

RESEARCH ARTICLE

Open Access



A microRNA molecular modeling extension for prediction of colorectal cancer treatment

Jian Li^{1,2,3*} and Ulrich R. Mansmann^{1,2}

Abstract

Background: Several studies show that the regulatory impact of microRNAs (miRNAs) is an essential contribution to the pathogenesis of colorectal cancer (CRC). The expression levels of diverse miRNAs are associated with specific clinical diagnoses and prognoses of CRC. However, this association reveals very little actionable information with regard to how or whether to treat a CRC patient. To address this problem, we use miRNA expression data along with other molecular information to predict individual response of CRC cell lines and CRC patients.

Methods: A strategy has been developed to join four types of information: molecular, kinetic, genetic and treatment data for prediction of individual treatment response of CRC.

Results: Information on miRNA regulation, including miRNA target regulation and transcriptional regulation of miRNA, is integrated into an *in silico* molecular model for colon cancer. This molecular model is applied to study responses of seven CRC cell lines from NCI-60 to ten agents targeting signaling pathways. Predictive results of models without and with implemented miRNA information are compared and advantages are shown for the extended model. Finally, the extended model was applied to the data of 22 CRC patients to predict response to treatments of sirolimus and LY294002. The *in silico* results can also replicate the oncogenic and tumor suppression roles of miRNA on the therapeutic response as reported in the literature.

Conclusions: In summary, the results reveal that detailed molecular events can be combined with individual genetic data, including gene/miRNA expression data, to enhance *in silico* prediction of therapeutic response of individual CRC tumors. The study demonstrates that miRNA information can be applied as actionable information regarding individual therapeutic response.

Keywords: MicroRNA regulation, Signaling pathway, Individualized medicine, Colorectal cancer

Background

With an average of 610,000 deaths per year, CRC has become the second most common cause of cancer death on a global scale. Because it is most commonly diagnosed at advanced stages, approximately 50 % of patients diagnosed with CRC will surrender to the disease [1]. From a molecular perspective, CRC is characterized by the accumulation of genetic alterations affecting the cellular functionalities of oncogenes and tumor suppressor genes, leading to genomic instability and cellular dysfunction [2]. A number of deregulated signaling pathways, most notably Wnt [3], Notch [4], Hedgehog [5] and others [6, 7], have

been identified as maintaining the malignant cellular growth and cancerous functional integration of CRC. The signaling network based on these deregulated pathways steers the oncogenetic development. Furthermore, miRNAs are deeply involved in the pathogenesis of CRC by affecting key components of those signaling pathways. They play significant roles in regulating cell growth, proliferation, invasion and metastasis in CRC [8–14]. Moreover, recent studies have demonstrated that miRNAs detected in blood serum, plasma and even in stool offer novel non-invasive approaches to diagnose CRC [13, 15–17]. Other recent studies revealed experimentally that expression levels of certain miRNAs were associated with specific prognosis and therapeutic outcomes in CRC, which provides compelling evidence that miRNAs have the potential to be prognostic and predictive biomarkers [18–21]. Further, detailed molecular

* Correspondence: lijian@ibe.med.uni-muenchen.de

¹Institute for Medical Informatics, Biometry and Epidemiology, Ludwig-Maximilians-University München, Munich, Germany

²German Cancer Consortium (DKTK), Heidelberg, Germany

Full list of author information is available at the end of the article

information related to miRNA function in clinical application was reported in the study of Melo & Kalluri [22]. Our study introduces an *in silico* model for the colorectal cancer cell which implements miRNA information and explores its potential for improved response prediction to specific treatment.

Methods

Colorectal Cancer Patients

The gene-expression and miRNA-expression data of the 22 CRC patients examined in this study can be downloaded from <https://tcga-data.nci.nih.gov/tcga/>, which is provided by the Cancer Genome Atlas, with the following filter setting configuration: Select a disease → *COAD- Colon adenocarcinoma*; Data type → *RNASeqV2/miRNASeq*; Center/Platform → *All*; Batch number → *All*; Sample → *Patient ID*; Data level → *Level 3*; Availability → *None*.

Each dataset was produced through the analysis of high-quality colon tumor samples from the participants. Each dataset was normalized using the trimmed mean of M-values normalization method proposed by Robinson & Oshlack [23], to remove systematic technical effects and minimize the sequencing technical bias on the data.

The Genomic Data of CRC Cancer Cell Lines from NCI-60

The CRC cancer cell lines examined in this study are COLO-205, HCC-2998, HCT-116, HCT-15, HT29, KM12, SW-620. The gene-expression data of these cancer cell lines can be downloaded via [24]. The miRNA-expression data can be accessed via [25].

The Non-Steroidal Anti-Inflammatory Drug (NSAID) Model

Our previous study [26] introduced the *in silico* NSAID model, which incorporates information of 20 diverse CRC-relevant signaling pathways, such as Wnt, Notch, BMP, beta-catenin and Hedgehog, and other molecular features. The model contains different types of biological components such as genes, RNAs, proteins and complexes. Components of the network are used to quantify specific aspects of tumorigenesis in terms of the cancer hallmarks [27]. The study demonstrated application for two therapeutic developmental strategies: synthetic lethality and miRNA biomarker discovery.

Molecular Addition of miRNA-Regulation (miRAO)

The algorithmic basis for our study is the NSAID model introduced by Li & Mansmann [26]. The following steps implement miRNA regulations:

1. Convert miRNA-target data into a data array (miRT) (with Ensembl-ID as key; miRNA-ID and references

(PubMed) as value); for example, miRT [ENSG00000236342] = (mir-1238, 17964270).

2. Convert the TransmiR data into a data array (TFmiR) (with miR-ID as key; Ensembl-ID, transcriptional regulation, references (PubMed) as value); for example, TFmiR[mir-223] = (ENSG00000159216, repression, 17996649).
3. Divide the NSAID model (XML file of NSAID) into different data arrays according to component type (such as gene array, mRNA array, protein array, reaction array, etc.)
4. Iterate all the gene components in the gene array; when a gene with Ensembl-ID matches a key of miRT, then the corresponding miRNA is created in the model by defining name, ID, location and other. Afterwards, the TFmiR is applied to identify the transcriptional factors that regulate the expression level of this miRNA. The corresponding type of transcription reaction is defined to link the miRNA gene with the miRNA. Afterwards, the miRNA is translocated into cytoplasm and modeling of its regulation on the corresponding mRNA is created and added into the model (Fig. 1). (The detailed molecular modeling is explained in the study of Li et al. [28].)

Model Initialization with miRNA and Gene Expression Data

The initial value of all components in the model is zero. The miRNA- and gene-expression data is available through the link from the Cancer Genome Atlas (as mentioned in the paragraph “Colorectal Cancer Patients”) or from the cell line data. These datasets are converted into a data array (similarly to the method explained above). The keys of this data array are the miRNA-IDs and Ensembl-IDs as well as the expression values. Afterwards, the expression levels of miRNA genes and normal genes in the model are set to the values as given by the miRNA-ID and Ensembl-ID. During Petri net simulation, signal fluxes of reactions are simulated in the model by transcription reactions and are expanded to the rest of the model by other reactions defined in the model. This data initialization is the input for the Flux Comparative Analysis explained in the following.

