Chemogenomics Mapping of Potential Drugs and Targets for Treatment of Multiple Myeloma

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Abstract

Multiple myeloma (MM) is the second common hematological malignancy affecting about 352,000 worldwide. Some subgroups of MM patients still cannot benefit from the currently available anti-MM drugs and therefore are at high risk of death. The pathological mechanism of MM remains to be unraveled. The identification of a global gene signature for MM might lead toward development of novel diagnostics and therapeutic interventions. Here, we identified common differentially expressed genes (DEGs) shared by 30 MM microarray data sets and compared the common DEGs with those induced by genetic or chemical perturbations. We found some potential therapeutic targets for MM treatment, for example RARA, FGFR1, PML, ROR1, SLAMF7, MTDH and Daxx. as modulating them can reverse the MM-induced gene signature. Based on our analysis results, we also predicted and validated some drug reposition, such as Imatinib, Decitabine, Dexamethasone, Vincristine, Paclitaxel, as well as Bortezomib plus Bafilomycin A1 combination for MM treatment by a literature search, data mining, and *in vitro* bioassays. This study could provide guidance and indications for the development of MM specific diagnostic biomarkers, indication predictors and therapeutic treatment.

Introduction

Multiple myeloma (MM)[1] is a blood cancer characterized by the abnormal proliferation of plasma cells accumulated in bone marrow. In a healthy condition, the plasma cells make antibodies that fight infections. But in multiple myeloma patients, they release too much abnormal protein into the bones and blood, releasing chemicals and causing organ damage and lytic lesions in the bone. MM is the second most prevalent hematological carcinogenesis currently affecting 86,000 patients in the US. It is estimated that currently there are 352,000 MM patients worldwide. In the US, about 30,280 new adult cases increase annually, and about 12,590 subjects are reported to die from MM each year, accounting for 0.9% of all cancer deaths (https://www.cancer.net/cancer-types/multiple-myeloma/statistics)

Unlike other hematological carcinogenesis with a single associated genetic aberration, MM is a heterogeneous disease with its major subtypes being classified, diagnosed and predicted according to several different genetic abnormalities of plasma cells[2]. With help from the development of techniques, large efforts have been made to advance our understanding of the complex multistep pathogenesis of MM, involving identification of abnormal gene expression profiles, bone marrow microenvironment change, cytogenetic rearrangement, cell survival signaling and other pathways. The enhanced insight of the molecular pathology of MM allows the development of increasing effective new-targeted therapies, such as thalidomide, bortezomib, and lenalidomide, which dramatically improve the clinical outcomes of MM by substantially increasing the overall survival of several subtypes of MM patients. Despite these dramatic improvements, unfortunately, this devastating disease is still incurable. Also, currently available MM drugs cannot benefit a large portion of newly diagnosed patients (15%) and these patients are still at high risk for relapse and death[3]. This indicates that some key aspects of the underlying pathobiology mechanism of MM remain elusive[2]. Furthermore, effective therapy is needed to treat this high-risk group.

The unsatisfied therapeutic effect on some subgroups of MM patients reveals the fact that some unknown underlying mechanism and complex genetic pathways are associated with the disease. In past decades, DNA microarray technology allowed us to interrogate a large number of genes and identify the disease-related genes. Analysis of the DNA microarray result often generates thousands of differentially expressed genes (DEGs), making it difficult to identify genes that are responsible for a particular disease condition from an individual experiment.

To address this problem, here we applied a meta-analysis approach on publicly accessible MM patient microarray datasets to generate a global gene profile for MM. As shown in **Figure 1**, the MM datasets were analyzed separately at first to discover the differentially expressed genes (DEGs) between the disease and health groups. Then DEGs obtained from each dataset were overlapped to find the common DEGs shared by different datasets, which is credible relevant to MM. Pathway enrichment analysis was done to identify the most MM associated biological processes and other disease conditions based on common DEGs. In addition, we went a step further by comparing each individual MM gene expression dataset with a gene signature induced by a drug or gene perturbation, and attaining the most correlated medications and genes for each dataset. The common MM correlated gene regulations shared by most datasets will help us find potential biomarkers for diagnosis and indication prediction as well as find future therapeutic targets that might have a capacity for MM treatment. The common MM negatively associated drugs appeared in most datasets might have a potential to be repurposed to treat patients with MM, as they can reverse the gene expression change in MM disease conditions. Finally, we will confirm our prediction about drug reposition by literature study and experiment validation. The findings can help to find genes that have the potential to be novel biomarkers for diagnostic and therapeutic interventions against MM, as well as discover drugs that can be repurposed to treat MM patients. This study offers a drug reposition approach based on meta-analysis of gene signature data.

Results

Enrichment Analysis for Common DEGs shared by MM datasets

We then applied 1439 common DEGs shared by at least 15 MM datasets as an input to do enrichment analysis for the biological process network (**Figure 2A**), the pathway map (**Figure 2B**), GO process (**Figure 2C**), and disease phenotypes (**Figure 2D**) using MetaCore. **Figure 2** displays the top 10 highly related pathways and the top 20 diseases. Not surprisingly, we found that more than half of the top 10 pathway maps and several process networks highly associated with MM datasets are tightly involved in cell proliferation, translation, transcription, and cell cycle, which are important biological pathways that are highly expressed in cancer cells due to the rapid and unlimited cell proliferation potential of cancer cells. In addition to these common pathways induced by multiple cancers, we identified several pathway maps, biological process networks and GO processes that are closely related to immune response and inflammatory signaling, especially IL-6 and B cell antigen receptor (BCR) signaling, which is specific for

the immune response caused by B cell. As we mentioned before, multiple myeloma is a type of cancer caused by malignant plasma cell, the DNA impairment in B cell make abnormal B cells, which will turn to malignant plasma cells in response to a stimulus, followed by the release of abnormal antibodies, leading to tissue damage. Thus, the BCR signaling is specific for MM, same as the IL-6 signaling in MM (**Figure 2B**). Moreover, viral process and parasitism symbiosis are identified as top listed GO process, which indicates a potential correlation between viral infection and multiple myeloma. Furthermore, ubiquitin-proteasome system is also emphasized here in the top ranked pathway maps, which further indicates a role of proteolysis via USP and autophagy in MM, in accordance with our previous analysis.

