018
Artesunate induces apoptosis and autophagy in HCT116 colon cancer cells, and autophagy inhibition enhances the artesunate-induced apoptosis	**Artesunate/HCT116**	Int J Mol Med.	2018
Chemopreventive activity of celastrol in drug-resistant human colon carcinoma cell cultures	**Celastrol/LoVo/Dx**	Oncotarget	2018
Interrogation of ethnomedicinal plants for synthetic lethality effects in combination with deficiency in the DNA repair endonuclease RAD1 using a yeast cell-based assay	**B. monneiri/RAD1**	J Ethnopharmacol.	2018
Lichen-derived caperatic acid and physodic acid inhibit Wnt signaling in colorectal cancer cells	**Caperatic acid and Physodic acid/ Wnt**	Mol Cell Biochem.	2018

TABLE II – Potential CRC-related drugs identified in PubMed articles, followed by related tumor targets and their respective journals and year of publication

Title	Compounds/Targets	Journal	Year of Publication
Gambogic Acid Efficiently Kills Stem-Like Colorectal Cancer Cells by Upregulating ZFP36 Expression	**Gambogic Acid/ZFP36**	Cell Physiol Biochem.	2018
Pristimerin exhibits in vitro and in vivo anticancer activities through inhibition of nuclear factor-κB signaling pathway in colorectal cancer cells	**Pristimerin/ Nuclear factor-κB**	Phytomedicine	2018
Vitex rotundifolia Fruit Suppresses the Proliferation of Human Colorectal Cancer Cells through Down-regulation of Cyclin D1 and CDK4 via Proteasomal-Dependent Degradation and Transcriptional Inhibition	**Vitex rotundifolia Fruit/ Cyclin D1 and CDK4**	Am J Chin Med.	2018
Antitumor effect and molecular mechanism of antioxidant polysaccharides from Salvia miltiorrhiza Bunge in human colorectal carcinoma LoVo cells	**Salvia miltiorrhiza Bunge polysaccharides/ LoVo**	Int J Biol Macromol.	2018
Macranthoidin B Modulates Key Metabolic Pathways to Enhance ROS Generation and Induce Cytotoxicity and Apoptosis in Colorectal Cancer	**Macranthoidin B/HCT-116**	Cell Physiol Biochem.	2018
Musa paradisiaca inflorescence induces human colon cancer cell death by modulating cascades of transcriptional events	**PIMET/HT29**	Food Funct.	2018
Andrographolide Antagonizes TNF-α-Induced IL-8 via Inhibition of NADPH Oxidase/ROS/NF-κB and Src/MAPKs/AP-1 Axis in Human Colorectal Cancer HCT116 Cells	**Andrographolide/ HCT116**	J Agric Food Chem.	2018
Polygonumins A, a newly isolated compound from the stem of Polygonum minus Huds with potential medicinal activities	**Polygonumins A/HCT116**	Scientific Reports	2018
Monochalcoplatin: An Actively Transported, Quickly Reducible, and Highly Potent PtIV Anticancer Prodrug	**Monochalcoplatin/ HCT116**	Angew Chem Int Ed Engl.	2018
Polymeric micelles for potentiated antiulcer and anticancer activities of naringin	**Naringin/Caco-2**	Int J Nanomedicine	2018
Discovery of a potent hedgehog pathway inhibitor capable of activating caspase8-dependent apoptosis	**Hh003/Hh**	J Pharmacol Sci.	2018
Cytotoxic activity of the bioactive principles from Ficus burtt-davyi	**Ficus burtt-davyi/Caco-2**	J Environ Sci Health B.	2018
Manzamine A Exerts Anticancer Activity against Human Colorectal Cancer Cells	**Manzamine A/HCT116**	Mar Drugs	2018

TABLE II – Potential CRC-related drugs identified in PubMed articles, followed by related tumor targets and their respective journals and year of publication

Title	Compounds/Targets	Journal	Year of Publication
Cytotoxic activity of the twigs of Cinnamomum cassia through the suppression of cell proliferation and the induction of apoptosis in human colorectal cancer cells	**Cinnamomum cassia/Cyclin D1**	BMC Complement Altern Med.	2018
Crosstalk Between Apoptosis and Autophagy Is Regulated by the Arginylated BiP/Beclin-1/p62 Complex	**Bortezomib combined with Mitomycin C/ BiP/ Beclin-1/p62 Complex**	Mol Cancer Res.	2018
Autophagy mediates cytotoxicity of human colorectal cancer cells treated with garcinielliptone FC	**Garcinielliptone FC/ HT29**	J Cell Physiol.	2018
Heterocyclic Analogs of Sulforaphane Trigger DNA Damage and Impede DNA Repair in Colon Cancer Cells: Interplay of HATs and HDACs	**Sulforaphane/ HAT/HDAC**	Mol Nutr Food Res.	2018

Source: Findings of Research.

At the end of the screening, the availability of the molecular forms of the drugs in the scope of the article was considered, and from that, 21 drugs were identified. From the 21 drugs that were initially analyzed for their pharmacokinetic characteristics, only 4 drugs were selected (pa > 0.6 in PASS Online and positive "druglikeness" characteristic in the SwissADME platform).

The 4 drugs screened were: α-amyrin (A), Lupeol (B), Magnolin (C) and Naphthoquinone (D) (Figure 1), with pa = 0.858, 0.836, 0.627 and 0.622, respectively, on PASS Online. Their 2D structures are shown below. After that, the structures passed through the SwissTargetPrediction (STP) filter, to evaluate the therapeutic targets in which these drugs act, and the probability of these targets in relation to carcinomas.

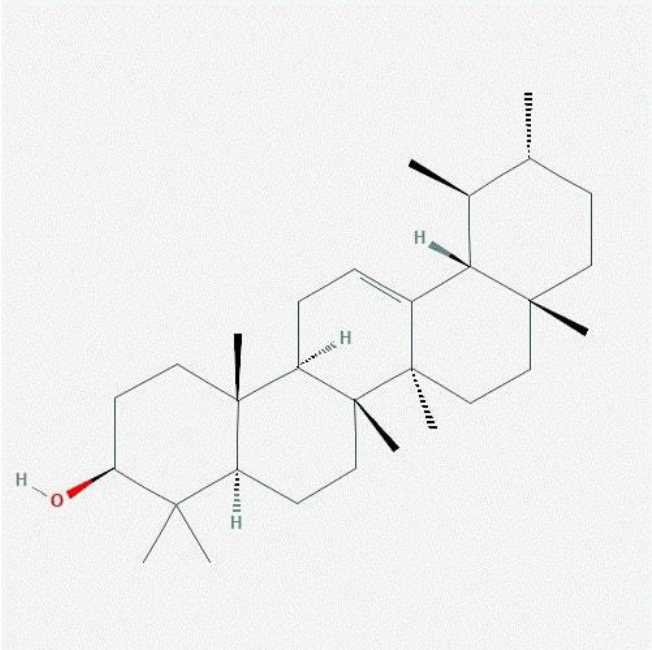

FIGURE 1A - Alpha-amyrin is a pentacyclic triterpenoid that is ursane which contains a double bond between positions 12 and 13 and in which the hydrogen at the 3beta position is substituted by a hydroxy group. It is a pentacyclic triterpenoid and a secondary alcohol. It derives from a hydride of an ursane.