Mathematical Implementation of Sirolimus and LY294002

We study the effect of sirolimus as a specific mTor inhibitor. According to Nashan [29], its dissociation constant is 0.65 nM indicating an inhibition of biochemical reactions catalyzed by the mTor protein complex. LY294002 is a potent inhibitor of PI3K, which catalyzes the conversion from PIP2 to PIP3. The dissociation constant of LY294002 is 210 nM [30]. Table 1 contains the

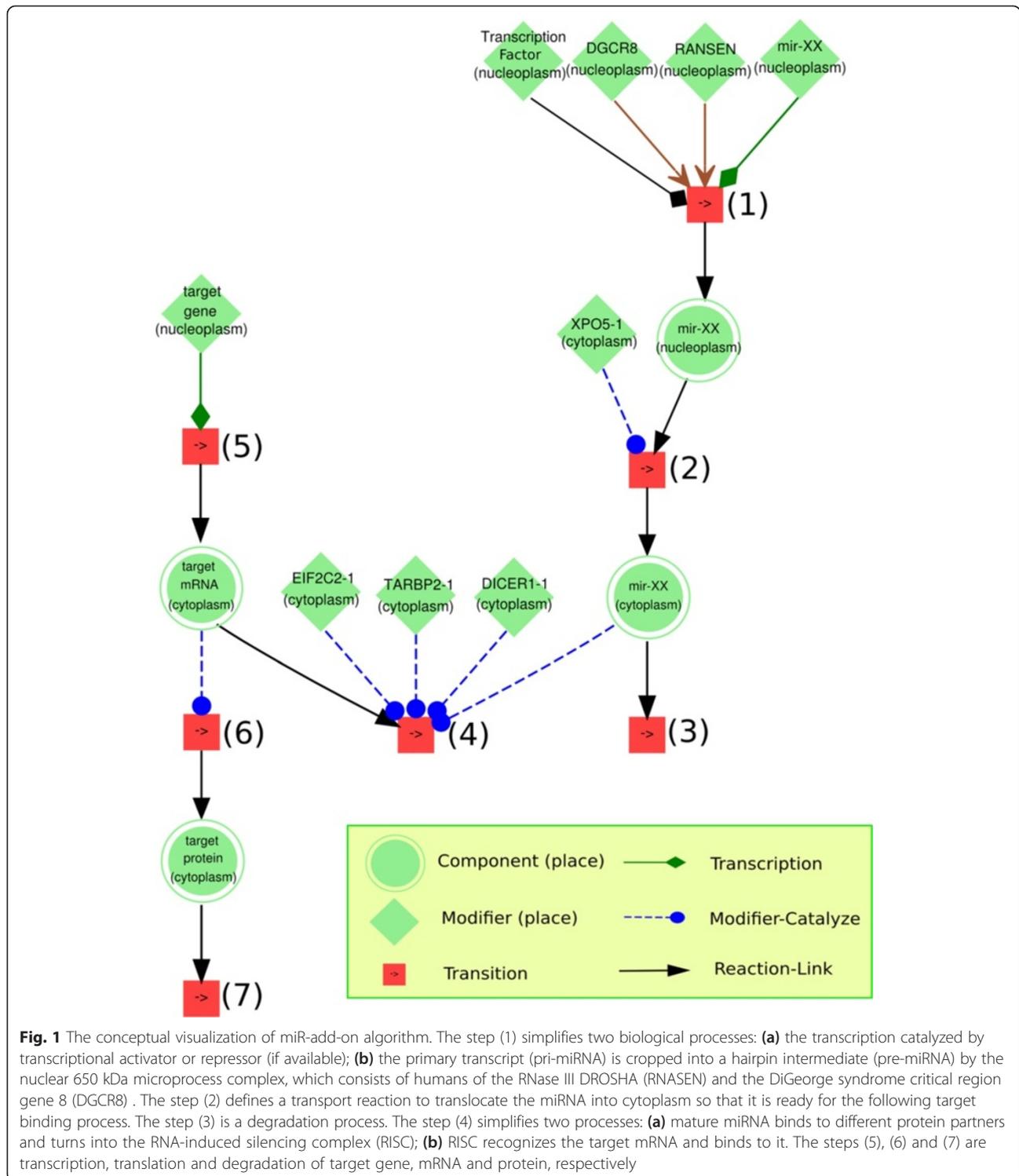


Fig. 1 The conceptual visualization of miR-add-on algorithm. The step (1) simplifies two biological processes: **(a)** the transcription catalyzed by transcriptional activator or repressor (if available); **(b)** the primary transcript (pri-miRNA) is cropped into a hairpin intermediate (pre-miRNA) by the nuclear 650 kDa microprocess complex, which consists of humans of the RNase III DROSHA (RNASEN) and the DiGeorge syndrome critical region gene 8 (DGCR8) . The step (2) defines a transport reaction to translocate the miRNA into cytoplasm so that it is ready for the following target binding process. The step (3) is a degradation process. The step (4) simplifies two processes: **(a)** mature miRNA binds to different protein partners and turns into the RNA-induced silencing complex (RISC); **(b)** RISC recognizes the target mRNA and binds to it. The steps (5), (6) and (7) are transcription, translation and degradation of target gene, mRNA and protein, respectively

molecular modeling of both drugs and the corresponding control state (without treatment).

Flux Comparative Analysis (FCA)

The FCA is an advanced Petri net simulation strategy. As the name suggests, it is an analysis of flux comparison

of two different states of a molecular model. The goal of FCA analysis is to detect whether a therapeutic intervention (drug treatment) can cause a significant flux change with regard to the structure of an entire molecular network, in order to predict how an individual would respond to a therapeutic intervention [26]. Essentially,

Table 1 'E': enzyme; 'I': Inhibitor. Currently, we have only applied the mass action law for implementing biochemical reaction

Sirolimus treatment	CRC patient + sirolimus	CRC patient
Molecular modeling	mTor complex II (E); Sirolimus (I)	mTor complex II (E)
	PRKCG + ATP $\rightarrow \rightarrow$ P-PRKCG + ADP	PRKCG + ATP $\rightarrow \rightarrow$ P-PRKCG + ADP
	mTor complex II (E); Sirolimus (I)	mTor complex II (E)
	SGK + ATP $\rightarrow \rightarrow$ P-SGK + ADP	SGK + ATP $\rightarrow \rightarrow$ P-SGK + ADP
	mTor complex II (E); Sirolimus (I)	mTor complex II (E)
	PRKCA + ATP $\rightarrow \rightarrow$ P-PRKCA + ADP	PRKCA + ATP $\rightarrow \rightarrow$ P-PRKCA + ADP
	mTor complex II (E); Sirolimus (I)	mTor complex II (E)
	AKT + ATP $\rightarrow \rightarrow$ P-AKT + ADP	AKT + ATP $\rightarrow \rightarrow$ P-AKT + ADP
	mTor complex II (E); Sirolimus (I)	mTor complex II (E)
	HIF1A + ATP $\rightarrow \rightarrow$ P-HIF1A + ADP	HIF1A + ATP $\rightarrow \rightarrow$ P-HIF1A + ADP
	mTor complex II (E); Sirolimus (I)	mTor complex II (E)
	PPARGC1 + ATP $\rightarrow \rightarrow$ P-PPARGC1 + ADP	PPARGC1 + ATP $\rightarrow \rightarrow$ P-PPARGC1 + ADP
	mTor complex II (E); Sirolimus (I)	mTor complex II (E)
	EIF4EBP + ATP $\rightarrow \rightarrow$ P-EIF4EBP + ADP	EIF4EBP + ATP $\rightarrow \rightarrow$ P-EIF4EBP + ADP
	mTor complex II (E); Sirolimus (I)	mTor complex II (E)
	PPARG + ATP $\rightarrow \rightarrow$ P-PPARG + ADP	PPARG + ATP $\rightarrow \rightarrow$ P-PPARG + ADP
LY294002 treatment	CRC patient + LY294002	CRC patient
Molecular modeling	Enzymes; LY294002 (I)	Enzymes
	PIP2 + ATP $\rightarrow \rightarrow$ PIP3 + ADP	PIP2 + ATP $\rightarrow \rightarrow$ PIP3 + ADP