Figure 2D shows the top 20 diseases that have a higher correlation with common DEGs genes shared by MM datasets. First, many of the other types of neoplasms and carcinoma are intensely connected to MM, as different cancer types often share a lot of similar pathological pathways. They occur in different tissues and organs, including uveal tracts, breast, ducts, lobules, endocrine gland, eye, and ovary. Additionally, we also find some diseases specifically related to multiple myeloma, such as the bone marrow diseases and hematological diseases. Multiple myeloma is a cancer of plasma cells t located in bone marrow, and it is the second most common hematologic malignancy. Interestingly, virus diseases, RNA virus infection, and immunological deficiency syndromes are ranked at the top of the diseases, indicating a tight association and potential common pathological pathways shared by viral infections like HIV and MM, which is consistent with our previous results.

Positive and negative correlated genetic perturbations

Top positively (**Figure 3A**) and negatively (**Figure 3B**) correlated genetic knockdown with MM datasets is identified. We identified the significant related genetic perturbations with each MM dataset using Knockdown Altas function in BaseSpace. We then took the highly connected gene perturbations with a gene perturbation score higher than 50 into account for summing up data of 30 MM datasets, finally to determine and rank the highly positive or negative correlated gene perturbations, including gene knockout, knockdown, mutation, and overexpression. Positive correlations between DEGs induced by specific single gene knockdown/knockout and MM induced DEGs may imply that the functions of these genes are impaired during the MM process, because knockdown/knockout of these genes will induce similar gene signature change as MM. Our findings are consistent with other literature reports. Negative correlations between a single gene knockdown/knockout DEGs and MM DEGs might suggest that this gene overexpresses in the MM disease condition, and blocking this single gene expression or inhibiting the protein encoded by this gene will reverse the differentially expressed genes in the pathological process of MM and help treat the disease.

The gene signature induced by ROR1 knockdown is highly negatively connected to the MM induced data. A functional study suggested that ROR1 is a part of Wnt signaling pathway and can promote cancer cell survival[1, 4]. Overexpression of the receptor tyrosine kinase-like orphan receptor 1 (ROR1) has been identified in B-cell chronic lymphocytic leukemia (B-CLL) and chronic myelogenous leukemia (CML), but

not in normal white blood cells and reactive lymph nodes, suggesting ROR1 might serve as a tumor-specific target for therapy[4, 5, 1].

SLAMF7 is negatively associated with MM by 16 negative associations and no positive association with 30 MM gene sets. This gene encodes Signaling Lymphocyte Activation Molecule Family member 7 (SLAMF7), a therapeutic target for MM treatment. In 2015, the FDA approved Elotuzumab (a humanized monoclonal antibody targeting SLAMF7) as a treatment for patients with multiple myeloma who have received one to three prior medications[6]. Now Elotuzumab is a global approved medication used in combination with other anti-MM drugs to treat relapsed/refractory and advanced MM. It demonstrates its efficacy to improve symptoms when added into other MM therapy without causing any significant additive toxicity [7].

Another top negatively associated knockdown gene is MTDH (Metadherin), which has shown a crucial role in several aspects of tumor progression, including transformation, evasion of apoptosis, invasion, metastasis, and chemoresistance. MTDH overexpression is frequently observed in different cancers, including melanoma, glioma, neuroblastoma, as well as liver, breast, prostate, and esophagus cancers. Overexpression of MTDH activates downstream PI3K/AKT, NF-κB, and Wnt/β-catenin signaling pathways to stimulate cancer cell proliferation, cell survival, invasion and chemoresistance. MTDH also mediates the transformation of oncogene Ha-Ras and c-Myc and the adhesion of tumor cells to remote organs to promote transformation and metastasis. MTDH can be a therapeutic target to block several processes of tumor progression[8].

Sixteen positive correlations were identified between MM datasets and DEGs induced by knock down of SATB1, “a cell-type specific global gene regulator” by folding chromatin into loop domains[9]. Scientists reported that SATB1 can regulate gene expression to promote growth and metastasis of breast tumors[10]. Here, we observe the reverse correlation between SATB1 and MM: the knockdown of SATB1 has a positive association with MM, indicating blocking of SATB1 might regulate genes to promote MM. Daxx, another gene knockdown with positive correlation with MM, is reached by 14 positive associations with only one negative one. Yang X et al. identified Daxx, as a Fas binding protein that can activate both Fas-mediated apoptosis and JNK signaling pathway[11]. As JNK is a key modulator for cell death, Daxx can activate cell death related pathways, and might have the potential to promote cancer cell death in MM. To summarize, most positive and negative related gene knockdown are in accordance with literature and clinical reports, specific for MM (ROR1 and SLAMF7), or for other types of cancers (MTDH and Daxx). Some highly correlated genes are still not reported with multiple myeloma, indicating a new direction for further investigation.