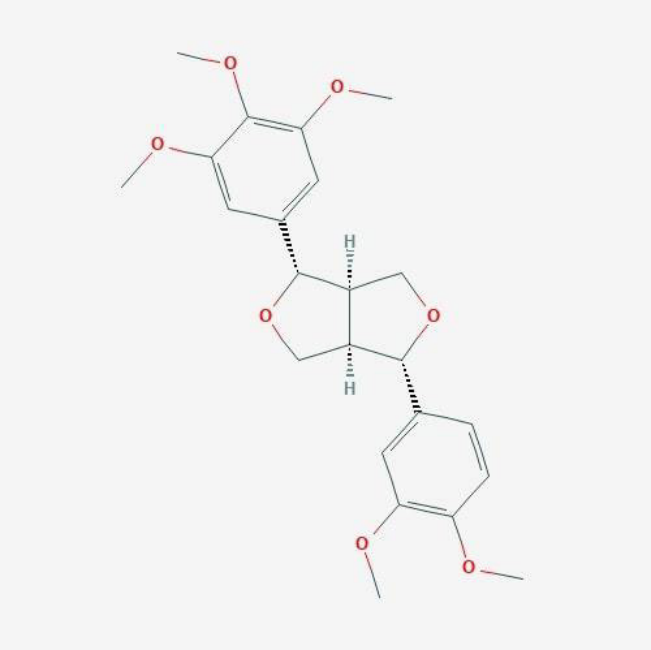

FIGURE 1C - Magnolin is a natural compound abundantly found in Magnolia flos, which has been traditionally used in oriental medicine to treat headaches, nasal congestion and anti-inflammatory reactions.

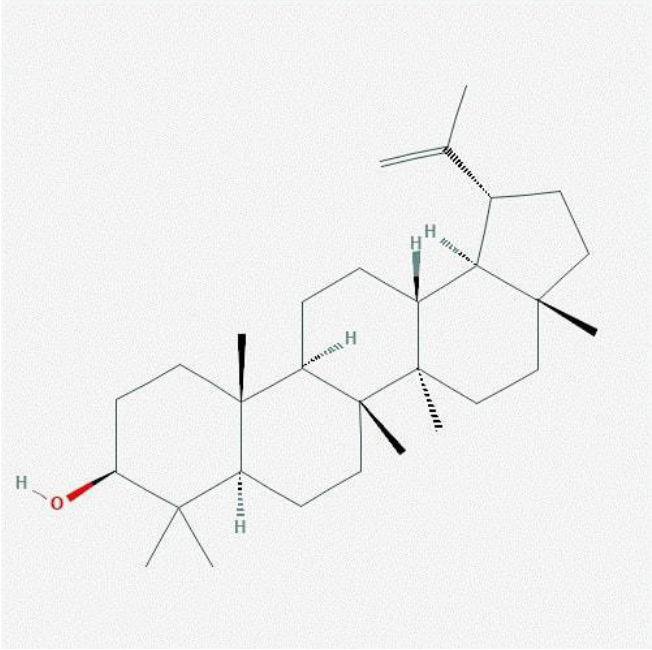

FIGURE 1B - Lupeol is a pentacyclic triterpenoid that is lupane in which the hydrogen at the 3beta position is substituted by a hydroxy group. It has a role as an anti-inflammatory drug and a plant metabolite. It is a secondary alcohol and a pentacyclic triterpenoid. It derives from a hydride of a lupane.

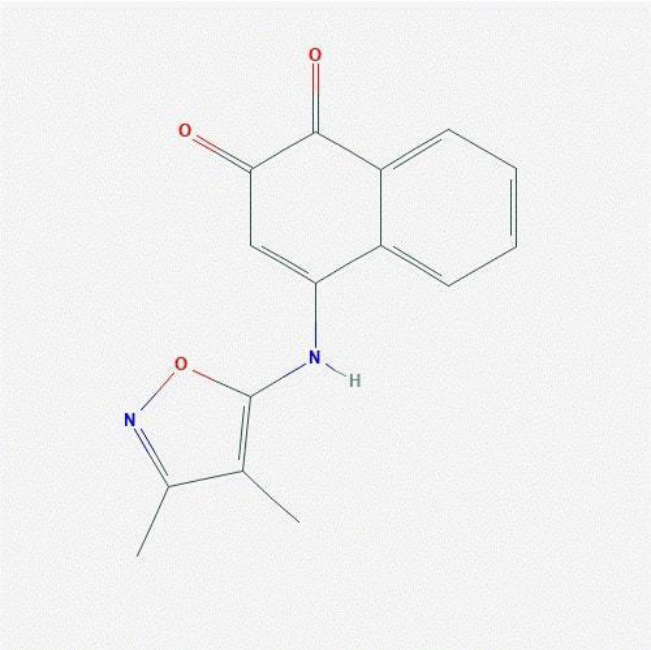

FIGURE 1D - Naphthoquinone is the major bioactive compound isolated from the alkanet plant. It was found that some naphthoquinone compounds have anticancer activity, but information about the structuralfunctional relationship of naphthoquinone compound is limited.

In the SwissTargetPrediction, among the targets identified in α-amyrin, the most related to colorectal carcinoma were CDC25A and CDC25B, with probability of 0.34 and 0.23, respectively. Among the identified targets of Lupeol, CDC25A and CDC25B were also among those involved with carcinoma cell proliferation, however with a lower probability (= 0.11), on a scale ranging from 0 to 1.

In Magnolin, the MCL1 target is related to the pathogenesis of leukemia, but it had a low probability (= 0.11). Finally, Naftoquinone had metalloproteinases in its target list, such as MMP3, MMP1 and MMP7, which play an extremely important role in the mechanism of tumor progression, although with a probability also below ideal (= 0.10).

Therefore, only α-amyrin was selected to perform molecular docking (Figure 2), whose ligand is the molecule in yellow, since it presented the highest probability among the 4 drugs evaluated in SwissTargetPrediction. The docking results showed that the CDC25B standard ligand obtained a value for interaction energy of -10.0 G (Kcal/mol), compared to that of α-amyrin, which obtained -7.6 G. Below are the figures resulting from the molecular interaction between ligand and protein (Figure 3) (Figure 4), obtained from Maestro version 12.1.013 and Discovery Studio 4.1, respectively.

