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Understanding and Manipulating Pathologic Splicing Preferences and Nonsense-mediated mRNA Decay (NMD) in Spliceosomal-mutant MDS

1/15/2017-1/14/2020

Mutations in the spliceosomal proteins SF3B1, SRSF2, U2AF1, and ZRSR2 constitute the most common class of genetic alterations in patients with myelodysplastic syndromes (MDS). Since the discovery of these mutations, data from multiple groups have revealed that mutations in SRSF2 and U2AF1 alter the normal RNA binding and splicing preferences of these proteins in a sequence-specific manner. In addition, we and others have identified that cells bearing spliceosomal gene mutations are preferentially sensitive to further genetic or pharmacologic perturbations to RNA splicing, compared with spliceosomal wild-type (WT) cells. However, it is not yet clear whether global inhibition of splicing will be clinically viable. The frequency of spliceosomal gene mutations in MDS, combined with the specific manner by which these mutations alter RNA splicing, make efforts to therapeutically target spliceosomal mutant cells with greater specificity imperative. Here we aim to develop two novel mechanism-based approaches targeting spliceosomal-mutant MDS based on: (1) selectively targeting the pathologic RNA-binding preferences of mutant spliceosomal proteins; and (2) restoring the expression of key transcripts targeted for nonsense-mediated decay (NMD) due to aberrant splicing. Understanding the mechanistic link between NMD and spliceosomal gene mutations will be critical for this latter aim. We therefore propose the following Specific Aims:

Aim I. Determine the precise mechanism by which mutant SRSF2 promotes NMD.

Aim II. Utilize antisense techniques to restore expression of mRNAs pathologically degraded by NMD in spliceosomal-mutant MDS cells.

Aim III. Determine the therapeutic potential of oligonucleotide decoys that exploit the RNA-binding preferences of WT versus mutant SRSF2 in SRSF2-mutant malignant cells.
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The Role of HIF-1α As a Central Pathobiologic Mediator of Myelodysplastic Syndromes
12/15/2016-12/14/2019

Myelodysplastic Syndromes (MDS) are characterized by multi-lineage dysplastic cytopenia. Every year, about 20,000 people are diagnosed with MDS in the United States. The leading causes of death are bleeding or infection resulting from refractory cytopenia. Generally, conventional chemotherapy is not effective for MDS. Transfusion dependence due to progressive anemia/thrombocytopenia also causes various complications. Further elucidation for the pathogenesis of MDS and establishment of novel therapeutic strategies are needed. To investigate the underlying mechanisms and preclinical targeting of MDS, we took an unbiased bioinformatic approach and found an elevated hypoxia inducible factor 1α (HIF-1α) signature in the CD34+ cells from primary MDS patient samples of various genotypes. We further validated HIF-1α protein (but not mRNA) upregulation with MDS patients’ BM biopsies. We have also established novel MDS-like mouse genetic model. Most importantly, using these models, we validated the critical role of HIF-1α in the disease development and MDS phenotypes. To molecularly dissect HIF-1α mediated pathobiology, we generated hematopoietic cell-specific tetracycline-inducible HIF-1α transgenic mice. Our preliminary data indicated that these mice developed MDS phenotypes which faithfully recapitulate a variety of human MDS phenotypes. Although MDS are heterogeneous diseases driven by a variety of mutations (genotypes), the key features of MDS (phenotypes) among patients are very similar. Thus, based on our preliminary data, we hypothesize that activated HIF-1α signaling, driven by clonal hematopoietic and oncogenic mutations, is a central pathobiologic mediator of Myelodysplastic Syndromes. We propose to determine, 1) the mechanism of HIF-1α activation by major MDS-associated mutations; 2) the essential and sufficient role of HIF-1α in MDS with multiple mouse MDS models; 3) therapeutic potential of targeting HIF-1α in human MDS xenograft models. Our study will not only provide stringent mechanistic tests of our hypotheses, but also lead to a better understanding the pathogenesis of MDS and to potential novel therapeutic targets.
There is evidence that alterations in the bone marrow microenvironment may contribute to the pathogenesis of myelodysplastic syndromes (MDS). CXCL12 expression in bone marrow stromal cells is increased in MDS. CXCL12 through interaction with its major receptor CXCR4 is a key regulator of hematopoiesis. It regulates neutrophil egress from the bone marrow and hematopoietic stem cell (HSC) quiescence and repopulating activity. Increased CXCR4 signaling in humans reproduces some features of MDS, including peripheral neutropenia despite an increased number of dysplastic neutrophils in the bone marrow. Increased CXCR4 signaling also decreases the competitive fitness of HSPCs. Our preliminary data suggest that pharmacologic or genetic attenuation of CXCR4 signaling in a murine model of MDS partially rescues some of the hematopoietic abnormalities. Based on these observations, we hypothesize that increased stromal expression of CXCL12 contributes to ineffective hematopoiesis and leukemic transformation in MDS.

