Research paper

Computational drug treatment simulations on projections of dysregulated protein networks derived from the myelodysplastic mutanome match clinical response in patients

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ABSTRACT

Although the majority of MDS patients fail to achieve clinical improvement to approved therapies, some patients benefit from treatment. Predicting patient response prior to therapy would improve treatment effectiveness, avoid treatment-related adverse events and reduce healthcare costs. Three separate cohorts of MDS patients were used to simulate drug response to lenalidomide alone, hypomethylating agent (HMA) alone, or HMA plus lenalidomide. Utilizing a computational biology program, genomic abnormalities in each patient were used to create an intracellular pathway map that was then used to screen for drug response. In the lenalidomide treated cohort, computer modeling correctly matched clinical responses in 37/46 patients (80%). In the second cohort, 15 HMA patients were modeled and correctly matched to responses in 12 (80%). In the third cohort, computer modeling correctly matched responses in 10/10 patients (100%). This computational biology network approach identified GCH overexpression as a potential resistance factor to HMA treatment and paradoxical activation of beta-catenin (through Csk1a1 inhibition) as a resistance factor to lenalidomide treatment. We demonstrate that a computational technology is able to map the complexity of the MDS mutanome to simulate and predict drug response. This tool can improve understanding of MDS biology and mechanisms of drug sensitivity and resistance.

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1. Introduction

The myelodysplastic syndromes (MDS) comprise a group of hematological malignancies characterized by ineffective hematopoiesis causing severe cytopenias, multiple genomic and epigenomic abnormalities, and progression to acute myeloid leukemia (AML). Molecular heterogeneity exists among MDS patients and is believed to cause variability in the syndromic phenotype and treatment response [1]. Only three drugs are approved by the U.S. Food and Drug Administration for MDS patients: azacitidine (AZA), decitabine (DEC), and lenalidomide (LEN). Despite these treatment options, failure to achieve hematopoiesis...
logical improvement (HI) is found in 60% of MDS patients treated with azacitidine or decitabine and 33% of deletion 5q (del[5q]) MDS patients [2,3]. No other standard therapies currently following failure of first line treatment, and as a result, nearly all MDS patients die of refractory disease [4,5]. Thus, there is a large unmet clinical need for (1) accurately predicting response to first line treatment and (2) identifying alternative therapies for non-responders.

Several investigators have identified single gene mutations associated with treatment response. For example, MDS patients with mutations in TET2 or DNMT3A mutation were more likely to achieve clinical improvement after HMA treatment [6–8]. In del(5q) MDS patients, the presence of a TP53 mutation was associated with relative resistance to lenalidomide treatment [9]. Whereas, these studies represent important incremental advances, the observations rely upon one-gene/one-drug analysis and censor tens to hundreds of other genomic abnormalities that co-exist within the MDS mutanome.

Therefore, we hypothesized that use of a computational biology technique that incorporates the totality of known genomic abnormalities and their predicted protein network disruptions would provide strong correlations with clinical outcome in MDS.

2. Materials and methods

2.1. Patients

The three MDS patient cohorts examined in this retrospective study were prospectively recruited to interventional treatment trials or institutional patient registries, where all patients consented to have tissue samples banked [6,9,10]. Their de-identified data were accessed via publication downloads or shared by investigators. This retrospective study was approved by University of Florida’s Institutional Review Board protocol IRB201602096.

2.2. Computational biology modeling

The computational biology computer modeling system utilized in this study was previously outlined and published in studies of glioblastoma multiforme and multiple myeloma [11–13]. Based on over 10 000 published PubMed references, this model considers signaling pathway interactions important in cancer including growth factor signaling cascades, cytokines, chemokines, mTOR regulators, cell cycle regulators, oxidative and ER stress responses, cancer metabolism, autophagy and pro-teosomal degradation, DNA damage repair, apoptosis cascades and p53 signaling to predict a patient’s response to a single drug or a combination of drugs. This modeling system includes more than 4 700 intracellular pathway elements that are capable of simulating 60 000 functional interactions, including comprehensive coverage of the kinome, transcriptome, proteome, and metabolome.

In this study, each MDS patient’s available genomic information (i.e., cytogenetic abnormalities and DNA sequencing data) was entered into the computational biology system, which utilized PubMed, STRING, HumanNet, and PathwayCommons online resources to determine whether the patient’s gene mutation generated an activated or inactivated protein.