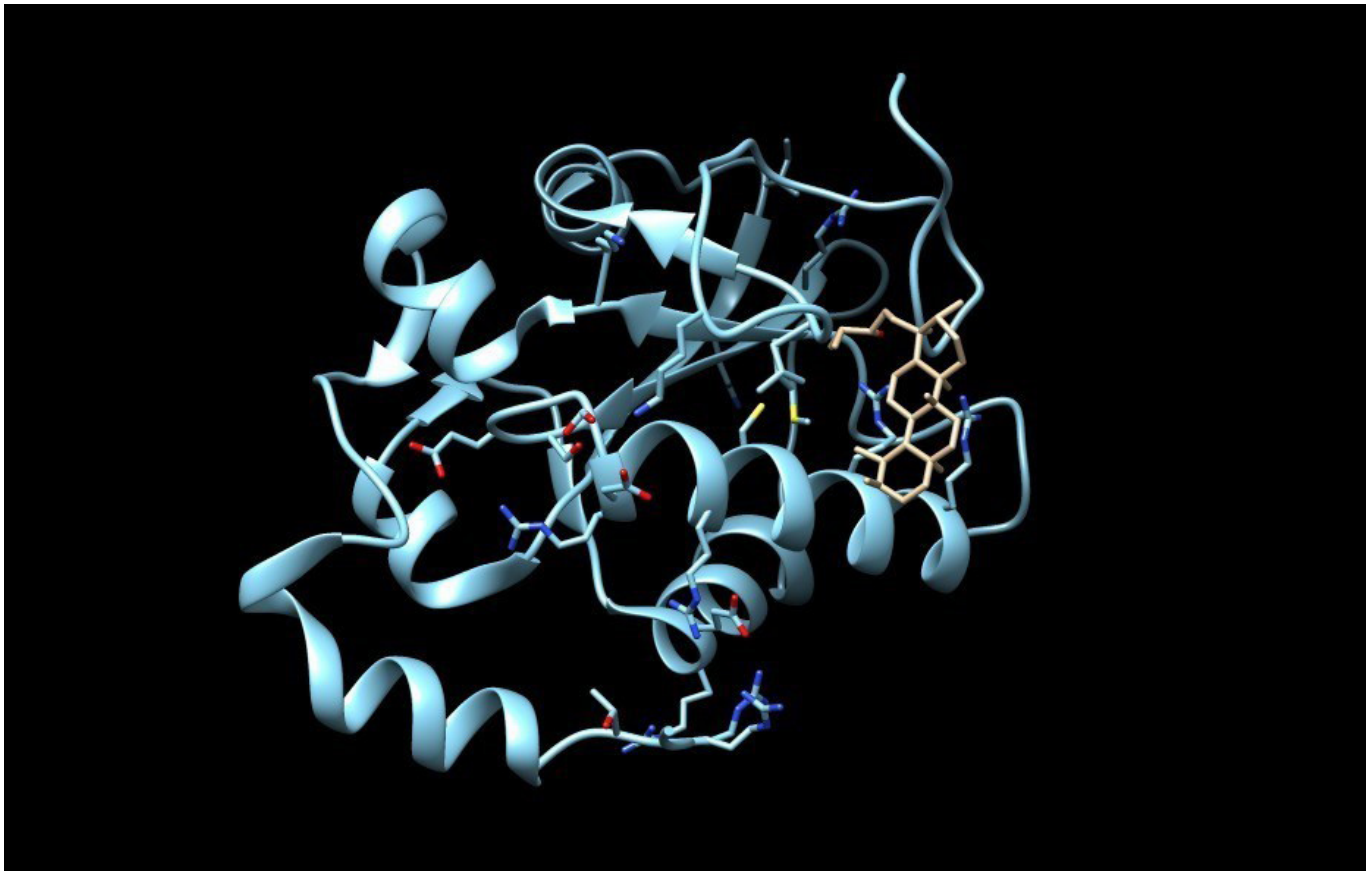

FIGURE 2 - Molecular Docking performed between the tumor target CDC25B and the ligand Alpha-amyrin, whose ligand is the molecule in yellow.