Current evidence suggests that stromal cells are not part of the MDS malignant clone. It follows that alterations in the bone marrow microenvironment in MDS, including increased CXCL12 expression, must be secondary to changes in a hematopoietic cell population(s). Macrophages are a prime suspect. Bone marrow macrophages are a key determinant of stromal CXCL12 expression. They regulate erythropoiesis through formation of blood islands, and they support osteoblasts, thereby potentially affecting hematopoiesis through regulation of the osteoblastic niche. Based on these observations, we hypothesize that abnormal macrophage function in MDS contributes to the altered bone marrow microenvironment in MDS, including increased stromal CXCL12 expression. These studies may provide the foundation for a clinical trial of CXCR4 inhibitors in MDS. The following specific aims are proposed.

Aim 1. To characterize the contribution of increased stromal CXCL12 expression to ineffective hematopoiesis and leukemic transformation in MDS.

Aim 2. To characterize the contribution of macrophages to the altered bone marrow microenvironment in MDS.
2016 Awardee

Ulrich Steidl, M.D., Ph.D.
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*Mechanisms of MDS Initiation at the Stem Cell Level*
12/15/2016-12/14/2019

Relapse and progressive disease remain the most common causes of death in MDS. The development of fundamentally novel therapeutic approaches is urgently desired. Recent evidence has indicated that initially formed pre-cancerous stem cells (pre-MDS-SC) precede the formation of fully transformed MDS stem cells (MDS-SC), and play a pivotal role in disease origination and progression. While the existence and essentiality of such pre-cancerous cell states has been demonstrated in mice and humans, almost nothing is known about the molecular mechanisms driving pre-MDS-SC formation and progression. We have recently reported a new mouse model of induction of pre-cancerous stem cells and progression to MDS, molecularly driven by heterozygous PU.1 enhancer deletion (PU.1 function is frequently disrupted in human MDS) combined with Msh2 deficiency, leading to an increased frequency of mutation types occurring during aging and in MDS patients. This model is hallmarked by definable pre-cancerous stem cells and closely resembles human disease in many phenotypic and molecular features, and now permits for the first time the longitudinal identification and molecular study of mechanisms driving the formation and progression of pre-cancerous stem cells in MDS. Such pre-MDS-SC in this model are characterized by key functional alterations, including a myeloid bias of early 'uncommitted' stem cells, a numerical expansion of phenotypic "myeloid biased" stem cells, and reduced quiescence. We propose to: 1. Identify and study mechanisms/pathways which are critical for the formation and the functional alterations of pre-MDS-SC; and 2. Delineate molecular determinants of the progression of pre-cancerous pre-MDS-SC to overt MDS. The research plan will employ our novel pre-MDS-SC to MDS progression model and longitudinal analysis of transcriptional and genetic dysregulation at the stem cell level. Targets will be identified and tested for functional relevance in vitro and in vivo, and validated on sorted stem cells from patients with MDS.
Familial cases of adult myelodysplastic syndrome (MDS) are likely to be more common than currently appreciated. Because MDS is diagnosed at 71 years old on average, we hypothesize that those patients who present at much younger ages are an ideal population in which to identify individuals with germline predisposition. Therefore, this study will determine the frequency of mutations in predisposition genes (e.g., RUNX1, GATA2, CEBPA, ANKRD26, SRP72, TP53, ETV6, DDX41, and genes that comprise the inherited bone marrow failure syndromes) in young patients with MDS (Specific Aim 1). This study will take advantage of a cohort of 100 MDS patients diagnosed at less than 40 years of age assembled at The University of Chicago and its affiliates. We also seek to define new familial syndromes using our collection of more than 300 families, each of which contains at least two cases of hematopoietic malignancies within two generations, and test remaining young MDS patients for these syndromes (Specific Aim 2). We anticipate that our results will demonstrate that many young patients with MDS have a genetic predisposition to their disease, a finding that will prompt the examination of germline susceptibility as a routine step in the management of these patients. The recognition of the germline nature of these patients’ disease is critical for proper clinical management. Because allogeneic stem cell transplant is the only curative treatment for MDS, and because young patients are candidates for allogeneic stem cell transplantation, determining those patients with a germline predisposition is critical to allow the selection of an appropriate allogeneic stem cell donor: either an unaffected HLA-matched sibling or an unrelated HLA-matched donor. Furthermore, the identification of genes that confer germline predisposition highlight critical cellular pathways that lead to MDS when disrupted and focus new research into areas previously unknown to contribute to MDS.
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Memorial Sloan-Kettering Cancer Center  
*Role of Cohesin Complex in Myelodysplastic Syndrome*  
11/1/2015-10/31/2018