To interpret the genomic signature of the patient, we used cytogenetic profiling by spectral karyotyping (SKY) to report chromosomal aberrations, including gain/loss of complete chromosomes or specific chromosomal regions resulting in monosomy/trisomy of the genes in the affected regions. In addition to deletions and duplications, other abnormalities such as derivative chromosomes, isochromosomes, and translocations may be incorporated into the system. Additionally, targeted gene panel sequencing or whole exome sequencing data can report copy number variations (CNV) and point mutation information that make up the genomic signature of each patient’s disease. The genomic aberration information derived from cytogenetics and sequencing data is used to create a list of genes with mutations and CNV in the patient’s genome. The genes found on the loci of the affected regions of the chromosomes are extracted from the human reference genome at ENSEMBL, and the complete list of genes is matched with the Cancer Technology Network to determine the subset of genes to be represented in the model.

Key assumptions are made when indicating the aberrations in each patient’s disease network: gain of function or amplification of tumor promoter genes, and loss of tumor suppressor genes drives cancer [14]. Gene variants with therapeutic implications are searched using public domain to determine each mutation’s functionality, represented as either a loss or gain of function. However, genes with mutations of unknown significance are parsed through a suite of variant calling algorithms to determine if the mutation is deleterious. For a deleterious mutation of unknown significance, a tumor promoter gene is assumed to have gain of function while a tumor suppressor gene is assumed to have loss of function at the protein activity level. Frameshift and missense mutations are assumed to cause a loss of gene function.

For CNV interpretation, amplifications are represented as an increase of gene expression while deletions are represented as knockdown of gene expression. Additionally, amplifications of tumor suppressor genes have lower contribution to the disease when compared to amplification of tumor promoter genes. A deletion of tumor suppressor genes has a higher dominance in the disease network when compared to deletion of tumor promoter genes.

Protein network maps were created for each patient based on their MDS mutanome data. In most cases, when multiple genomic abnormalities co-exist, a complex map of intersecting protein networks was created that represented the MDS patient’s disease physiology. Using the patients’ maps, cell proliferation was simulated for each patient’s disease (Fig. 1). The proliferation index is an average function of the active CDK-cyclin complexes that define cell cycle checkpoints, and is determined by calculating permutations in the biomarkers CDK4-CCND1, CDK2-CCNE, CDK2-CCNA, and CDK1-CCNB1. The drug(s) of interest (e.g., AZA, DEC, LEN, AZA+LEN) were then introduced at various concentrations (i.e., C, 0.5C, and 4C) using relevant in vitro data reported in PubMed. If the drug’s target and downstream mediators were present, then decreases in cell proliferation were observed (Fig. 1).

A viability index based on survival and apoptosis is also generated for each patient. The biomarkers constituting the survival index include AKT1, BCL2, MCL1, BIRC5, BIRC2, and XIAP, while the apoptosis index includes BAX, CASP3, NOXA, and CASP8. The overall viability index of a cell is calculated as a ratio of survival index/apoptosis index, and the weightage of each biomarker is adjusted to achieve a maximum correlation with the experimental trends for the endpoint. The virtual patient disease network is created by overlaying the patient’s genomic signature onto the control network, as per the rules and assumptions stated earlier, and running it thought the simulation technology to achieve a dynamic disease state.

If MDS cell growth characteristics (proliferation, viability, apoptosis) normalized in a dose dependent manner, then the patient’s disease was scored as responsive (Fig. 1). If the drug in the MDS model did not decrease cell proliferation or viability, then the disease was scored as non-responsive.
2.3. Statistical analyses

To determine the extent by which computer modeled predictions of drug sensitivity significantly linked with actual clinical response, we utilized association analyses of each independent variable of interest with each of the patient clinical outcomes. This analysis was carried out using Fisher’s Exact Test. P values < 0.05 were statistically significant.

3. Results

3.1. Del(5q) MDS cohort

A total of 52 patients confirmed to have del(5q) MDS were treated with LEN (Table 1)[9]. Conventional G-banding cytogenetics, single nucleotide polymorphism arrays (SNP-A) and gene sequencing of 6 recurrently mutated myeloid genes (TET2, ASXL1, IDH1, IDH2, TP53, CBL) were used to molecularly characterize each patient. Clinical response to treatment was assessed using International Working Group (IWG) response criteria revised in 2006[15]. All patients received a minimum of two cycles of LEN and responses were evaluated after four cycles of treatment. Of the 52 del(5q) MDS patients treated with LEN, 46 with both clinical outcomes and available genomic data were included (Fig. 2A).