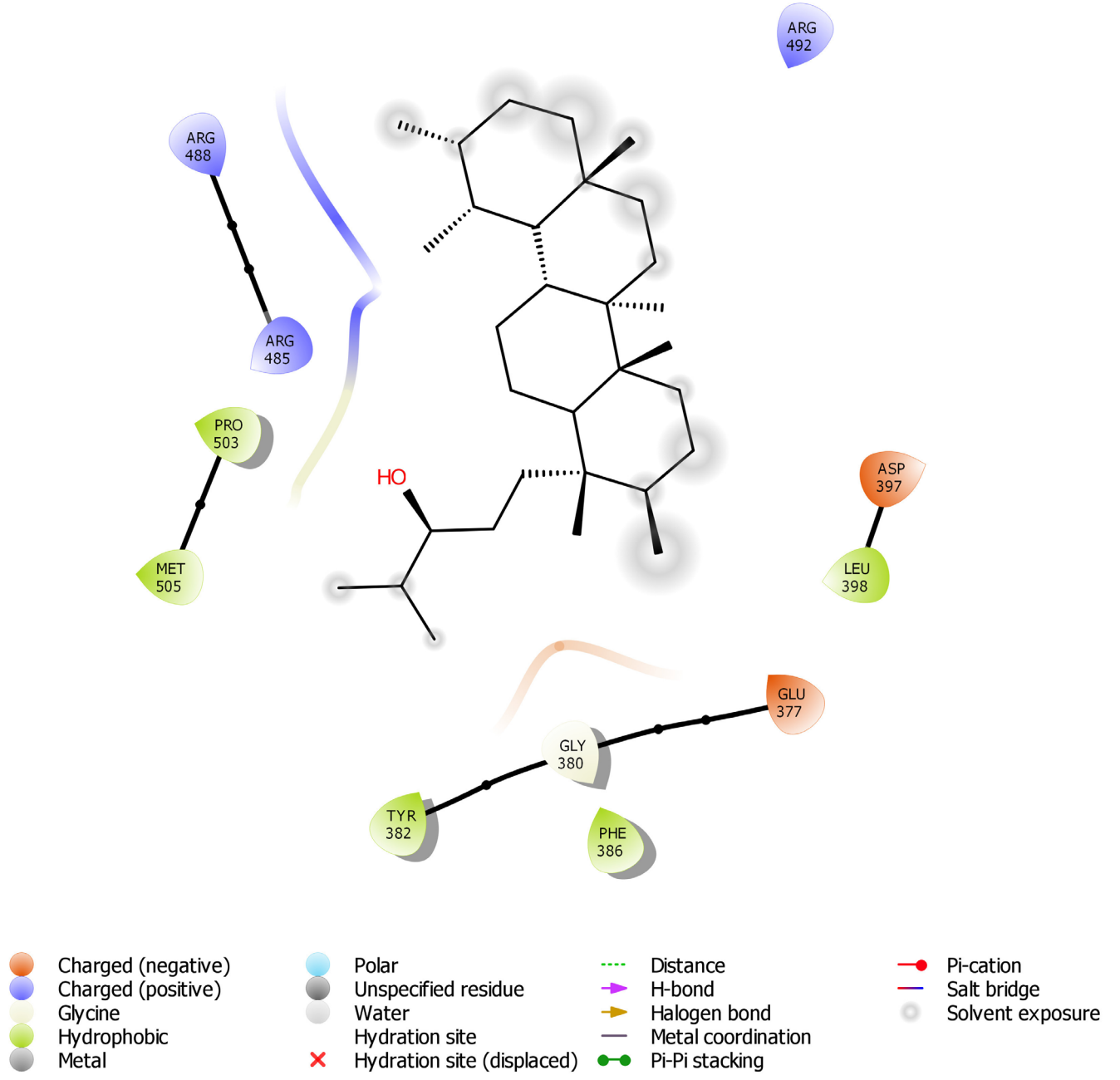

FIGURE 3 - 2D representation of the protein versus ligand interaction (CDC25B and α-amyrin), obtained from Maestro version 12.1.013, with their respective physical-chemical characteristics pointing to the stability of the structure. Such characteristics are crucial for the future development of viable drugs for the treatment of CRC.

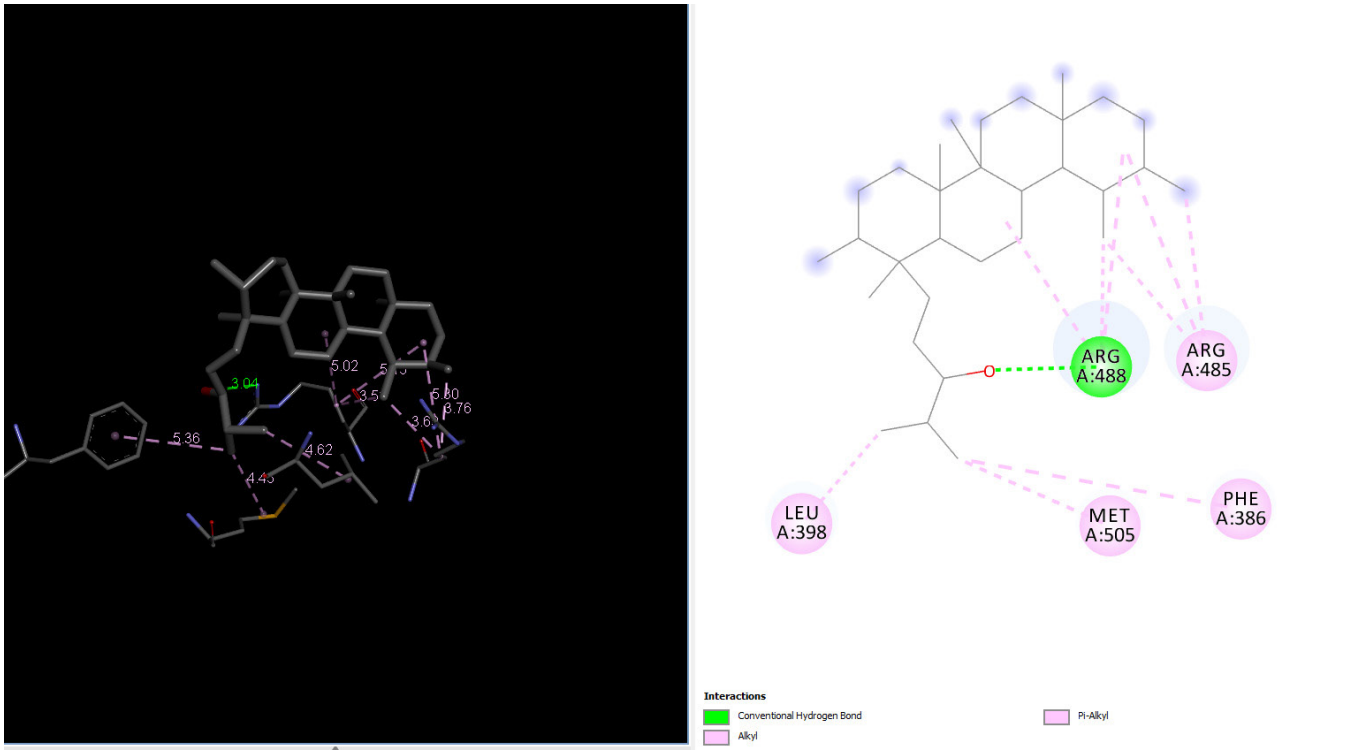

FIGURE 4 - Protein versus ligand interaction (CDC25B and α-amyrin), obtained from Discovery Studio 4.1. Hydrogen and alkyl bonding can be seen in ligand-protein interactions.

As illustrated in the figures above, hydrogen and alkyl bonding can be seen in ligand-protein interactions.

Hence, the lower (more negative) the interaction energy, the greater the attraction forces acting between the receptor and the ligand and, consequently, the greater the stability of the complex (receptor plus ligand), generally making the candidate more promising to be a drug (ligand).

On the other hand, it was not possible to perform molecular docking with CDC25A, as it did not have a cocrystallized structure with any ligand so that potential bonding could be performed.

Finally, no relationship was found between the molecules identified in the first phase of this research (CTNNB1, BAX, HMSH6, HMLH1, PTGS2, CCND1, KRAS, EGFR, PIK3CA and BRAF) and CDC25B on Thomson Reuters Integrity, justifying the non-continuation of the investigation of such markers, to the detriment of α-amyrin-related markers.

CDC25B

Subsequently, the identification of the CDC25B target related to α-amyrin on SwissTargetPrediction, the search for the proteins of that target in the PDB database (Protein Data Bank) continued, in which 17 protein structures were listed, being the 4CDC25B (Figure 5) used for molecular docking, as it was the only one that was cocrystallized with a ligand (α-amyrin), a necessary condition for comparison between possible interaction of the new ligand and the molecule considered a reference (8H8)

FIGURE 5 - 4WH7 Protein (3D structure). Structure of the CDC25B Phosphatase Catalytic Domain with Bound Ligand.

There are several transcription variants for this gene. Below is a summary of the protein and RNA expression of CDC25B divided by systems (Figure 6), its protein expression being significantly higher in the gastrointestinal tract, and in Figure 7 there is an overview of its expression in the various organs, in which it is observed that the colon and the rectum occupy third and fourth places, respectively, representing a fundamental fact in the search for new forms of inhibition of this gene in search of alternatives to the treatment of CRC.