MM positively correlated gene mutations and gene overexpression are overlapped with each other. Among the top ranked positive related overexpression displayed in **Figure 3C**, RARA, FGFR1, PML, CD247, MYH11 and CBFB are also found in the top list of positively associated gene mutations (**Supplementary Figure 3**), which are also demonstrated to be involved in carcinogenesis pathologies. Our analysis suggested that FGFR1 mutations might cause FGFR1 overexpression in tumor cells. Fibroblast growth

factor receptor 1 (FGFR1) plays an essential role in the regulation of cell proliferation, differentiation and migration via mediating the downstream signaling, such as MAPK, AKT/PI3K, and Ras. Amplification of FGFR1 was reported in breast cancer[12], non-small[13] and squamous cell lung cancer[14], urinary bladder cancer[15], and prostate cancer[16]. Several tyrosine kinase inhibitors targeting FGFR1 are currently applied for cancer treatment. Regorafenib, Sorafenib, Lenvatinib, Nintedanib and Pazopanib are approved by the FDA to treat carcinoma that occurs in different tissues and organs, such as hepatocellular carcinoma, advanced renal cell carcinoma, non-small cell lung carcinoma and differentiated thyroid carcinoma (DTC) that is refractory to radioactive iodine[17]. Negative correlated overexpression also shares some common gene with negative correlated mutations, such as ZBTB16 and MAPT. ZBTB16 plays a role in myeloid maturation and serves as a component of E3 ligase that mediates protein ubiquitination and subsequent proteasomal degradation for abnormal proteins, which might be associated with acute leukemia and multiple myeloma[18]. MAPT encodes the microtubule-associated protein tau (MAPT), and its mutations have been associated with some neurodegenerative disorders such as Alzheimer's disease, Pick's disease, and frontotemporal dementia.

Positive and negative associations between MM- and drug-induced gene profiles

Significant differentially expressed genes with a score over 50 compared to each MM datasets were collected and summarized for a total of 30 datasets. Top ranked drugs, which induced DEGs with more than 15 negative associations compared to MM induced DEGs among 30 datasets, are listed in **Table 1**, with their therapeutic targets and number of negative and positive associations. Drugs that induced DEGs with negative correlations with MM, might be able to reverse the gene expression change caused by myeloma, and have a beneficial effect to treat MM. Most of the highly negatively related medications are anticancer agents, anti-infective drugs, dietary supplements and immune regulators.

Table 1. Associations between MM-induced and drug-induced gene profiles

Drug	Target	Negative Association	Positive Association
Brefeldin A	Protein Transport	21	6
Bafilomycin A	Vacuolar-Type H^+-ATPase	19	6
Ambroxol	Neuronal Na^+ Channels.	18	1
GDC-0941	PI3K Inhibitor	18	5
Lindane	Hair Lice	18	4
Imatinib	ABL, C-Kit, PDGFR	18	3
Ergocalciferols	Vitamin D2	18	1
Dexamethasone	GCR	18	8
Decitabine	Nucleic Acid Synthesis Inhibitor.	17	10
Clonidine	A2 Receptors	17	2
Clinafloxacin	Topoisomerases DNA Gyrase and Topoisomerase IV	17	7
Gentamicin C	30s Subunit of The Bacterial Ribosome	17	0
Geldanamycin	HSP90	17	4
Letrozole	Orally Active Nonsteroidal Aromatase Inhibitor	17	4
Zardaverine	PDE3/4 Phosphodiesterase Inhibitor	17	3
Tetracycline	30S Subunit of Microbial Ribosomes	17	10
Perhexiline	Mitochondrial Carnitine Palmitoyltransferase-1	17	2
Acrolein	Cyclophosphamide metabolite	16	5
Amphotericin B	Ergosterol	16	3
Bamipine	H1 Histamine Receptor	16	7
Caffeine		16	7
Meptazinol	Opioid Analgesic	16	2
Mephentermine	ADRB1	16	5
Lithium Carbonate		16	5
Strophanthidin	Na^+/ K^+ ATPase In Muscle Tissue	16	9

Sirolimus	mTOR, F_k Binding Protein-12	16	9
Vincristine	Tubulin	15	10
Trihexyphenidyl	Antimuscarinic	15	1
Tolazoline	Non-Selective A-Adrenergic Receptor Antagonist	15	2
Sotalol	Non-Selective Beta-Adrenergic Receptor Blocker	15	3
Solanine	Cholinesterase	15	2
Paclitaxel	Tubulin	15	11
Monobenzone	Increases the Excretion of Melanin	15	6
Lanatoside C	Cardiac Glycoside	15	9
Isoproterenol	Non-Selective B Adrenoreceptor Agonist	15	6
Folic Acid	Vitamin B9	15	7
Cyclosporine	Immunosuppressant with A Specific Action on T-Lymphocytes	15	10

Among the top listed drugs with negative associations (**Table 1**), several drugs have already been approved and applied to treat MM or investigated in clinical trials. **Table 2** lists nine currently used anti-MM drugs that are also identified from the top list of MM gene negatively associated drugs with their development stage, regimen, number of positive and negative associations, as well as reference. For example, both Imatinib and Ergocalciferols have 18 negative correlations with MM gene datasets and only a few positive correlations (3 for Imatinib, 1 for Ergocalciferols). Imatinib, a tyrosine kinase inhibitor, is now in clinical trial Phase II in a single drug form and in combination use for MM treatment. Ergocalciferol, also known as Vitamin D2, is used in combination with other anti-tumor drugs as a nutrient supplement to treat MM [20]. Another dietary supplement, Folic Acid, also known as Vitamin B9, was also identified to have potential for treating MM, which is consistent with the presence of this drug in clinical trial Phase II for combination use in MM. Dexamethasone, a type of corticosteroid medication with 18 negative associations and 8 positive ones, was approved by the FDA to treat patients with MM in combination with other drugs [21]. Additionally, four other antitumor agents, including Sirolimus, an mTOR inhibitor, Decitabine, a nucleic acid synthesis inhibitor, as well as Vincristine and Paclitaxel, tubulin inhibitors, are in different clinical trial phases (Phase I for Sirolimus and Decitabine, Phase III for Vincristine, Phase II for Paclitaxel) to treat MM along or with other medications.