Fig. 2B shows a simplified schematic of dysregulated protein networks in one of the correctly predicted responders to LEN treatment. Lenalidomide is known to interact with the E3 ubiquitin ligase cereblon (CRBN), which forms a complex with Cullin-4A (CUL4A), damaged DNA binding protein 1 (DDB1), and regulator of cullins 1 (ROC1) [16]. Lenalidomide has been shown to target this enzyme and alter its activity [17]. The computational technique projected that a patient’s chromosome del(5q) abnormality caused a knockdown of IRF1, NR3C1 and ARHGAP26. The modeling software further predicted this chromosomal abnormality to cause a dominant Nfkb pathway that is effectively targeted through lenalidomide redirection of CRBN. Of note, it was discovered in the computational biology model that lenalidomide paradoxically upregulated beta-catenin, a known resistance factor for lenalidomide therapy, through inhibition of CSNK1A1. This observation was inferred by the computational model, which was informed by Pubmed literature that CSNK1 could be regulated by CRBN. The model then made the connection between the drug and the target, identifying the feedback to CSNK1.

Conversely, Fig. 2C is a 2-D schematic of a correctly predicted non-responder, which displays a more complex network of protein perturbations. In addition to the known gene knockdowns resulting from del(5q), this patient also showed knockdowns of down-regulators of beta-catenin signaling (APC, CTNNAA1, CDH1), which predicted dominance in this resistance pathway. Additionally, the CHMP1A knockdown was predicted to decreases TP53 activity, thereby weakening the intended targeted pathway of lenalidomide and suggesting drug resistance.

In the clinical trial, 37/46 patients achieved clinical response to LEN as defined by CR or HI according to IWG 2006 criteria and 33/37 were correctly matched using the computational biology modeling system, yielding a test sensitivity of 89% (p = 0.03586). Nine out of 46 patients did not respond to LEN treatment and 4/9 were correctly matched using the computational biology modeling system, yielding a test specificity of 44%. Therefore, the positive predictive value (PPV) of the computational biology system for the cohort of del(5q) MDS patients was 87%, the negative predictive value (NPV) was 50%, and the overall test accuracy was 80%.

3.2. HMA Cohort

A total of 213 MDS patients were treated with an HMA (AZA or DEC) (Table 1)[6]. A subset of 15 patients with abnormal karyotype was randomly selected for this study. Conventional cytogenetics and next-generation sequencing of 40 recurrently mutated myeloid genes were used. Clinical response to treatment was assessed using IWG criteria revised in 2006[15].

Of the 15 patients, 7 achieved response as defined by CR, PR or HI to HMA treatment and 7/7 were correctly matched using the computational biology modeling system, yielding a test sensitivity of 100%. Of the 8 patients who did not respond to HMA treatment, 5 were correctly matched, yielding a test specificity of 63% (p = 0.02564). Therefore, the PPV for this cohort of higher-risk MDS patients treated with HMAs was 70%, the NPV was 100%, and the overall test accuracy was 80%.

Fig. 3B shows a simplified 2D schematic of a responder patient’s dysregulated protein networks. As a hypomethylating agent, azacitidine efficacy was predicted when a dominant methylation pathway was present, as is seen through DNMT mediated CpG methylation. The representative responder patient in Fig. 3B had a loss of function of ASXL1, which was predicted to decrease methylation and has previously been associated with lack of response to therapy [18]. However, the patient’s downstream overexpression of EZH2 was predicted by the computational model to nullify the loss of ASXL1 and allow methylation to persist as a target for azacitidine.

In contrast, the non-responder MDS patient shown in Fig. 3C demonstrated a loss of ASXL1 and TET2, which was predicted to decrease PRC2-mediated function and DNMT recruitment for CpG methylation. This is in agreement with the finding in Bejar et al. that TET2 mutations do not serve as a marker for drug sensitivity if accompanied by an ASXL1 mutation [19]. Additionally, this non-responder patient was predicted to have overexpression of GGH, which inhibits S-adenosyl methionine synthesis required for methylation, downregulating the methylation pathway, and tilting the balance towards non-response. This GGH discovery in this
Table 1
Patient Cohorts and Statistics.