Recent studies have identified loss-of-function mutations in the cohesin complex in a spectrum of malignancies, including most commonly in high risk MDS. Cohesin is a multi-protein complex which regulates sister chromatid alignment during mitosis; leading to the suggestion that cohesin mutations lead to chromosomal instability. However, in MDS, cohesin mutations are not associated with aneuploidy or complex cytogenetics, suggesting an alternate mechanism of transformation. To elucidate the pathophysiologic mechanism underlying cohesin mutations in MDS we have developed novel cohesin conditional mouse models. Our initial studies have shown that haploinsufficiency for Smc3 leads to increased stem cell self-renewal, and can cooperate with known leukemia disease alleles to induce myeloid transformation. Cohesin has been identified to have a role in regulating DNA loop formation, gene expression, and pluripotency. Young and colleagues discovered that a subset of transcriptional enhancers, which they denote as super-enhancers (SEs), is highly occupied by nuclear co-activator complexes including cohesin, mediator, and BRD4. This chromatin state is most common at genes that govern cell identity and differentiation status. In normal cells, cohesin maintains cell-type specific gene expression programs by facilitating chromatin looping of promoters and distal enhancers. Our preliminary data suggests that cohesin haploinsufficiency may promote aberrant chromatin architectures that promote tumorigenesis through the loss of innate nuclear topology, resulting in loss of cellular identity and in impaired hematopoietic differentiation. These data suggest that cohesin mutations reversibly alter the transcriptional landscape in MDS, which can be therapeutically targeted. Our proposal aims to use well characterized, genetically accurate models of MDS and patient samples to elucidate the role of cohesin alterations in MDS and to identify specific therapeutic targets in cohesin mutant MDS.
Eirini Papapetrou, M.D., Ph.D.
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A Novel Genotype-to-Phenotype Platform to Study MDS Pathogenesis and Treatment
11/1/2015-10/31/2018

With near-complete catalogues of the genetic alterations that drive MDS now available, the ushering of a new era of precision medicine in MDS care is within reach. Nearly all MDS patients have at least one genetic abnormality (mutation or chromosomal deletion) and genomics analyses are rapidly becoming routine. The next big challenge lies in the translation of this information into biomarkers to predict response to current disease-modifying therapies and into new therapies targeting specific genetic alterations. However, many of the recurrently mutated genes are not leading directly to obvious targets. A most promising avenue to uncover genotype-specific drug susceptibilities is the identification of common pathways and cellular processes in which different mutations converge. To this end we will develop MDS models based on patient-derived and genetically engineered induced pluripotent stem cells (iPSCs). This platform allows us for the first time to model each of a number of genetic abnormalities commonly found in MDS in a pure population of human cells in a controlled and defined isogenic setting and draw associations between genotypes and cellular phenotypes, transcriptome and methylome profiles of hematopoietic cells derived in vitro. We will then use these iPSC lines and this information to identify common drug susceptibilities and for in silico drug discovery approaches. This work will aid our ability to stratify MDS patients into genetically described subgroups towards more rational treatments. It can generate new hypotheses for follow up studies to discern the disease mechanisms and for clinical translation and potentially new therapeutic targets for drug development. This work is aligned with the mission of the Taub Foundation as it is high-impact, innovative translational research to understand the underlying causes of MDS and to advance its treatment and prevention.
2015 Awardee

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Curing MDS Using Hematopoietic Cells Reprogrammed From Autologous, Normal Endothelial Cells
2/1/2016-1/31/2019

