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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 GGH overexpression as a potential resistance factor to HMA treatment and paradoxical activation of beta-catenin (through Csnk1a1 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.

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 hemato-

Abbreviations: MDS, myelodysplastic syndromes; AML, acute myeloid leukemia; AZA, azacitidine; DEC, decitabine; LEN, lenalidomide; HI, hematological improvement; SKY, spectral karyotyping; CNV, copy number variation; IWG, International Working Group; HMA, hypomethylating agent; PPV, positive predictive value; NPV, negative predictive value; CR, complete response; PR, partial response.

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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 exist 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 proteosomal 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.