For instance, the substance A and B participate in a reaction catalyzed by an enzyme and inhibitor to produce the products C and D: enzyme; inhibitor
 $A + B \rightarrow \rightarrow C + D$
 where the mathematical implementation: $[C] = [D] = [A] * [B] * [\text{enzyme}] * [iKd] / [\text{inhibitor}] * [eKd]$, eKd: enzymatic dissociation constant; iKd: inhibitor dissociation constant

during FCA, two states are generated for each cell line/patient for each treatment: one is the control state (without treatment) and the other is the perturbation state (with treatment). During the Petri net simulation, the fluxes generated for each state in the model are compared for each patient. The following simulation algorithm code is applied to generate the steady state of each state:

R_i = the i -th reaction in the molecular model; Parameters of R_i include speed (S), kinetic parameter (k), product (p), reactant (a), enzyme (e)
 $C_{j,t}$ = the concentration of the j -th bio-object (such as gene, protein) in the model at time step t
 $S_t = C_{a,t} * C_{e,t} * k$
 N, M = the number of reactions and bio-objects in the model, respectively.

1. Input: Gene-expression data and miRNA-expression data
2. For each j (from 1 to M) at time step t :
3. if $C_{j,t} - C_{j,t-5} > 0.001$:
4. then reachSteadyState = False
5. If not reachSteadyState:
6. for each i (from 1 to N) at time step t :

7. if $C_{p,t-1} < C_{a,t-1}$ & $S_t < C_{a,t-1} * 0.75$:
8. then evaluate R_i as Petri net firing rule at t :
9. $C_{p,t} = C_{p,t-1} + S_t$
10. $C_{a,t} = C_{a,t-1} - S_t$
11. If reachSteadyState:
12. select the readout components
13. Output: compare readout components between two states (Control vs. Treatment)

Definition of Sensitivity Score for Drug Response

Experimentally based sensitivity score (experimental data) = GI_{50}

(GI_{50} : the $-\log$ mol/L drug/concentration yielding a growth inhibition of 50 %, [31])

Model-based sensitivity score (prediction data) = $\log(P) + K$

(P : relative change value of readout component 'proliferation' hallmark in the treatment state compared to that in the control state. In this case, the hallmark 'proliferation' is selected as the readout component for the FCA analysis; K : constant value, currently estimated as 5.2. the hallmark "proliferation", as a mirror of proliferative ability, is taken as the primary outcome, since the cell line models

quantify response on treatment by its impact on cellular growth)

Correlation Between Experimentally Measured and Computationally Simulated Scores

It is of interest to calculate the Pearson correlation between observed and predicted response of cell lines under a specific treatment. Aggregating correlation over all treatments was calculated following the principles of Bland and Altman as presented in [32].

The Availability of the Model

The XML file of the NSAID-miR model is available under [33].

Results

A strategy for prediction of individual treatment response is proposed which is based on an *in silico* environment in which the molecular regulation effect of miRNAs combined with other molecular information can be utilized. A flowchart depicts the work-flow of this concept (Fig. 2). There are four major sources of input information: molecular, kinetic, individual genetic (miRNA/mRNA expression data) and treatment data.

The microRNA Extension for the Non-Steroidal Anti-Inflammatory Drug (NSAID) Model

The NSAID model depicts a consolidated molecular basis of CRC which is extended in this study by including miRNA regulation. An algorithm, the miRNA-add-on (miRAO), is proposed which automatically adds miRNA regulation into molecular models such as the NSAID model. The miRAO checks whether each miRNA has

been validated with targets according to a specified miRNA-target database (Additional file 1) and whether each miRNA has validated transcription factors (TFs) according to the TransmiR database (version 1.2), which provides detailed information regarding type and effect of transcriptional regulations on miRNAs with corresponding literature [34]. If such validated gene targets or miRNA transcription factors are available, then the miRAO adds the molecular miRNA regulation to the model (Fig. Methods;). In this way, the NSAID model is extended with available validated miRNA-target and TF-miRNA information, which strengthens the model with detailed molecular regulation mechanisms related to miRNA. The new version of the model is named NSAID-miR; a summarization is given in Table 2.

Therapeutic Prediction of Ten Signaling Agents on CRC Cancer Cell Lines

In order to validate the NSAID-miR model, the inhibition effects of ten signaling agents on seven CRC cancer cell lines are simulated and compared to experimentally measured inhibition effects (sensitivity scores) from the study of Holbeck et al. [31]: COLO-205, HCC-2998, HCT-116, HCT-15, HT29, KM-12, and SW-620. The NSAID-miR model is initialized with gene-expression and miRNA-expression data of individual CRC cancer cell lines (Methods). The effects of ten signaling agents are studied: dasatinib, erlotinib, everolimus, gefitinib, imatinib, lapatinib, nilotinib, sorafenib, sunitinib, and temsirolimus. The inhibition potential of these tyrosine-kinase inhibitors can be specified through corresponding dissociation constants. They were measured experimentally by Karaman and

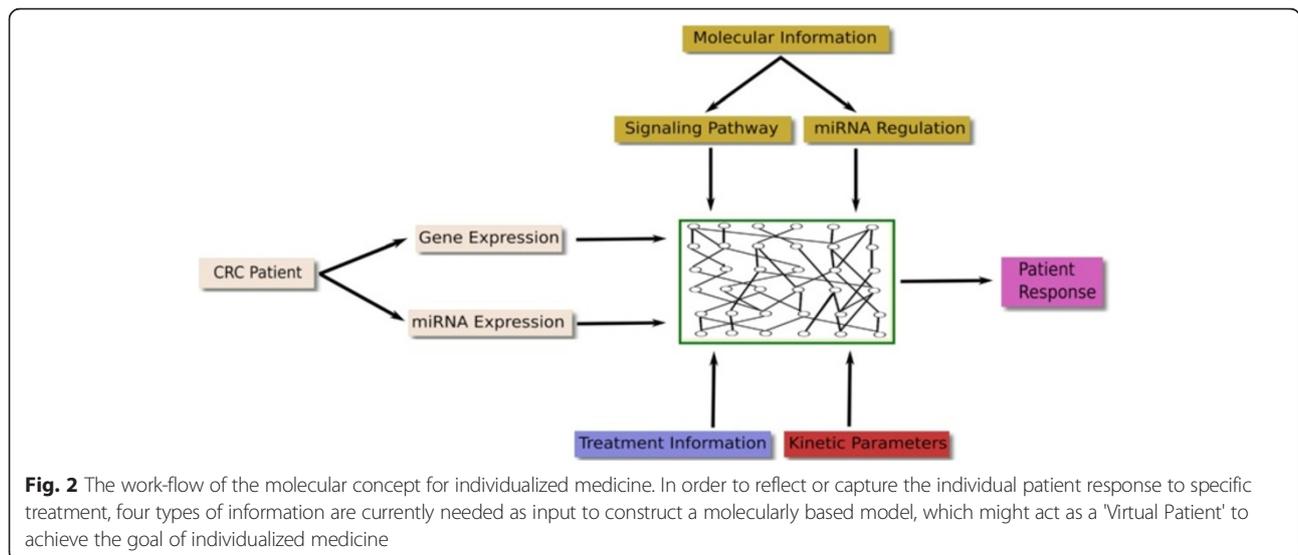


Fig. 2 The work-flow of the molecular concept for individualized medicine. In order to reflect or capture the individual patient response to specific treatment, four types of information are currently needed as input to construct a molecularly based model, which might act as a 'Virtual Patient' to achieve the goal of individualized medicine