Currently, MDS can only be cured by transplanting normal hematopoietic stem cells (HSCs). The problem is that transplanting HSCs from other people (allogeneic) is very toxic and not always possible. Re-transplanting a recipient’s own HSCs (autologous) just returns MDS. However, autologous transplants could treat and possibly cure patients with MDS if normal, autologous HSCs could be produced from these patients. The chance for cure would increase if the transplanted HSCs had specialized functions that allowed them to outcompete residual MDS cells. We’ve recently found that normal human endothelial cells (ECs) can be efficiently reprogrammed into hematopoietic stem and progenitor cells (HSPCs) that are potentially suitable for autologous transplantation. This is important because ECs and HSPCs reprogrammed from ECs (rEC-HSPCs) do not arbor the genetic/epigenetic lesion causing MDS. Our long-term goal is to use autologous, rEC-HSPCs to cure MDS. Our objective here is to provide an essential proof-of-concept demonstrating that rEC-HSPCs with customized functions can be manufactured and used for HSCT. The rationale for the proposed studies is that to get to MDS therapies we must define the optimal timing of EC reprogramming, identify a feasible tissue source for donor ECs and show that customized, transplantable rEC-HSPCs can be manufactured. Our objective will be met by completion of the following specific aims: Aim 1 - Define and optimize the kinetics with which engraftable human rEC-HSPCs emerge during cellular reprogramming of ECs; Aim 2 - Identify the extent to which the EC tissue of origin alters the reprogramming efficiency and long-term engraftment potential; Aim 3 - Design and manufacture human rEC-HSPCs with customized function that can reconstitute hematopoiesis in immune-deficient mice (NSG). The proposed studies provide critical steps towards translating this research into new, potentially superior, cellular therapies for MDS.
Janis Abkowitz, M.D.
University of Washington School of Medicine
The Pathogenesis of Anemia in MDS
12/1/2014-11/30/2017

Myelodysplastic syndrome (MDS) is characterized by ineffective hematopoiesis resulting in peripheral blood cytopenias. Anemia, and especially macrocytic anemia, is a prominent manifestation. At the time of diagnosis, over 80% patients with MDS are anemic, and 50% have a hemoglobin level < 10 g/dL. Although this results from the apoptosis of early erythroid precursors, its pathophysiology is not known. The goal of this application is to characterize and discern the reason for erythroid marrow failure in MDS. Specifically, we will test the hypothesis that ineffective erythropoiesis occurs when heme exceeds globin. Intracellular free heme is toxic, leading to increased reactive oxygen species (ROS) and cell death. An in vitro culture system has been optimized that phenocopies in vivo differentiation, including the uptake and use of iron and the signaling and onsets of heme and globin synthesis as erythroid cells mature. In initial studies of marrow cells from two patients with MDS resulting from the deletion of chromosome 5q (characterized by ribosomal protein S14 (RPS14) haploinsufficiency) and three patients with Diamond Blackfan anemia (an inherited anemia characterized by the haploinsufficiency of RPS19 and other ribosomal proteins), we have shown that heme is in excess in early erythroid (CD36neg/GlyAneg) cells and apoptosis occurs. As only those cells that epigenetically down-regulate heme synthesis (have less ALAS2 by RT PCR) or upregulate heme export (have increased FLVCR) survive, we established that heme toxicity induces the erythroid marrow failure. Here we propose to test the relevance of this hypothesis to other MDS subtypes using marrow cells from early and intermediate grade MDS patients with anemia. These studies should provide novel insights into heme-globin coordination in MDS. Also, our hypothesis, if confirmed, would suggest that slowing heme synthesis (e.g., by aggressive iron chelation) or facilitating heme export could help ameliorate anemia in early stage MDS.
Myelodysplastic syndrome (MDS) is an incurable hematopoietic malignant disease. Lenalidomide (LEN) has emerged as the only targeted therapeutic approved for the treatment of a small subpopulation of patients with chromosome 5q deletion (del5q). However, the majority of non-del(5q) patients do not benefit from LEN. We reported that LEN acts by inhibiting the activity of two haplodeficient phosphatases, PP2Ac and Cdc25C, encoded within the 5q region. They account for the disease’s karyotype selective specificity. Small interfering RNA suppression of these genes’ expression recapitulates del(5q) susceptibility to LEN with induction of apoptosis in non-del(5q) MDS cells. In combination of our novel finding that MDS progenitors overexpress TLR9, which bind and internalize its native ligand unmethylated CpG oligonucleotides, we developed an innovative disease-specific targeting approach using a single molecule containing: CpG, siRNAs to Cdc25C-PP2A and LEN, which renders non-del5q progenitors sensitive to clonal suppression by the drug. In the first aim, we will exploit the novel concept of applied synthetic lethality in non-del5q progenitors using this TLR9 targeted siRNA payload drug delivery. In the second aim, we will verify the results of Aim 1 by characterizing the in vivo activity of the proposed CpG-siRNA-LEN conjugates in an MDS mouse model. Alternatively, CpG-linked lytic peptide that can only induce cell death upon receptor mediated internalization will be tested both in vivo and in vitro, permitting the combination of CpG-mediated specificity direct towards the malignant MDS clone with the internal delivery of cytotoxic agent. This strategy aims to have higher therapeutic efficiency with lower side effects as compared to current standard of care. The strategies proposed are innovative with strong clinical application potential and with broad applicability to other malignancies. Accomplishment of the goals proposed here will have a direct clinical impact on the improvement of MDS patient care.
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The Contribution of Aging to the Pathogenesis of the Myelodysplastic Syndromes
11/1/2014-10/31/2017