Table 2. Currently anti-MM drugs induce DEGs highly negatively related with MM

Drug	Negative association	Positive association	Development stage	Reference	Regimen
Imatinib	18	3	Phase II	PMID: 16321825	Single and combination
Ergocalciferols	18	1	Phase II	NCT00171925	Combination
Dexamethasone	18	8	Approved		Combination
Decitabine	17	10	Phase I	NCT00002980	Single
Sirolimus	16	9	Phase I	NCT0069343, NCT01303965	Combination
Vincristine	15	10	Phase III	NCT00002556, PMID:15005346, PMID: 8370422	Combination
Paclitaxel	15	11	Phase II	NCT01646762	Single and combination
Cyclosporine	15	10	Phase III	PMID: 11843823	Combination
Folic Acid	15	7	Phase II	NCT00171925	Combination

For some of these drugs listed in **Table 1**, they have the potential to be repurposed for the treatment of multiple myeloma because of their possible capacity to reverse the mRNA level change in pathological conditions. We checked the literature first and did some simple *in vitro* assays using some easily accessible medications to verify the gene signature analysis prediction for drug reposition.

As shown in **Figure 5A**, although Folic Acid and Bafilomycin A1 themselves did not have much anti-proliferation effect, the combination use of Folic Acid or Bafilomycin A1 with Bortezomib will improve the anti-proliferation activity of Bortezomib (a well-known FDA approved anti-MM drug) in MM.1.S cells (a multiple myeloma cell line) with 16h medication treatment. The other two drugs predicted to be repurposed for MM treatment in our study, Sorafenib and Doxorubicin, also showed some anti-proliferation effects on the MM cell line. These results indicate that our prediction via gene signature analysis might be reliable on the cellular level.

To further investigate the signaling pathway these medications go through to kill the tumor cells, we treated MM1.S cells with these drugs and their combinations for 16h at their effective concentrations. Then we collected the cell lysate samples and ran Western blot to measure the caspase-3 level changes, a well-known apoptosis marker, influenced by different drugs. As shown in **Figure 5B** we found that co-treatment of Bortezomib with folic acid or Bafilomycin A1 induced more caspases 3 cleavage (19kDa and 17kDa) than treatment with Bortezomib alone. Bafilomycin A1 and Folic acid also induce caspase 3 cleavage compared with DMSO alone, but the effect is much weaker than bortezomib. In addition, in

accordance with our MTT result, treatment of Sorafenib also induces the cleavage of caspase 3, induces apoptosis signaling and causes tumor cell death.

To summarize, both the common DEGs from the MM datasets and the drugs that induce negative correlated transcriptional profiles are involved in the pathogenesis of infection, immune, and inflammatory pathways or are indicated to treat viral, bacterial and fungal infection, inflammation and immune diseases. This result suggests a tight relationship between multiple myeloma and these diseases, from molecular mechanism to therapeutic treatment. It was reported that infections with the HIV and hepatitis C virus appeared associated to an enhanced multiple myeloma risk. Infections are a major threat to multiple myeloma patients, with majority of morbidity and mortality due to infections[31, 32]. In a nationwide population-based study in Sweden, multiple myeloma patients showed a 7-fold risk to develop a bacterial infection and a 10-fold risk for viral infection compared to matched controls ($P<0.001$)[33, 32]. The complications of MM are the major cause for the death of patients. It is necessary to introduce the anti-infective agents in combination with anti-tumor therapy for the treatment of infectious complications in MM patients, which is consistent with our drug reposition prediction[32].

As for the immune response, multiple myeloma is a cancer of plasma cells, which are cells responsible for producing antibodies in response to foreign antigen and debris stimulations. Multiple myeloma disease will lead to the dysfunction of plasma cells, which is followed by immunodeficiency. It has been shown that MM patients have a low immune response to infection, which is one of the causes for a higher risk of infection[34, 35]. “Managing the complications of the disease and its treatment, such as infections, thrombosis and neuropathy, has become more important as MM patients survive longer[32].” Drugs with negative associated gene signature compared to MM in our study also include cardiovascular agents and CNS medications, suggesting possible medication reposition to treat the complications of the disease.

Declarations

Compliance with Ethical Standards

Conflict of interest: The authors declare that they have no competing interest.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study

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Author Contribution

SM with the idea of writing this manuscript. XX supports the work. SM wrote the first draft. All authors read and approved the final version.

Available of data and material

We collected the AD-induced DEGs from commercial software Illumina BaseSpace software (Santa Clara, CA, USA, http://www.nextbio.com). The data can also be reached using GEO ID in **Table 1** in http://www.ncbi.nlm.nih.gov/geo/. The associated gene and chemical perturbations from each dataset were reached and downloaded through Pharmaco Atlas search and Knockdown Atlas search in BaseSpace. It's a commercial software, we will upload the data in this study we downloaded using the software.

References

1. Daneshmanesh AH, Porwit A, Hojjat-Farsangi M, Jeddi-Tehrani M, Tamm KP, Grandér D et al. Orphan receptor tyrosine kinases ROR1 and ROR2 in hematological malignancies. Leukemia & lymphoma. 2013;54(4):843-50.
2. Lentzsch S, ebrary I. Genetic and molecular epidemiology of multiple myeloma. 1. Aufl.;2013;1; ed., vol Book, Whole. New York, NY: Springer; 2013.
3. Hsu P, Lin T-W, Gau J-P, Yu Y-B, Hsiao L-T, Tzeng C-H et al. Risk of Early Mortality in Patients With Newly Diagnosed Multiple Myeloma. Medicine. 2015;94(50):e2305. doi:10.1097/MD.0000000000002305.
4. Hudecek M, Schmitt TM, Baskar S, Lupo-Stanghellini MT, Nishida T, Yamamoto TN et al. The B-cell tumor–associated antigen ROR1 can be targeted with T cells modified to express a ROR1-specific chimeric antigen receptor. Blood. 2010;116(22):4532-41.
5. Fukuda T, Chen L, Endo T, Tang L, Lu D, Castro JE et al. Antisera induced by infusions of autologous Ad-CD154-leukemia B cells identify ROR1 as an oncofetal antigen and receptor for Wnt5a. Proceedings of the National Academy of Sciences. 2008;105(8):3047-52.
6. Administration USFaD. Press Announcement—FDA approves Empliciti, a new immune-stimulating therapy to treat multiple myeloma. 3 December 2015.
7. van de Donk NWCJ, Moreau P, Plesner T, Palumbo A, Gay F, Laubach JP et al. Clinical efficacy and management of monoclonal antibodies targeting CD38 and SLAMF7 in multiple myeloma. Blood. 2015:blood-2015-10-646810.
8. Hu G, Wei Y, Kang Y. The multifaceted role of MTDH/AEG-1 in cancer progression. Clinical cancer research. 2009;15(18):5615-20.
9. Yasui D, Miyano M, Cai S, Varga-Weisz P, Kohwi-Shigematsu T. SATB1 targets chromatin remodeling to regulate genes over long distances. Nature. 2002;419(LBNL–51500).
10. Han H-J, Russo J, Kohwi Y, Kohwi-Shigematsu T. SATB1 reprogrammes gene expression to promote breast tumour growth and metastasis. Nature. 2008;452(7184):187.