Table 2 The component/reaction summary of the NSAID-miR model

Component	No.	Reaction	No.
Gene	1284	Transcription	1933
mRNA	2360	Translation	898
Protein	1473	Decay	2172
miRNA	367	Complex-formation	579
Compound	44	Translocation	1361
Complex	856	Phosphorylation	749
Pseudo-object	21	Dephosphorylation	357
SiRNA	1	Activation	341
		miRNA-binding	1516
Sum:	6406	Sum:	9906

colleagues [35]. How these inhibition effects are modeled is described in Table 3. Subsequently, we performed the FCA to calculate the simulated sensitivity scores of the cell lines (Methods) and supplies kinetic data. The Pearson correlation is used to compare the model-based sensitivity scores to the experimentally based sensitivity scores measured under the *in vitro* condition by Holbeck et al. [31]. Among these signaling agents, the dasatinib (0.964, $p = 2.78e-03$), everolimus (0.929, $p = 6.75e-03$), imatinib (0.893, $p = 1.23e-02$), and sunitinib (0.821, $p = 3.41e-02$) have high correlations of sensitivity scores (>0.80 , Fig. 3a-c, Additional file 2). We also quantified the correlation between everolimus and temsirolimus treatment response of CRC cell lines measured by GI-50 and the model-based sensitivity score using the R^2 measure. Under everolimus, $R^2 = 0.9713$, and under temsirolimus, $R^2 = 0.9824$. (Fig. 3d). These high correlations provide evidence that the NSAID-miR model captures drug effects in the CRC cellular system.

Lapatinib (0.679, $p = 1.10e-02$) has the lowest correlation of sensitivity scores (Fig. 3b, Additional file 2). The reason for this relatively low prediction rate could be that the inhibition effect of lapatinib in the NSAID-miR is only determined by the inhibition of three members of the ERBB-family. The dissociation constant between lapatinib and ERBB4 is relatively high (54 nM), which further weakens the effect of lapatinib in the NSAID-miR (Table 3). The overall correlation of the sensitivity scores of all ten drug treatments is 0.947. However, without use of miRNA expression data as input data for the *in silico* model, the same approach only achieved an overall correlation of 0.838 (Fig. 4). This difference (p -value: 0.021) indicates the value of miRNA expression profiles in better understanding the molecular mechanisms of cellular systems and their essential role in the prediction of therapeutic responses.

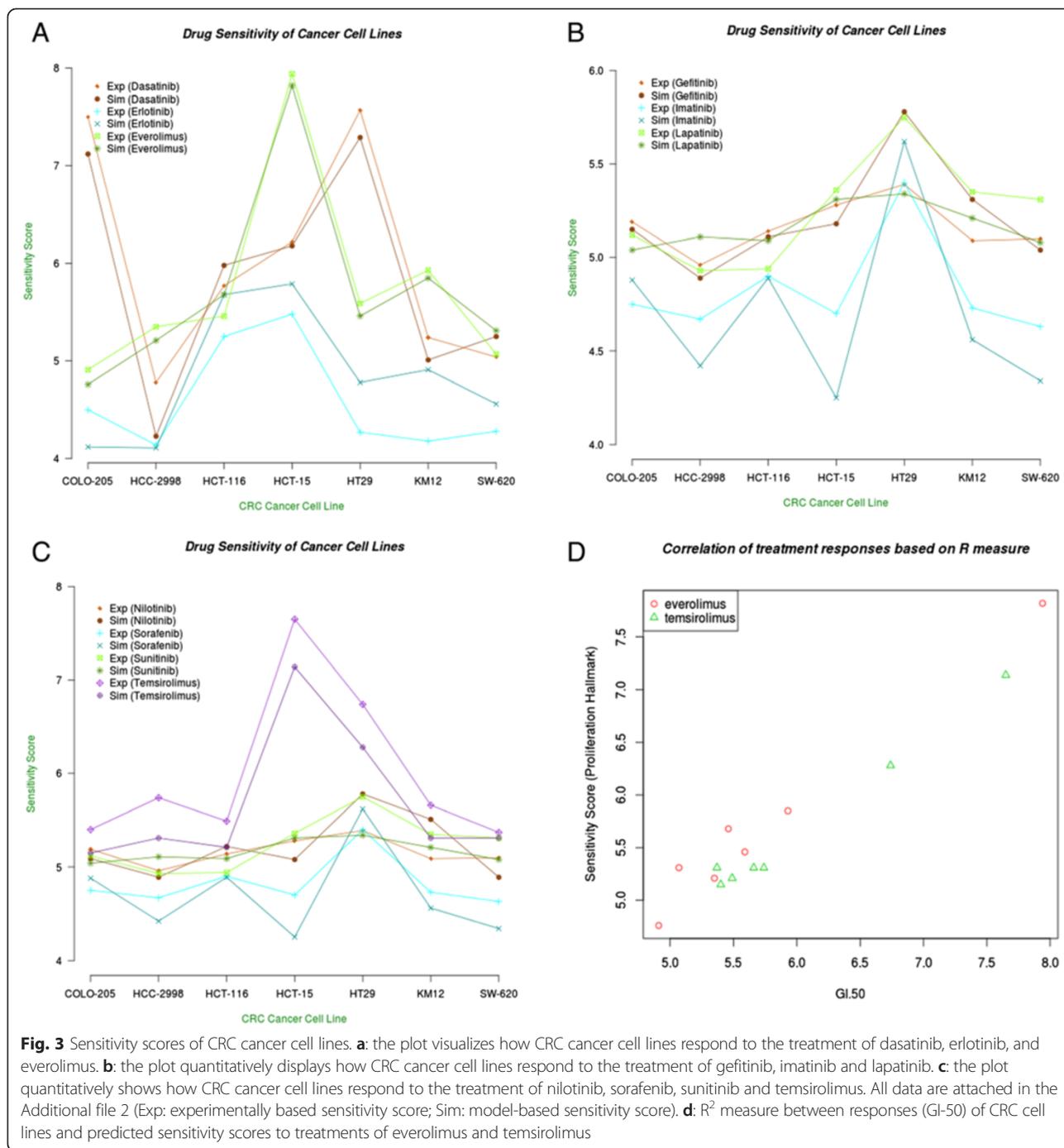
Table 3 Ten signaling agents and their targets with dissociation constant

Drug	Target	Dissociation constant (nM)
Dasatinib	ABL1	0.53
	EPHA3	0.09
	EPHA5/8	0.24
	PDGFRA	0.47
	LYN	0.57
Erlotinib	KIT	0.62
	SRC	0.21
	EGFR	0.67
	ERBB4	230
	LYN	530
Everolimus	SRC	700
	MTOR	2.2
	EGFR	1.0
Gefitinib	ERBB2	3500
	ERBB4	410
	LYN	990
	ABL1	12.0
Imatinib	ABL2	10.0
	KIT	14.0
	PDGFRA	31.0
	PDGFRB	14.0
Lapatinib	EGFR	2.4
	ERBB2	7.0
	ERBB4	54.0
Nilotinib	KIT	22
	PDGFRB	22
Sorafenib	DDR1	1.5
	DDR2	6.6
Sunitinib	FLT3	0.47
	KIT	0.37
	PDGFRA	0.79
	PDGFRB	0.08
Temsirolimus	MTOR	2.2
	VEGFR	0.75

The experimentally measured dissociation constants of these signaling agents were mainly taken from Karaman et al. [35]