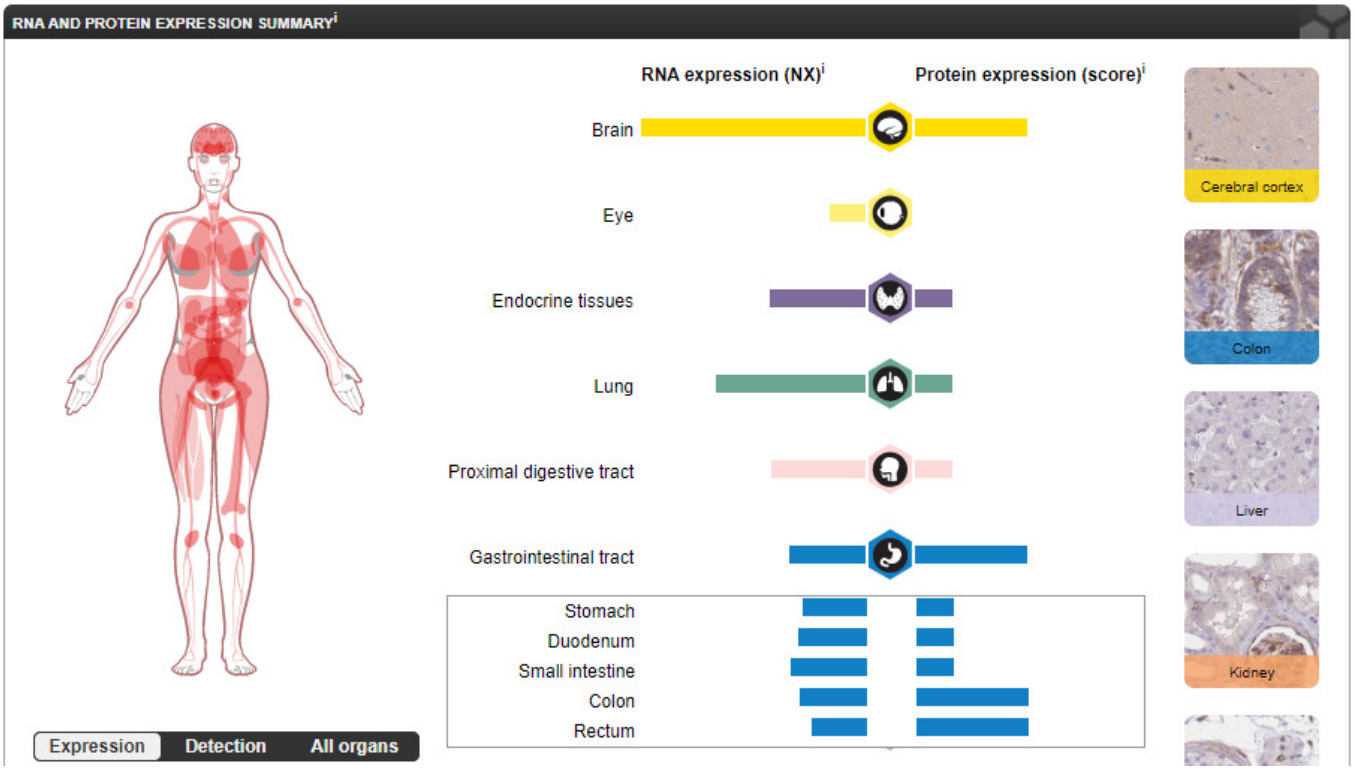

FIGURE 6 - Expression of CDC25B in organic systems. Nuclear and cytoplasmic expression in several tissue types, most abundant in the gastrointestinal tract and lymphoid tissues.

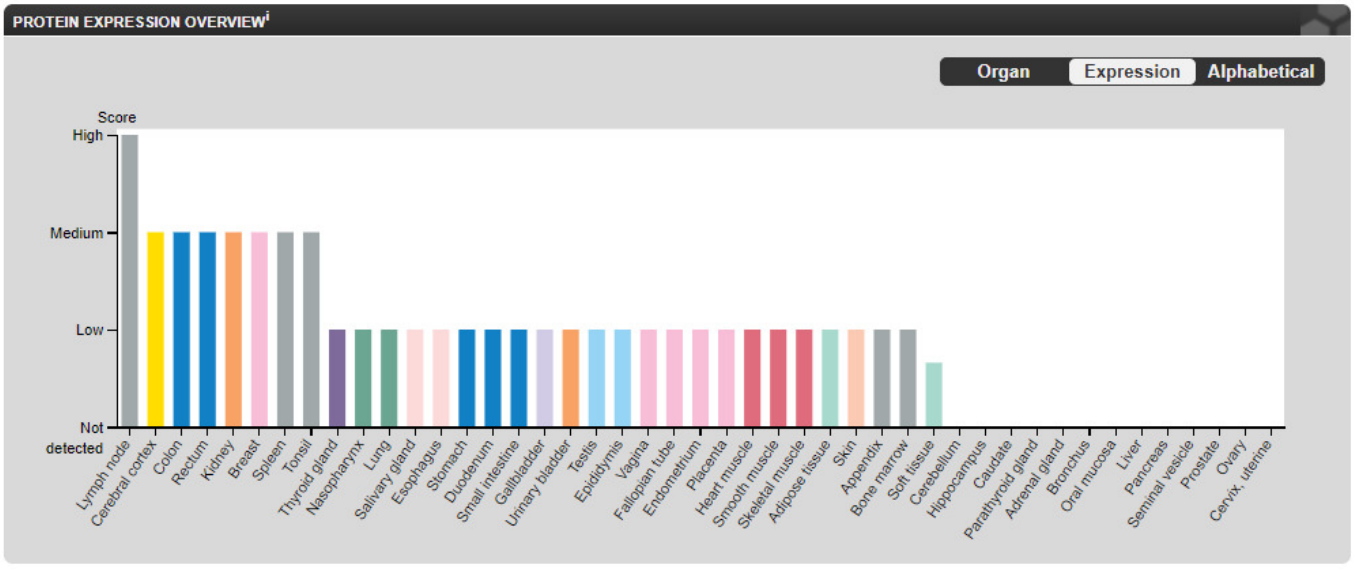

FIGURE 7 - Expression of CDC25B in organs. Nuclear and cytoplasmic expression in several organs, most abundant in Lymph node, Cerebral cortex, Colon and Rectum.

There is suggestive evidence that CDC25B phosphatase is an oncogenic protein. According to Takemasa *et al.* (2000)reported data on smoking behaviors for PLWH by gender, CDC25B is an oncogenic protein involved in the process of tumorigenesis. They also state that in human colorectal carcinoma, activation of the CDC2/cyclin B complex occurs due to overexpression of CDC25B, which improves growth conditions and maintains these tumors (Figure 8).

It also highlights the protein-protein interaction network to which the CDC25B belongs, taken from the STRING database, which has information from several sources, such as data from experimental studies, computational forecasting methods and public domain reviews (Figure 9).