11. Yang X, Khosravi-Far R, Chang HY, Baltimore D. Daxx, a novel Fas-binding protein that activates JNK and apoptosis. Cell. 1997;89(7):1067-76.
12. Turner N, Pearson A, Sharpe R, Lambros M, Geyer F, Lopez-Garcia MA et al. FGFR1 amplification drives endocrine therapy resistance and is a therapeutic target in breast cancer. Cancer research. 2010;70(5):2085-94.
13. Dutt A, Ramos AH, Hammerman PS, Mermel C, Cho J, Sharifnia T et al. Inhibitor-sensitive FGFR1 amplification in human non-small cell lung cancer. PloS one. 2011;6(6):e20351.
14. Weiss J, Sos ML, Seidel D, Peifer M, Zander T, Heuckmann JM et al. Frequent and focal FGFR1 amplification associates with therapeutically tractable FGFR1 dependency in squamous cell lung cancer. Science translational medicine. 2010;2(62):62ra93-62ra93.
15. Simon R, Richter J, Wagner U, Fijan A, Bruderer J, Schmid U et al. High-throughput tissue microarray analysis of 3p25 (RAF1) and 8p12 (FGFR1) copy number alterations in urinary bladder cancer. Cancer research. 2001;61(11):4514-9.
16. Yang F, Zhang Y, Ressler SJ, Ittmann MM, Ayala GE, Dang TD et al. FGFR1 is essential for prostate cancer progression and metastasis. Cancer research. 2013;73(12):3716-24.
17. Grillo F, Florio T, Ferraù F, Kara E, Fanciulli G, Faggiano A et al. Emerging multitarget tyrosine kinase inhibitors in the treatment of neuroendocrine neoplasms. Endocrine-Related Cancer. 2018;25(9):R453-R66. doi:10.1530/erc-17-0531.
18. Furukawa M, He YJ, Borchers C, Xiong Y. Targeting of protein ubiquitination by BTB-Cullin 3-Roc1 ubiquitin ligases. Nat Cell Biol. 2003;5(11):1001-7. doi:http://www.nature.com/ncb/journal/v5/n11/suppinfo/ncb1056_S1.html.
19. World Health Organization WHF, World Stroke Organization. Global atlas on cardiovascular disease prevention and control Policies, strategies and interventions. 2011.
20. Hamon M, Mallat M, Herbet A, Nelson DL, Audinot M, Pichat L et al. [3H]Metergoline: A New Ligand of Serotonin Receptors in the Rat Brain. Journal of Neurochemistry. 1981;36(2):613-26. doi:10.1111/j.1471-4159.1981.tb01634.x.
21. Miller KJ, King A, Demchyshyn L, Niznik H, Teitler M. Agonist activity of sumatriptan and metergoline at the human 5-HT1Dβ receptor: further evidence for a role of the 5-HT1D receptor in the action of sumatriptan. European Journal of Pharmacology: Molecular Pharmacology. 1992;227(1):99-102. doi:https://doi.org/10.1016/0922-4106(92)90149-P.
22. Carew JS, Nawrocki ST, Krupnik YV, Dunner K, McConkey DJ, Keating MJ et al. Targeting endoplasmic reticulum protein transport: a novel strategy to kill malignant B cells and overcome fludarabine resistance in CLL. Blood. 2006;107(1):222-31. doi:10.1182/blood-2005-05-1923.
23. Wu YC, Wu WKK, Li Y, Yu L, Li ZJ, Wong CCM et al. Inhibition of macroautophagy by bafilomycin A1 lowers proliferation and induces apoptosis in colon cancer cells. Biochemical and Biophysical Research Communications. 2009;382(2):451-6. doi:https://doi.org/10.1016/j.bbrc.2009.03.051.