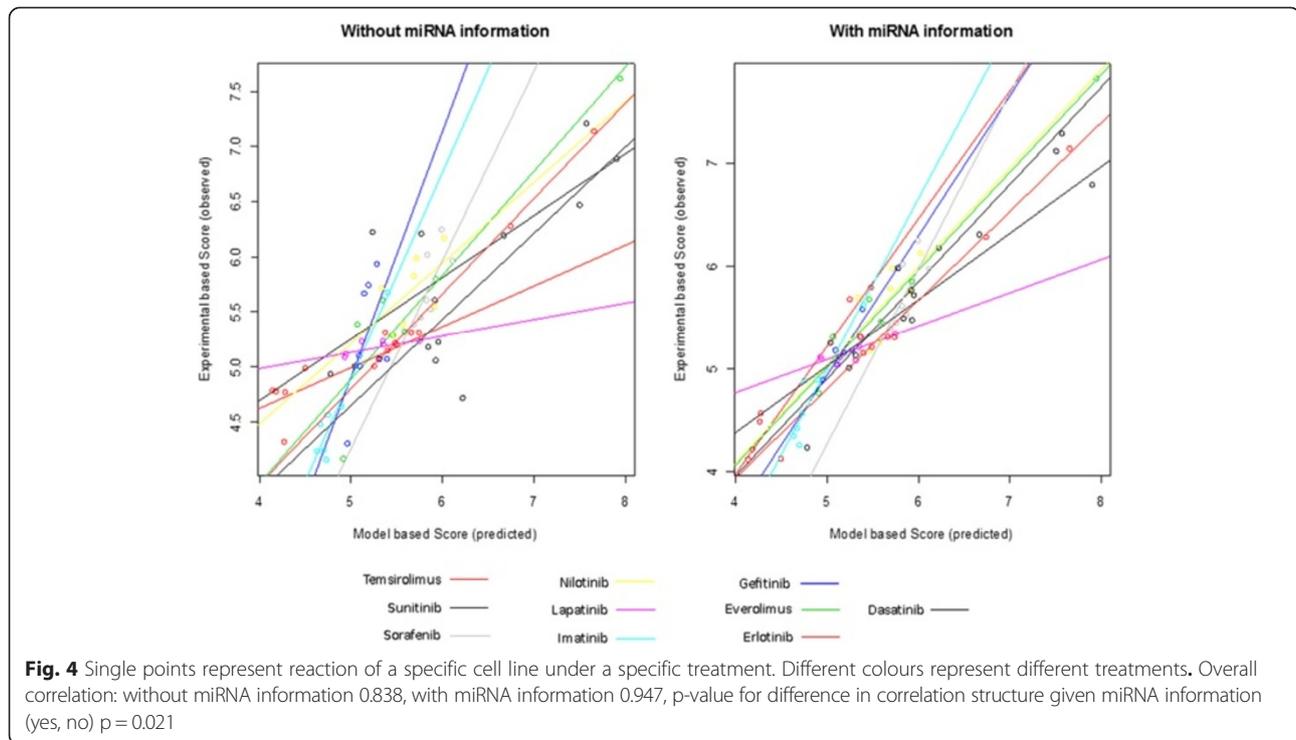
Therapeutic Prediction of CRC Patients with Microsatellite Instability (MSI) and Microsatellite Stability (MSS)

Microsatellites are short repetitive DNA sequences that are prone to frameshift mutations and base-pair substitutions during duplication. MSI is one of the most extensively investigated genetic phenotypes, and is detected in approximately 15 % of CRC cases [36]. Many studies provide evidence that CRC with MSI status is associated with favorable clinical outcomes [37–41].



In contrast, other studies show controversial data related to the predictive value of MSI [42–45]; clearly, there is uncertainty as to the predictive ability of this genetic phenotype in clinical practice. In response to this, we apply the NSAID-miR model to predict the therapeutic responses of MSI/MSS patients. Moreover, we investigate the molecular mechanisms leading to the discrepancy between the clinical outcomes of individual colon cancer patients with MSI status versus

those with MSS status. From the Cancer Genome Atlas [46], we obtained genetic data, including miRNA/gene-expression data and patient information (age, sex, race, cancer stage and other) of 22 colon cancer patients (Additional file 3), of which eleven have MSS status, and eleven MSI; the patients' cancer stages range from I to III. Further, two recent studies demonstrated that both drugs sirolimus and LY294002 (targeting mTor- and PI3K-signaling pathways, respectively) clearly reduced the



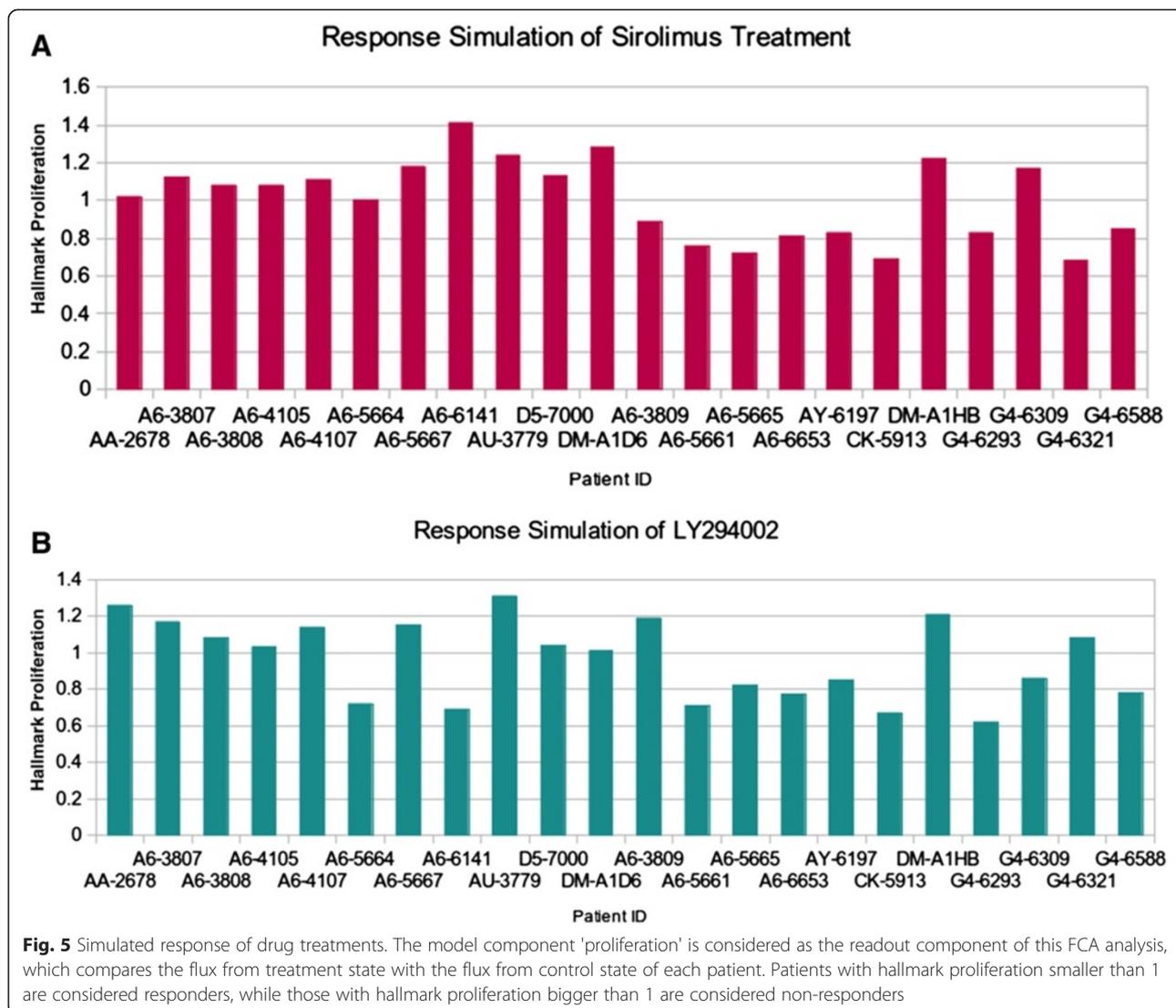
growth of MSI tumors, but not MSS tumors [47, 48]. In order to investigate this issue with the application of the NSAID-miR model, we initialized the model with the gene-expression and miRNA-expression data of these patients individually (Methods). Subsequently, we performed the FCA to predict how these individual patients would respond to drug treatments.