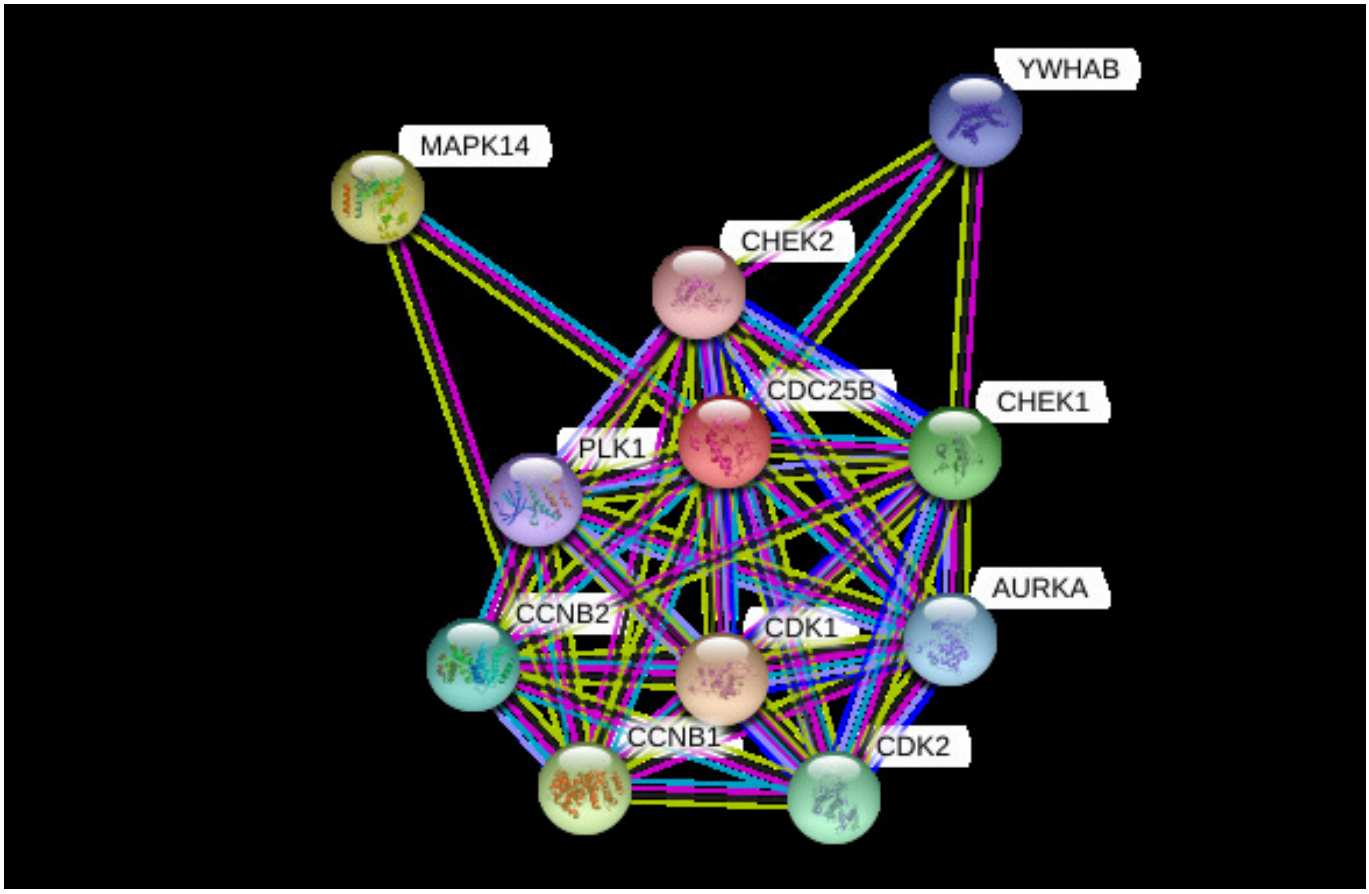

FIGURE 8 - CDC25B protein-protein interaction network. Network nodes represent proteins: For Review Only splice isoforms or post-translational modifications are collapsed, i.e. each node represents all the proteins produced by a single, protein-coding gene locus. Edges represent protein-protein associations: associations are meant to be specific and meaningful, i.e. proteins jointly contribute to a shared function; this does not necessarily mean they are physically binding each other.