24. Lu X, Chen L, Chen Y, Shao Q, Qin W. Bafilomycin A1 inhibits the growth and metastatic potential of the BEL-7402 liver cancer and HO-8910 ovarian cancer cell lines and induces alterations in their microRNA expression. Experimental and Therapeutic Medicine. 2015;10(5):1829-34. doi:10.3892/etm.2015.2758.
25. Ohta T, Arakawa H, Futagami F, Fushida S, Kitagawa H, Kayahara M et al. Bafilomycin A1 induces apoptosis in the human pancreatic cancer cell line Capan-1. The Journal of pathology. 1998;185(3):324-30.
26. Li L-q, Xie W-j, Pan D, Chen H, Zhang L. Inhibition of autophagy by bafilomycin A1 promotes chemosensitivity of gastric cancer cells. Tumor Biology. 2016;37(1):653-9. doi:10.1007/s13277-015-3842-z.
27. Wallen E, Sellers RG, Peehl DM. Brefeldin A induces p53-independent apoptosis in primary cultures of human prostatic cancer cells. The Journal of urology. 2000;164(3):836-41.
28. Kawaguchi T, Miyazawa K, Moriya S, Ohtomo T, Che X-F, Naito M et al. Combined treatment with bortezomib plus bafilomycin A1 enhances the cytocidal effect and induces endoplasmic reticulum stress in U266 myeloma cells: crosstalk among proteasome, autophagy-lysosome and ER stress. International journal of oncology. 2011;38(3):643-54.
29. Niwa S, Ishibashi O, Inui T. Brefeldin A inhibits osteoclastic bone resorption through induction of apoptosis. Life sciences. 2001;70(3):315-24.
30. Numan M. Ambroxol hydrochloride, a chaperone therapy for Paget's disease of bone and other common autophagy-mediated aging diseases?
31. Nucci M, Anaissie E. Infections in Patients With Multiple Myeloma. Seminars in Hematology. 2009;46(3):277-88. doi:https://doi.org/10.1053/j.seminhematol.2009.03.006.
32. Blimark C, Holmberg E, Mellqvist U-H, Landgren O, Björkholm M, Hultcrantz M et al. Multiple myeloma and infections: a population-based study on 9253 multiple myeloma patients. Haematologica. 2014;100(1):107.
33. Kristinsson SY, Landgren O, Dickman PW, Derolf ÅR, Björkholm M. Patterns of Survival in Multiple Myeloma: A Population-Based Study of Patients Diagnosed in Sweden From 1973 to 2003. Journal of Clinical Oncology. 2007;25(15):1993-9. doi:10.1200/JCO.2006.09.0100.
34. Karlsson J, Andréasson B, Kondori N, Erman E, Riesbeck K, Hogevik H et al. Comparative study of immune status to infectious agents in elderly patients with multiple myeloma, Waldenstrom's macroglobulinemia, and monoclonal gammopathy of undetermined significance. Clinical and vaccine immunology. 2011;18(6):969-77.
35. Hargreaves R, Lea J, Griffiths H, Faux J, Holt J, Reid C et al. Immunological factors and risk of infection in plateau phase myeloma. Journal of clinical pathology. 1995;48(3):260-6.

Figures

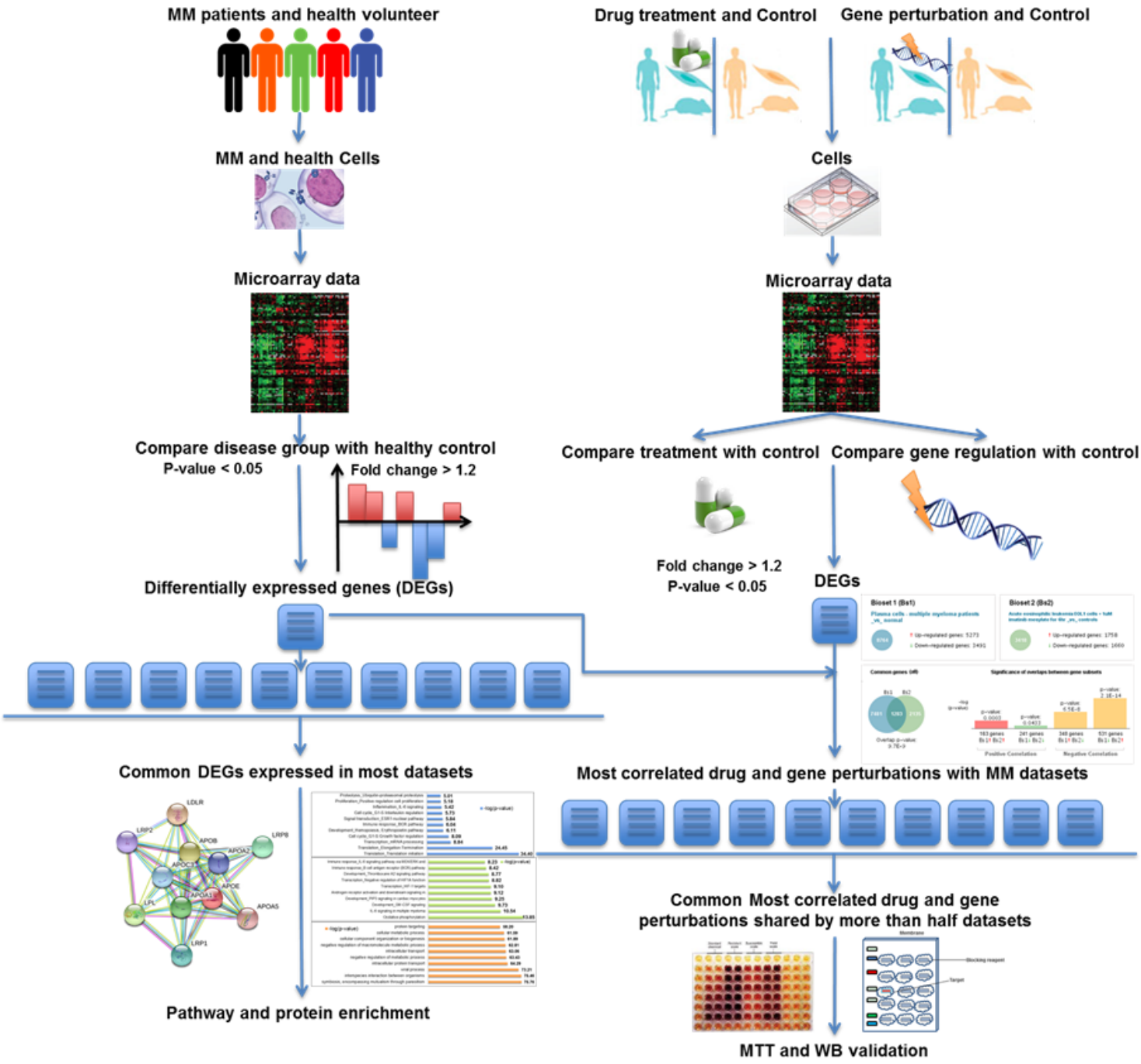


Figure 1

Workflow for this meta-analysis study.