The results show that all MSS patients would not respond to the sirolimus treatment, regardless of colon cancer stage (Fig. 5). Among the eleven MSI patients, we found that two patients with advanced cancer stage III would not respond to the sirolimus treatment, while the remaining nine MSI patients would. In general, the predictions are in agreement with the results from the aforementioned studies [47, 48]. Further, our results support the finding that the prognosis for MSI tumors in stage III CRC is poor [49]. Taking model based miRNA level as readouts of model NSAID-miRNA, FCA results between MSI and MSS patients after treatment show that the expression levels of miR-18a, -19a, -203, -224, and -92 are downregulated on average by >15 % ($p = 3.49e-06$) in patients with stages I and II. The expression levels of miR-181b, -183, -20a, -21, -31 and -96 are downregulated on average by >15 % ($p = 6.09e-04$) in patients with stage III (Fig. 6a; Additional file 4). The expression levels of miR-30a, -143, -145, -200b and -378 are upregulated on average by >1.8 fold ($p = 7.84e-06$) in patients of all stages (Additional file 4).

Two male African Americans in cancer stages IIA and IIIC among eleven MSS patients would respond to the LY294002 treatment. Further investigation of the FCA result shows that the expression levels of mir-21, -140, -188, -216, -224, -374, -503 and -675 were reduced in these two patients after treatment (Fig. 6b; Additional file 4). Eight among eleven MSI patients would respond to the LY294002 treatment. Interestingly, we found that the three MSI patients with CRC stage III, who would not respond to the LY294002 treatment, showed high activity of the cell-cycle pathway, and the expression levels of mir-21, -34a, -95, -135a and -320 remained nearly unchanged after this treatment (Additional file 4). This result might reveal the key miRNA-regulators that negatively contribute to the clinical outcome of MSI patients. Furthermore, by using a ROC curve and the corresponding AUC, we quantified the discrimination between response between MSS and MSI patients through the *in silico* prediction given by our model. The AUC for response prediction under sirolimus is 0.876, The AUC for response prediction under LY294002 is 0.715. (Fig. 7)

Conclusion & Discussion

This study introduces a concept which integrates different types of molecular data for individualized medicine. It uses an *in silico* environment to capture the molecular



regulation effect of miRNAs within individual cancerous cellular systems (Fig. 2). Four major sources of input information are used to calculate the individual response of the system: molecular, kinetic, individual genetic (miRNA/mRNA expression data) and treatment data. The internal network structure of the NSAID-miR model covers molecular signaling pathways (including transcription and translation, protein-protein interaction, and protein modification) and miRNA regulation. The kinetic data describes kinetic values of different types of reactions (such as transcription, phosphorylation, complex formation, receptor-ligand-binding) and allows to implement the treatment effects. The kinetic data impacts the signal flow (defining classical chemical reactions of substrates for producing products with or without modifiers) throughout the network. Contrasting the flux in the untreated cell with the flux of the treated cell allows

quantifying changes in the cancer hallmarks. These changes can be used to predict treatment response of the system.

During this study, data of CRC cell lines as well as patients were used for the validation. For ten agents, we simulated the responses of seven CRC cell lines and compared them to their *in vitro* drug response data. There is high correlation, which indicates the reliability and precision of the predictions of the proposed model. In order to give a first demonstration of the potential clinical usefulness of this concept, we received the miRNA and gene expression data of 22 MSI/MSS patients provided by the Cancer Genome Atlas [46] for predicting the clinical outcome of the sirolimus and LY294002 treatments. The prediction results show that most MSI patients would respond to both drug treatments, however most MSS patients would not. At the

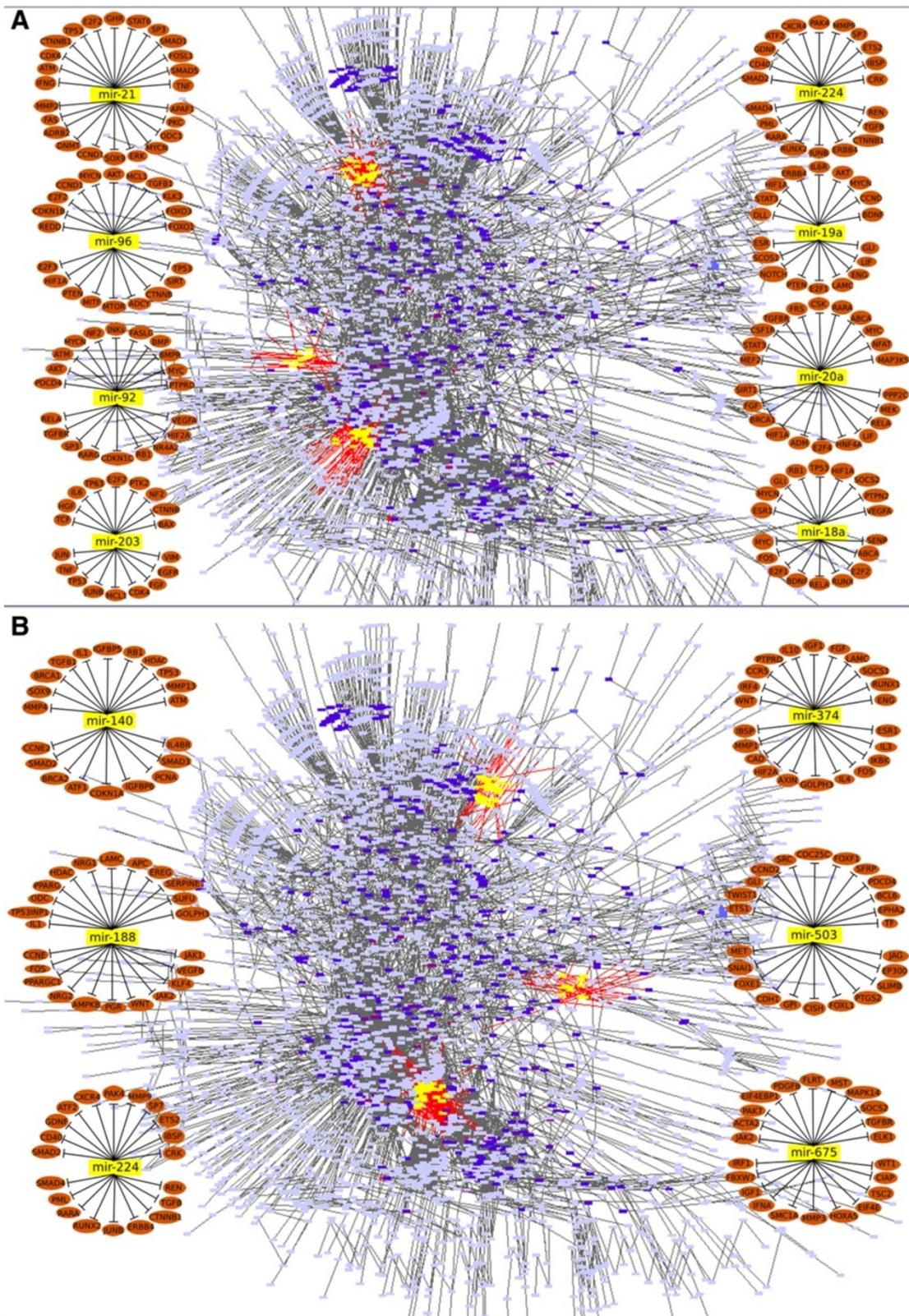


Fig. 6 Simulated miRNA expression pattern after treatments. The simulated result reveals that global miRNA expression profile can be changed due to the drug treatment. According to the expression patterns, both treatments have more significant impact on the MSI patients than MSS patients. However, specific patients may have individual treatment responses