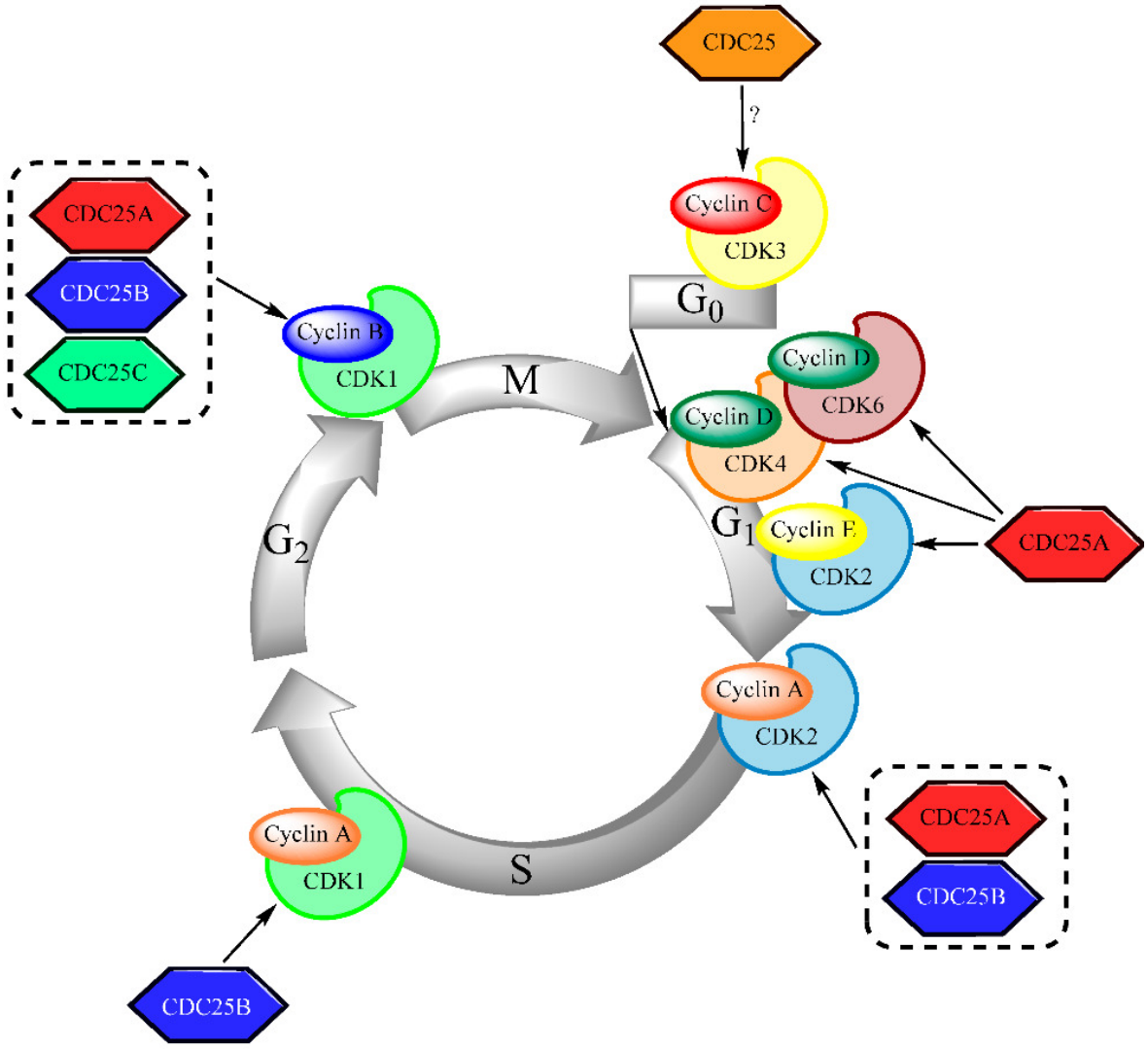

FIGURE 9 - An overview of the regulatory function of CDC25s in the progression of the cycle. M-phase inducer phosphatase 2; Tyrosine protein phosphatase which functions as a dosage-dependent inducer of mitotic progression. Required for G2/M phases of the cell cycle progression and abscission during cytokinesis in a ECT2-dependent manner. Directly dephosphorylates CDK1 and stimulates its kinase activity. The three isoforms seem to have a different level of activity (580 aa).

DISCUSSION

CDC25

Cell Division Cycle 25 phosphatases (CDC25) are members of the family of double specific protein phosphatases (DSPases). Among its main functions, the progression of the cell cycle stands out by activating cyclin-dependent serine/threonine-protein-kinase (CDKS). Overexpression of CDC25 is often associated with many types of cancers (Moura, Conde, 2019).

CDC25B is one of the members of CDC25 phosphatases and appears to be essential in the transition of the G2/M phase in human cells and in the separation during cytokinesis in an ECT2-dependent manner, in addition to dephosphorylating directly to CDK1 and stimulate your kinase activity (Zhao *et al.*, 2013).

As follows, CDC25B is an adequate target for drug intervention, since it has shown to be an oncogene when overexpressed, although its role in the formation of tumors has not been fully determined (Pruitt *et al.*, 2009)transcripts and proteins. RefSeq records integrate

information from multiple sources and represent a current description of the sequence, the gene and sequence features. The database includes over 5300 organisms spanning prokaryotes, eukaryotes and viruses, with records for more than 5.5 × 106 proteins (RefSeq release 30. This elucidates the fact that the labeling of CDC25 isoforms by inhibiting their protein-protein interactions with the substrate CDK2/Cyclin A demonstrates to be a new possible way of reaching this class of molecules as new therapeutic targets for CRC (Lund *et al.*, 2015).

Overexpression of CDC25B was also identified in 43% of patients with CRC and this fact implies a poor prognosis of the disease. The increase in CDC25B levels also interferes with the recognition of DNA damage repair points, while increasing spontaneous mutagenesis and impairing the onset of mitosis (Hassan *et al.*, 2014) and validation was performed using multiplex ligation probe amplification method. Genome-wide expression profiling was performed on 15 paired samples from the same group of patients using the Affymetrix Human Gene 1.0 ST array. Significant genes obtained from both array results were then overlapped. To identify molecular pathways, the data were mapped to the KEGG database. Whole genome CNV analysis that compared primary tumor and non-cancerous epithelium revealed gains in 1638 genes and losses in 36 genes. Significant gains were mostly found in chromosome 20 at position 20q12 with a frequency of 45.31% in tumor samples. Examples of genes that were associated at this cytoband were PTPRT, EMILIN3 and CHD6. The highest number of losses was detected at chromosome 8, position 8p23.2 with 17.19% occurrence in all tumor samples. Among the genes found at this cytoband were CSMD1 and DLC1. Genome-wide expression profiling showed 709 genes to be up-regulated and 699 genes to be down-regulated in CRC compared to non-cancerous samples. Integration of these two datasets identified 56 overlapping genes, which were located in chromosomes 8, 20 and 22. MLPA confirmed that the CRC samples had the highest gains in chromosome 20 compared to the reference samples. Interpretation of the CNV data in the context of the transcriptome via integrative analyses may provide more in-depth knowledge of the genomic landscape of CRC. . Thus, CDC25B presents itself as an important prognostic marker of colorectal carcinoma and can be clinically useful in the selection of patients who could benefit from adjuvant therapy.

Xiao *et al.* (2019)we show that cell division cycle 25B (CDC25B noted that CDC25B induces CDK2 dephosphorylation and activation, an event required for entry into mitosis, and is overexpressed in several tumors, including colorectal carcinoma. Therefore, further studies with CDC25B as a therapeutic target for CRC are justified, since there are still few studies published in this line of research, demonstrated through the search for "CDC25B AND colorectal cancer" in the PubMed database and only 14 articles found.

Drugs

Compound 7 (3 - [(1,4-dioxonaphthalen-2-yl) sulfanyl] propanoic acid) and sulfur-containing derivatives 4 and 6-8 represent some of the drugs that exhibit inhibitory activity against CDC25A and CDC25B (Li *et al.*, 2020). The natural compound HB-21, on the other hand, is able to bind irreversibly to cys473 through a covalent bond and inhibit CDC25B (Zhang *et al.*, 2018). In addition to these, sulforaphene promoted apoptosis of colon cancer cells and the interruption of the cell cycle in the G2 / M phase, while also phosphorylating CDK1 and CDC25B in inhibitory site (Byun *et al.*, 2016).

Alternative Splicing

An analysis of functional enrichment in 285 genes, 25% of which were mutated, demonstrated that the cancer cells of the colorectal segment present numerous forms of reprogramming of their transcriptome, which induces the control of the cell cycle stages involved in oncogenesis. The overexpressed genes that relate to CRC physiopathology and have alternative splicing were as follows: CCND1, CDC25B, MCM2 and MCM3 (Pira *et al.*, 2020).

When investigating the types of mRNA splicing in 32 transcriptomes, Pira *et al.* (2020) detected 12,800 important Alternative Splicing (AS) events present in the CRC cells in relation to the normal colorectal segment. A total of 148 SA genes were identified in the CRC, of

which 17 genes were shown to be new splicing events. Remarkably, 9 new genes out of 17 splicing have been shown to be involved in the pathogenesis of CRC, including CDC25B.