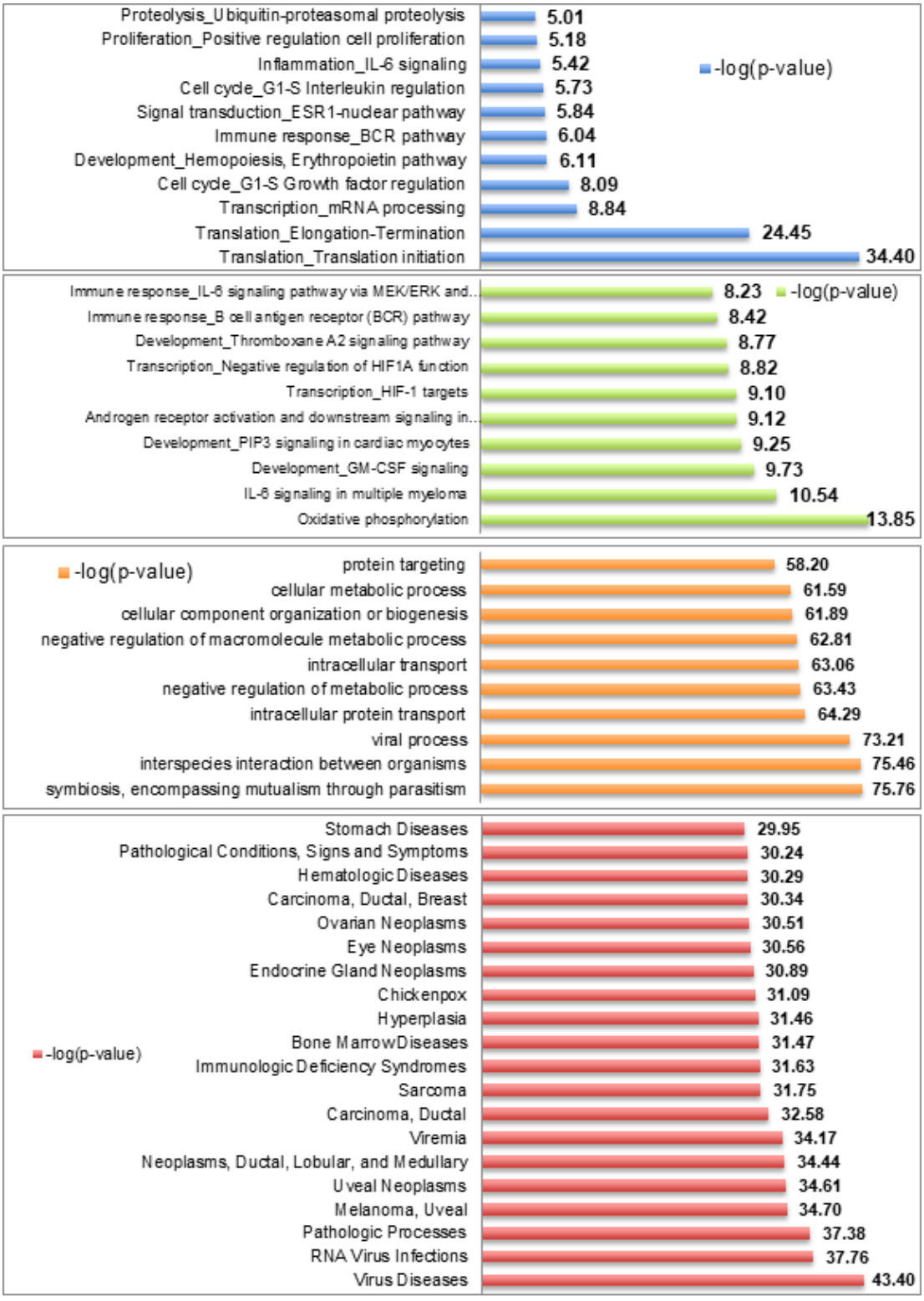


Figure 2

Enrichment Study for common MM DEGs (A) Enrichment by biological process network. (B) Enrichment by pathway map. (C) Enrichment by GO process. (D) Enrichment by Disease (by biomarkers).