<table>
<thead>
<tr>
<th>Patient Cohort</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>Patients Modeled</td>
<td>46</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Patients Excluded</td>
<td>6</td>
<td>98</td>
<td>26</td>
</tr>
<tr>
<td>MDS Risk Category</td>
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<tr>
<td>Low: 48%</td>
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<td>Int-1: 50%</td>
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<tr>
<td>Int-2: 2%</td>
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<tr>
<td>High: 0%</td>
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<tr>
<td>Cyto genetic Abnormalities</td>
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<td></td>
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<tr>
<td>del(5q)= other abnormalities</td>
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<tr>
<td>Treatment</td>
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<tr>
<td>Lenalidomide 10 mg PO QD D1-21 every 28-day cycle</td>
<td>Azacitidine 75 mg/m2 PO QD daily 5–10 days for 21 days of a 28-day cycle or Decitabine 20 mg/m2 IV on days 1–5 of each 28 day cycle</td>
<td>Lenalidomide 5–10 mg PO QD 14–21 Days + Azacitidine 75 mg/m2 PO QD daily 5–10 days for 21 days of a 28-day cycle.</td>
<td></td>
</tr>
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- Patients who Achieved Response (N, %): 37 (80%) | 8 (53%) | 7 (46%) |
- Patients Who Did Not Achieve Response (N, %): 9 (19%) | 8 (20%) | 2 (100%) |
- Correctly Predicted Responders (N): 33 (89%) | 7 (100%) | 8 (100%) |
- Correctly Predicted Non-Responders (N): 4 (44%) | 5 (63%) | 2 (100%) |
- Positive Predictive Value (%): 87% | 70% | 100% |
- Negative Predictive Value (%): 50% | 100% | 100% |
- Sensitivity/Specificity (%): 89%/44% | 100%/63% | 100%/100% |
- Prediction Accuracy: 80% | 80% | 100% |
- P value: 0.03586 | 0.02564 | 0.022 |

Patient specific information for cohorts 1, 2 and 3, showing number of patients modeled, risk categories, cytogenetics, treatment and predicted response rates.

Fig. 2. Predictive percentages and two simplified 2-D schematics of a correctly predicted responder and non-responder from lenalidomide treated del(5q) patients. Schematic of patients included in cohort 1, with 46/52 patients being modeled. 37/46 patients responded to lenalidomide treatment with 33/37 correctly predicted, giving our modeling system a test sensitivity of 89%. 9/46 patients did not respond to lenalidomide treatment with 4/9 correctly predicted, giving our modeling system a test specificity of 44%. The PPV is 87%, NPV is 50%, and overall test accuracy is 80%. This analysis is statistically significant (p = 0.03586), calculated using a Fisher’s Exact Test. A. Simplified schematic of dysregulated protein networks in a positively predicted responder to lenalidomide treatment. Red lines show knockdown in patient proteins, dark red lines show knockdown from Lenalidomide treatment, blue lines show positive effects and black lines are normal protein networks. Lenalidomide induces ubiquination and proteosomal degradation of CRBN resulting in the mapped downstream effects. Knockdown of IRF1, NR3C1 and ARH GAP26 are classic characteristics of del(5q) patients and create a dominant NFkB pathway that is effectively targeted through lenalidomide inhibition of CRBN. B. Simplified schematic of dysregulated protein networks in a correctly predicted non-responder to lenalidomide treatment. In addition to the classic knockdown of del(5q) characteristics, there are knockdowns of beta-catenin signaling (APC, CTNNAL1, CDH1), causing dominance in this pathway. Additionally, the CHMP1A KD decreases activity of TP53, weakening the intended targeted pathway of lenalidomide and making this individual refractory to treatment.
A total of 36 high-risk MDS patients were enrolled in a phase I/II study to evaluate the effectiveness of AZA and LEN in patients with higher-risk MDS (Table 1) [10]. Because clinical and genomic data were required for this computational technology, only 10/36 were eligible for study (Fig. 4A). Clinical response to AZA and LEN treatment, as defined by CR+HI using IWG 2006 criteria, was achieved by 8/10 patients, all 8 of who were correctly matched using the computational biology modeling system; yielding a test sensitivity of 100% (p = 0.022) [15]. The 2 patients who did not respond to AZA and LEN treatment were also correctly matched using the computational biology modeling system, yielding a test specificity of 100%. Therefore, the computational biology PPV and the NPV for this higher-risk MDS patient cohort treated with AZA and LEN was 100%.

Fig. 4B shows a simplified schematic of a positively predicted responder to AZA and LEN treatment with mutations in TET2, IDH1/2, SRSF2, and other cytogenetic abnormalities. Azacitidine efficacy is facilitated by DNMT mediated CpG methylation and lenalidomide is dependent on inhibition of NFκB dominance, translation and activation of TP53. The responder patient schematic shows overexpression of DNMT1 and CDH1 with a knockdown of TET2, indicating sensitivity to azacitidine treatment. Additionally, overexpression of CARD11, STK11, TP53, STUB1 and IRF7 indicated sensitivity to lenalidomide treatment. Notably, increased activity of TP53, BAX and BBC3 favor apoptotic pathways targeting MDS cells.