Alternative Splicing allows individual genes to produce multiple protein isoforms - thus playing a central role in the generation of complex proteomes (Nilsen, Graveley, 2010)breathing and, sometimes, thinking organism is staggeringly complex. Where do all of the parts come from? Early estimates stated that about 100,000 genes would be required to make up a mammal; however, the actual number is less than one-quarter of that, barely four times the number of genes in budding yeast. It is now clear that the 'missing' information is in large part provided by alternative splicing, the process by which multiple different functional messenger RNAs, and therefore proteins, can be synthesized from a single gene. . Many AS changes induce cancer-associated phenotypes and contribute to their pathophysiology by promoting angiogenesis, cell proliferation or stopping apoptosis (Climente-González, 2017)their functional impact and relevance to tumorigenesis remain mostly unknown. We carried out a systematic analysis to characterize the potential functional consequences of alternative splicing changes in thousands of tumor samples. This analysis revealed that a subset of alternative splicing changes affect protein domain families that are frequently mutated in tumors and potentially disrupt protein-protein interactions in cancer-related pathways. Moreover, there was a negative correlation between the number of these alternative splicing changes in a sample and the number of somatic mutations in drivers. We propose that a subset of the alternative splicing changes observed in tumors may represent independent oncogenic processes that could be relevant to explain the functional transformations in cancer, and some of them could potentially be considered alternative splicing drivers.

Circular RNAs

Accordingly, growing evidence has revealed that circular RNA (circRNA) plays critical roles in the development and progression of diseases, especially in cancers, and in the discovery of new tumor biomarkers as a research target for the development of target therapies. Li *et al.* (2019) investigated the role of circRNA in carcinogenesis and were able to identify a greater amount of hsa_circRNA_102958 in the CRC compared to the control group. They also demonstrated that hsa_circRNA_102958 promoted the expression of CDC25B by inhibiting miR-585 in the CRC.

Circular RNAs can be defined as a curious group of RNA due to their closed structure by covalent bonds, significant stability and crucial role in gene regulation (Vo *et al.*, 2019)high stability, and implicated roles in gene regulation. Here, we used an exome capture RNA sequencing protocol to detect and characterize circRNAs across >2,000 cancer samples. When compared against Ribo-Zero and RNase R, capture sequencing significantly enhanced the enrichment of circRNAs and preserved accurate circular-to-linear ratios. Using capture sequencing, we built the most comprehensive catalog of circRNA species to date: MiOncoCirc, the first database to be composed primarily of circRNAs directly detected in tumor tissues. Using MiOncoCirc, we identified candidate circRNAs to serve as biomarkers for prostate cancer and were able to detect circRNAs in urine. We further detected a novel class of circular transcripts, termed read-through circRNAs, that involved exons originating from different genes. MiOncoCirc will serve as a valuable resource for the development of circRNAs as diagnostic or therapeutic targets across cancer types.Through bioinformatics and molecular sequencing techniques, it was possible to expand knowledge about the expression of circRNAs in different species. It has, among others, great diversity and diverse participation in the cell cycle, in addition to playing an important role in transcription and splicing events (Bach, Lee, Sood, 2019)abundance, and evolutionary conservation among species points to their distinct properties and diverse cellular functions as efficient microRNAs and protein sponges; they also play important roles in modulating transcription and splicing. Additionally, most circRNAs are aberrantly expressed in pathological conditions and in a tissue-specific manner such as development and progression of cancer. Herein, we highlight the characteristics, functions, and mechanisms of action of circRNAs in cancer; we also provide an overview of recent progress in the circRNA field and future application of circRNAs as cancer biomarkers and novel therapeutic targets.

Ligant interactions

Using the molecular docking method, a 3D structure of the CDK2/Cyclin A set with CDC25B was created and validated. The researchers synthesized a ligand and concluded, through computer simulation, that the inhibitor comp #1 had the necessary characteristics to bind to CDC25B and to deregulate the interactions between CDC25B and CDK2/cyclin A (Li *et al.*, 2017) the most optimized 3D structure of CDK2/Cyclin A in complex with CDC25B was constructed and validated using two methods: 1. In that event, it is clear that CDC25B may be involved in the transition from adenoma to cancer. The expression of CDC25B in colorectal cancer might accelerate the transformation of the cell cycle and promotes metastases in distant organs.

As illustrated in the Figure 2, 3 and 4, hydrogen and alkyl were found in ligand-protein interactions of this research. Since hydrogen (H) bonds are often used to facilitate the protein-ligand bond, when using targeting as protein-ligand interactions itself, it is also related that Links H promote a binding affinity to the displacement ligand of water molecules filtered by the protein at the binding site (Ross, Morris, Biggin, 2012; Barillari *et al.*, 2007).

Alkyl halides are known to be highly flexible substances. Its reactive characteristic is generally researched in the fields of medicinal chemistry, as well as in biochemistry (Carey, Sundberg, 2007; Kambe, Iwasaki, Terao, 2011). This set can result in several experiments, such as protein purification, protein stabilization and translocation. This is because an alkylase chloride ligand binds rapidly, through covalent bonding, with the protein marker under physiological conditions (Nicolaou, Edmonds, Bulger, 2006).

Final Considerations

Given the importance of in silico studies in the evaluation of study objects in millimeter-controlled conditions, its fundamental importance in determining new drugs against tumor markers is observed. Thus, this research resulted in relevant findings, such as the α-amyrin, Lupeol, Magnolin and Naphthoquinone ligands.

The drugs mentioned above, especially α-amyrin, are shown as potential objects of study for further research, with the CDC25B as a target, since this marker was important in the pathophysiology of colorectal carcinoma.

Additionally, the union of different platforms was useful in elucidating the analyzes, since they all complement each other. SwissADME, for example, identified the pharmacokinetic characteristics of molecules, while Thomson Reuters Integrity made it possible to assess the interactions between possible targets and drugs. Finally, GeneCards® and MalaCards served to identify genes and other tumors related to CRC.

Considering that the extremely strict control of experimental conditions observed in in silico studies does not occur in the same way as in *in vitro* and *in vivo* studies, it is beneficial to invest resources and research in this area of concentration. Another advantage is that in silico methods make their prediction based on the structure of a compound even before it is synthesized, promoting greater savings in resources, agility in the delivery of results, virtual and high-throughput screening of candidate drugs and less possibility of errors and unfavorable research outcomes.

In this sense, the investigation of CDC25B through molecular docking, using its CDC25B protein, allowed for greater elucidation of its structure, in addition to understanding the pharmacokinetic and pharmacodynamic viability of its ligand through its pharmacokinetic and pharmacodynamic characteristics, and also protein-protein interactions and protein-ligand found in this work using in silico analysis software.

Therefore, CDC25B is an important therapeutic target involved in the pathogenesis of CRC, and its α-amyrin ligand has potential clinical viability as a future drug for targeted therapy, since it demonstrated favorable protein-ligand interactions in molecular docking assays. However, *in vitro* and *in vivo* studies are still needed to determine toxicity patterns, therapeutic concentrations and cell specificity.

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