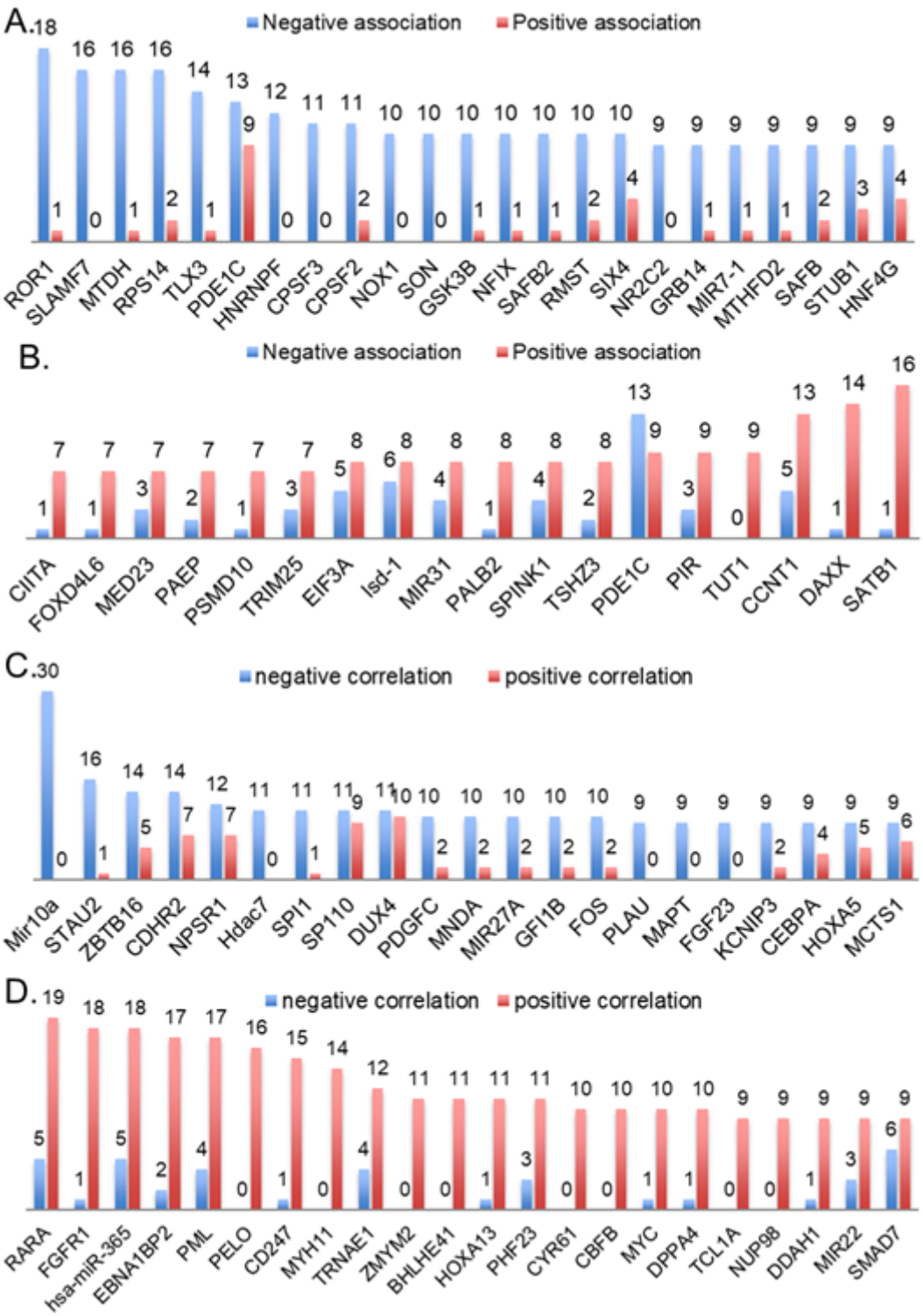
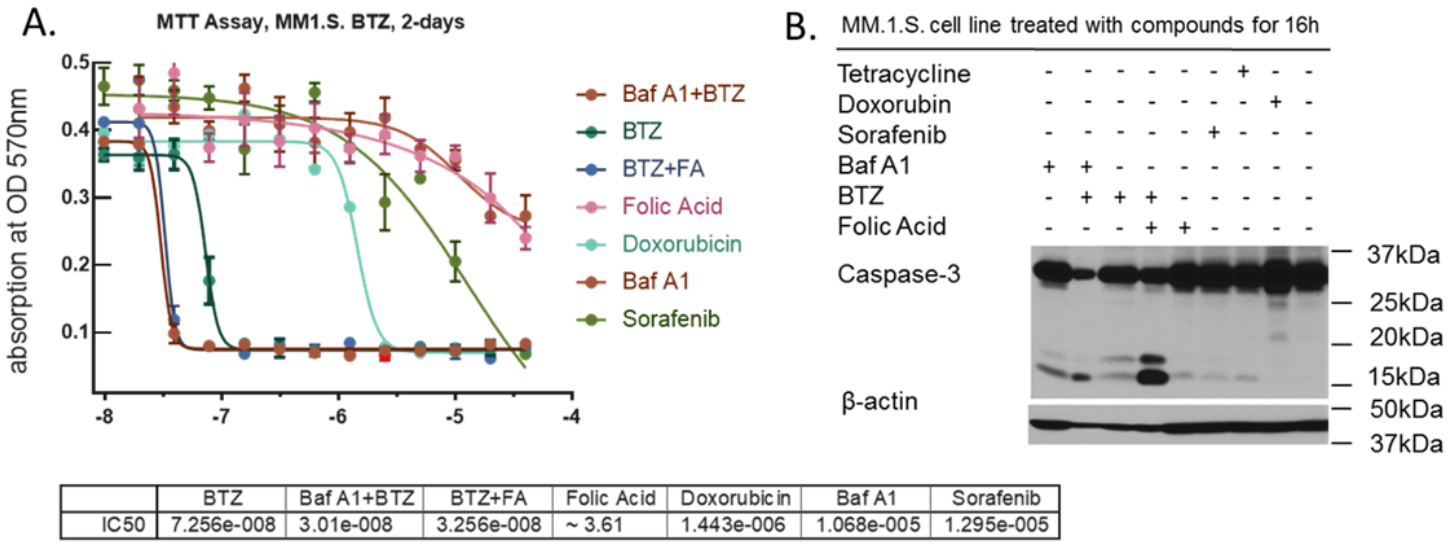


Figure 3

The associations between MM induced and gene knockdown (A/B) and overexpression (C/D) induced gene profiles.



	BTZ	Baf A1+BTZ	BTZ+FA	Folic Acid	Doxorubicin	Baf A1	Sorafenib
IC50	7.256e-008	3.01e-008	3.256e-008	~ 3.61	1.443e-006	1.068e-005	1.295e-005

Figure 4

Some predicted medications showed potency to inhibit myeloma cell growth through apoptosis signaling. (A) Anti-proliferation effects of several medications and some of their combinations on multiple myeloma cell line. MM.1.S cells were seeded on 96-well plates and incubated with indicated doses of drugs and drug combinations for 48h. The percentage of survival was determined by MTT assay. Data is represented as mean ± SD of three experiments carried out in duplicate. The medications include Bafilomycin A1 (Baf A1), bortezomib (BTZ), folic acid (FA), Doxorubicin, and Sorafenib, as well as Bortezomib and Bafilomycin A1 combination, and Bortezomib and Folic Acid combination. (B) Immunoblotting results for MM.1.S cells treated with different drugs and their combinations to measure the caspase-3 level by caspase 3 antibody.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupportinginformationMMGene01162021.docx