In contrast, Fig. 4C shows a simplified schematic of a correctly matched non-responder with no gene mutations, but who harbors abnormalities of chromosome 5, 7, and other aberrations. This patient showed significantly greater knockdown of key proteins needed for AZA and LEN treatment efficacy. Knockdown of DNMT3B, EZF1, MAT2B, and CDH1 indicated resistance to azacitidine through downregulation of the target methylation pathway. Knockdown of STUB1, IKZF1/3, APC, AXIN1, CAV1, TSC2, PRKCB, HIPK2, CARD11, CDH1, and CTNNNA1 indicated resistance to lenalidomide through weakened targets of therapy and strengthening of the beta-catenin resistance loop. The combination of these
protein–protein network aberrations was predicted to result in resistance to both AZA and LEN treatment.

4. Discussion

In this study we show the feasibility of using computer-modeling capabilities to map the MDS mutanome and mathematical simulations to predict drug response. We showed that the incorporation of chromosomal abnormalities, copy number variations, and gene mutations from each patient’s MDS culminates in a unique nexus of dysregulated protein networks, which can be used for simulating drug treatments. Using data from three published clinical trials, our computational biology method accurately matched clinical response in 80–100% of patients, which was higher than would be expected from empiric prescription of chemotherapies. The test specificities of each cohort ranged from 44%-100%. This range in specificity may be due to small sample sizes, the limited genomics datasets available, and the small mutation panel utilized in the LEN and HMA cohorts.

This technology represents an improvement upon conventional attempts to identify patient responders and non-responders. Investigators have used large datasets and multivariable regression analyses to identify single gene abnormalities, such as mutations in TET2 or DNMT3A, that are associated with response to HMAs or lenalidomide [19–21]. Whereas these one-gene/one-drug retrospective studies are vitally important in building the base framework in disease modeling, we show that a method incorporating all known genomic abnormalities and their interactions within individual MDS patients is capable of faithfully simulating drug response. More specifically, we show that patients whose MDS harbors a single gene mutation predictive of response may also harbor uncooperative gene mutations that portend drug resistance. Thus, our results support a multidimensional simulation method when interpreting the malignant mutanome. This data is consistent with previous reports of using predictive simulation technology to create personalized therapeutics [13].

Our technology also identified novel biomarkers worthy of further exploration, and expands upon a previous study using reverse phase protein array and computational modeling to investigate the complex role of p38 MAPK mechanisms involved in MDS pathogenesis [22]. For instance, GGH overexpression was identified in one MDS patient as one of the resistance factors responsible for HMA drug resistance (Fig. 3C). GGH catalyzes the hydroly-
sis of anti-folylpoly-gamma-glutamates, removing gamma-linked polyglutamates, and functions to inhibit S-adenosyl methionine synthesis required for methyl group transfers [23]. The loss of GGH function was predicted to inactivate azacitidine’s targeted methylation pathways. Activated GGH represents a novel candidate biomarker of resistance to HMA that is worthy of further examination, and adds rigor to the modeling software’s ability to identify disease-relevant biomarkers [24,25]. Furthermore, this finding warns against combining anti-folates with HMAs in MDS patients with wild type GGH. Another biomarker suggestion was lenalidomide’s paradoxical activation of beta-catenin, a noted resistance factor, through lenalidomide’s inhibition of CSNK1A1. The CSNK1A1 kinase is part of the beta-catenin destruction complex that phosphorylates and induces proteasomal degradation of beta-catenin. Therefore inhibition of CSNK1A1 results in increased levels of beta-catenin, strengthening the resistance loop against lenalidomide [26]. This finding demonstrates the pivotal regulatory role of beta-catenin in lenalidomide response. Further studies of the beta-catenin destruction complex may provide insight into the beta-catenin resistance mechanism and clinical outcomes in patients with an altered complex. Thus, through computational protein network mapping it is possible to trace the paths for potential targets of therapy as well as to better understand the cancer physiology of MDS and its potential treatments.

Conflicts of interest

Taher Abbasi and Shireen Vali are employees of Cellworks Group, Inc. CRC has received consulting fees from Cellworks Group, Inc. Mikkael Sekeres serves on an Advisory Board for Celgene Corp. Rafael Bejar is a consultant to Celgene, Genoptix, and Foundation Medicine. All other authors declare no relevant conflicts of interest.

Author contributions

CRC, LD, RM, CM conceived the concept of the study, designed the experiments, analyzed the results, and wrote the manuscript. SV, AK, NKS and TA performed the experiments and edited the manuscript. KEH, MAS, MM, FS and RB edited the manuscript. All authors reviewed and approved the manuscript.

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