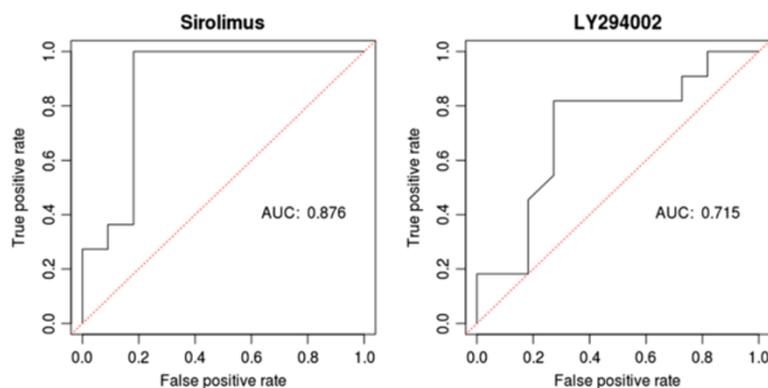


Fig. 7 ROC analysis of discrimination of treatment responses between MSS and MSI patients

moment, data on clinical response for these patients is still not available. But our result is in accordance with clinical knowledge that MSI status is related to the response of the treatment under study [47,48]. Based on our results, we strongly hypothesize that one molecular reason for better therapeutic outcomes of MSI patients could be the upregulation of tumor-suppressor miRs and downregulation of oncogenic miRs, which drives the cellular system of patients with MSI status away from the full-fledged malignant cellular state with strong drug resistance and uncontrolled proliferation.

In a recent study, Ellwanger and colleagues [50] decipher the role of miRNA on a large scale, which provides knowledge for the implementation of miRNA regulation in molecular *in silico* models. We see that many of their findings are already implemented in our NSAID-miR model, for instance, the regulation mechanisms of mir-21, mir-181 and let-7. However, the NSAID-miR model might be the first molecular signaling model which contains not only validated miRNA-target relationship information but also includes literature-referenced relationships between transcription factors and miRNAs. In addition, the NSAID-miR model can be applied to investigate therapeutic response of patients with cancers beyond CRC. For instance, we are studying a genome-scale model of acute myeloid leukemia (AML) to predict individual response to AML clinical treatments; the results achieved thus far are promising (data not shown), which indicates that our approach also possesses the potential to be extended to diverse other cancer types. However, one limitation of our model is the applied kinetic data, which is mainly determined through empirical experience. How to perform appropriate estimates in patient groups (depending on age, sex, etc.) is the issue of our future research. Furthermore, our concept does not consider metabolic molecular information. This is another challenge of future research.

Additional file

Additional file 1: Validated miRNA target with literature reference.

Additional file 2: Experimentally based sensitivity score and model-based sensitivity score for treatments of ten signaling agents with and without consideration of miRNA expression data.

Additional file 3: Patient Information.

Additional file 4: Comparison result between treatment state and control state from the FCA analysis.

Competing Interest

The authors declare no conflict of interest with regard to the content of this manuscript.

Authors' contribution

JL made the conception and design of the study; JL collected the data and performed the simulation and analysis; JL, UM did evaluation and interpretation of the read-out data; JL drafted the manuscript; UM revised the manuscript critically; UM gave final approval for publication. All authors read and approved the final manuscript.

Acknowledgement

This study is funded by the German Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ). We thank the Cancer Genome Atlas for the collaboration and professional technical support. We thank Rory Wilson for help with the manuscript revision.

Author details

¹Institute for Medical Informatics, Biometry and Epidemiology, Ludwig-Maximilians-University München, Munich, Germany. ²German Cancer Consortium (DKTK), Heidelberg, Germany. ³German Cancer Research Center (DKFZ), Heidelberg, Germany.

Received: 11 July 2014 Accepted: 14 May 2015

Published online: 18 June 2015

References

- Edwards BK, Ward E, Kohler BA, Ehemann C, Zauber AG, Anderson RN, et al. Annual report to the nation on the status of cancer, 1975–2006, featuring colorectal cancer trends and impact of interventions (risk factors, screening, and treatment) to reduce future rates. *Cancer*. 2010;116(3):544–73.
- Armaghany T, Wilson JD, Chu Q, Mills G. Genetic alterations in colorectal cancer. *Gastrointest Cancer Res*. 2012;5(1):19–27.
- de Sousa EM, Wermeulen L, Richel D, Medema JP. Targeting Wnt signaling in colon cancer stem cells. *Clin Cancer Res*. 2011;17(4):653–7.
- Qian L, Wong B. Role of Notch signaling in colorectal cancer. *Carcinogenesis*. 2009;30(12):1979–86.

5. Gulino A, Ferretti E, De Smaele E. Hedgehog signaling in colon cancer and stem cells. *EMBO Mol Med*. 2009;1(6–7):300–2.
6. Fang JY, Richardson BC. The MAPK signaling pathways and colorectal cancer. *Lancet Oncol*. 2005;6(5):322–7.
7. Wang S, Liu Z, Wang L, Zhang X. NF-kappaB signaling pathway, inflammation and colorectal cancer. *Cell Mol Immunol*. 2009;6(5):327–34.
8. Schetter AJ, Okayama H, Harris CC. The Role of microRNAs in Colorectal Cancer. *Cancer J*. 2012;18(3):244–52.
9. Akao Y, Nakagawa Y, Naoe T. MicroRNA-143 and-145 in colon cancer. *DNA Cell Biol*. 2007;26(5):311–20.
10. Asangani IA, Rasheed SA, Nikolova DA, Leupold JH, Colburn NH, Post S, et al. MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor Pcdcd4 and stimulates invasion, intravasation and metastasis in colorectal cancer. *Oncogene*. 2008;27(15):2128–36.
11. Chen X, Guo X, Zhang H, Xiang Y, Chen J, Yin Y, et al. Role of miR-143 targeting KRAS in colorectal tumorigenesis. *Oncogene*. 2009;28(10):1385–92.
12. Gregersen LH, Jacobsen AB, Frankel LB, Wen J, Krogh A, Lund A. MicroRNA-145 targets YES and STAT1 in colon cancer cells. *PLoS One*. 2010;5(1), e8836.
13. Ng EK, Chong WW, Jin H, Lam EK, Shin VY, Yu J, et al. Differential expression of microRNAs in plasma of patients with colorectal cancer: a potential marker for colorectal cancer screening. *Gut*. 2009;58(10):1375–81.
14. Zhang J, Guo H, Zhang H, Wang H, Qian G, Fan X, et al. Putative tumor suppressor miR-145 inhibits colon cancer cell growth by targeting oncogene Friend leukemia virus integration 1 gene. *Cancer*. 2011;117(1):86–95.
15. Huang Z, Huang D, Ni S, Peng Z, Sheng W, Du X. Plasma microRNAs are promising novel biomarkers for early detection of colorectal cancer. *Int J Cancer*. 2010;127(1):118–26.
16. Cheng H, Zhang L, Cogdell D, Zheng H, Schetter A, Nykter M, et al. Circulating plasma miR-141 is a novel biomarker for metastatic colon cancer and predicts poor prognosis. *PLoS One*. 2011;6(3), e17745.
17. Link A, Balaguer F, Shen Y, Nagasaka T, Lozano JJ, Boland CR, et al. Fecal MicroRNAs as novel biomarkers for colon cancer screening. *Cancer Epidemiol Biomarkers Prev*. 2010;19(7):1766–74.
18. Hotchi M, Shimada M, Kurita N, Iwata T, Sato H, Morimoto S, et al. microRNA expression is able to predict response to chemoradiotherapy in rectal cancer. *Mol. Clin Oncol*. 2013;1(1):137–42.
19. Kulda V, Pesta M, Topolcan O, Liska V, Treska V, Sutnar A, et al. Relevance of miR-21 and miR-143 expression in tissue samples of colorectal carcinoma and its liver metastases. *Cancer Genet Cytogenet*. 2010;200(2):154–60.
20. Nielsen BS, Jorgensen S, Fog JU, Sokilde R, Christensen IJ, Hansen U, et al. High levels of microRNA-21 in the stroma of colorectal cancers predict short disease-free survival in stage II colon cancer patients. *Clin Exp Metastasis*. 2011;28(1):27–38.
21. Shibuya H, Linuma H, Shimada R, Horiuchi A, Watanabe T. Clinicopathological and prognostic value of microRNA-21 and microRNA-155 in colorectal cancer. *Oncology*. 2010;79(3–4):313–20.
22. Melo SA, Kalluri R. Molecular Pathways: MicroRNAs as Cancer Therapeutics. *Clin Cancer Res*. 2012;18:4234.
23. Robinson MD, Oshlack A. A scaling normalization method for differential expression analysis of RNA-seq data. *Genome Biol*. 2010;11:R25.
24. NCI-60 RNA Expression Data Repository. <http://discover.nci.nih.gov/datasets.jsp>
25. NCI-60 CellMiner. <http://discover.nci.nih.gov/cellminer>
26. Li J, Mansmann U. Modeling of Non-Steroidal Anti-Inflammatory Drug Effect within Signaling Pathways and miRNA-Regulation Pathways. *PLoS One*. 2013;8(8), e72477.
27. Hanahan D, Weinberg R. Hallmarks of Cancer: The Next Generation. *Cell*. 2011;144:646–74.
28. Li J, Pandey V, Kessler T, Lehrach H, Wierling C. Modeling of miRNA and drug action in the EGFR signaling pathway. *PLoS One*. 2012;7(1), e30140.
29. Nashan B. Review of the proliferation inhibitor everolimus. *Expert Opin Investig Drugs*. 2002;11(12):1845–57.
30. Walker EH, Pacold ME, Perisic O, Stephens L, Tawkins PT, Wymann MP, et al. Structural determinants of phosphoinositide 3-kinase inhibition by Wortmannin, LY294002, Quercetin, Myricetin, and Staurosporine. *Mol Cell*. 2000;6:909–19.
31. Holbeck S, Collins JM, Doroshov JH. Analysis of food and drug administration-approved anti-cancer agents in the NCI60 panel of human tumor cell lines. *Mol Cancer Ther*. 2010;9:1451–60.
32. Bland JM, Altman DG. Calculating correlation coefficients with repeated observations: Part 1 – correlation within subjects. *BMJ*. 1995;310:446.
33. NSAIID-miR model. ftp://138.245.80.137/NSAIID_miR.xml
34. Wang J, Lu M, Qiu C, Cui Q. TransmiR: a transcription factor-microRNA regulation database. *Nucleic Acids Res*. 2010;38(Database issue):D119–22.
35. Karaman MW, Herrgard S, Treiber DK, Gallant P, Atteridge CE, Campbell BT, et al. A quantitative analysis of kinase inhibitor selectivity. *Nat Biotechnol*. 2008;26(1):127–32.
36. Tejpar S, Bertagnoli M, Bosman F, Lenz HJ, Garraway L, Waldman F, et al. Prognostic and predictive biomarkers in resected colon cancer: current status and future perspectives for integrating genomics into biomarker discovery. *Oncologist*. 2010;15(4):390–404.
37. Halling KC, French AJ, McDonnell SK, Burgart LJ, Schaid DJ, Peterson BJ, et al. Microsatellite instability and 8p allelic imbalance in stage B2 and C colorectal cancers. *J Natl Cancer Inst*. 1999;91(15):1295–303.
38. Gryfe R, Kim H, Hsieh ET, Aronson MD, Holowaty EJ, Bull SB, et al. Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. *N Engl J Med*. 2000;342(2):69–77.
39. Ribic CM, Sargent DJ, Moore MJ, Thibodeau SN, French AJ, Goldberg RM, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *N Engl J Med*. 2003;349(3):247–57.
40. Lanza G, Gafa R, Santini A, Maestri I, Guerzoni L, Cavazzini L. Immunohistochemical test for MLH1 and MSH2 expression predicts clinical outcome in stage II and III colorectal cancer patients. *J Clin Oncol*. 2006;24(15):2359–67.
41. Roth AD, Tejpar S, Delorenzi M, Yan P, Fiocca R, Klingbiel D, et al. Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: results of the translational study on the PETACC-3, EORTC 40993, SAKK 60–00 trial. *J Clin Oncol*. 2010;28(3):466–74.
42. Jernvall P, Maekinen MJ, Karttunen TJ, Maekelae J, Vihko P. Microsatellite instability: impact on cancer progression in proximal and distal colorectal cancers. *Eur J Cancer*. 1999;35(2):197–201.
43. Feeley KM, Fullard JF, Heneghan MA, Smith T, Maher M, Murphy RP, et al. Microsatellite instability in sporadic colorectal carcinomas is not an indicator of prognosis. *J Pathol*. 1999;188(1):14–7.
44. Salahshor S, Kressner U, Fischer H, Lindmark G, Glimelius B, Pahlman L, et al. Microsatellite instability in sporadic colorectal cancer is not an independent prognostic factor. *Br J Cancer*. 1999;81(2):190–3.
45. Gafa R, Maestri I, Matteuzzi M, Santini A, Ferretti S, Cavazzini L, et al. Sporadic colorectal adenocarcinomas with high-frequency microsatellite instability. *Cancer*. 2000;89(10):2025–37.
46. Cancer Genome Atlas. <http://cancergenome.nih.gov>
47. Zhang J, Roberts TM, Shivdasani RA. Targeting PI3K signaling as a therapeutic approach for colorectal cancer. *Gastroenterology*. 2011;141(1):50–61.
48. Thompson C, Leong S, Messersmith M. Promising targets and drugs in development for colorectal cancer. *Semin Oncol*. 2011;38(4):588–97.
49. Tejpar S, Delorenzi R, Fiocca R, Yan P, Klingbiel D, Dietrich D, et al. Microsatellite instability (MSI) in stage II and III colon cancer treated with 5FU-LV or 5FU-LV and irinotecan (PETACC 3-EORTC 40993-SAKK 60/00 trial). *J Clin Oncol*. 2009;27:155.
50. Ellwanger DC, Leonhardt JF, Mewes HW. Large-scale modeling of condition-specific gene regulatory networks by information integration and inference. *Nucleic Acids Res*. 2014;42:21.

Submit your next manuscript to BioMed Central and take full advantage of:

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at
www.biomedcentral.com/submit