Aging is associated with changes in the hematopoietic system including anemia, reduced cellular immunity, and increased innate immune system activation. Recent data indicate that many of these changes may be due to alterations in hematopoietic stem cells (HSCs), as aged HSCs exhibit a myeloid-lineage bias as well as decreased long-term reconstitution potential and self-renewal. Aging is also associated with increased risk of developing malignancies originating from HSCs including the myelodysplastic syndromes (MDS). While the presence of somatic mutations and karyotypic alterations in MDS indicate a significant cell-intrinsic contribution to MDS pathogenesis, it is likely that cell-extrinsic factors modify MDS biology. Indeed, the hematologic changes observed in MDS patients are similar to those seen in the normal elderly (e.g. anemia, decreased lymphoid output), and thus, we hypothesize that MDS HSC function is modified by cell-intrinsic and cell-extrinsic factors present in the normal aging background.

Given that MDS arises from HSCs and elimination of MDS HSCs is required for cure, it is important to identify all the factors that regulate MDS HSC function. Thus, we will investigate the contribution of the physiologic context in which MDS arises - aging - to the development and severity of MDS phenotypes. We will use a combination of our recently described MDS HSC xenograft model and our genetically faithful MDS mouse model, the Tet2/Asxl1 double knockout, in order to: (1) determine the role of aging on cell-intrinsic alterations in MDS HSC function, (2) determine the role of the aging niche on MDS HSCs, and (3) determine whether anti-aging interventions that reverse/inhibit normal HSC aging can improve MDS HSC phenotypes. We hope this approach will elucidate the age-related factors that contribute to MDS pathogenesis and will allow us to develop novel strategies to inhibit disease progression, improve hematologic parameters, or even prevent disease.
Mutations in genes for ribosomal proteins (RPs) or for processing factors are found in patients with Diamond Blackfan anemia (DBA), 5q- syndrome, a subtype of myelodysplastic syndrome (MDS), and Shwachman-Diamond syndrome (SBDS), among others. Major complications of these diseases include defective erythropoiesis, craniofacial abnormalities and the increased cancer risk. RP mutations cause activation of p53, through accumulation of free RPs that bind and sequester MDM2, a negative regulator of p53. A large percentage of mortalities in DBA are treatment-related. To find more effective therapies, our lab performed an in vivo chemical screen in a zebrafish model of DBA, and found that inhibitors of calmodulin (CaM), and of the CaM-dependent kinase CHK2, block p53 activity and rescue the erythroid defect. However, although many CaM inhibitors are already FDA-approved as antipsychotics, they cross the blood brain barrier (BBB) and their use is associated with dyskinesia, making them too risky for treating children. Our goal is to find CaM or CHK2 inhibitors that do not cross the BBB, and thus have the potential to be used in clinical trials for patients with DBA. We will leverage a novel chemical scaffold approach, developed by Dr. James Bradner, to generate 400 new CaM inhibitors, and will test these molecules for BBB permeability and hemoglobin rescue in zebrafish. Compounds will then be further characterized in a human and mammalian DBA model. The technology we propose to use - rapid generation and screening of compounds excluded from the BBB - may also be applicable to other diseases in which treatment toxicity related to BBB permeability is a problem. Overall, this work will identify potential compounds to test in clinical trials for DBA, MDS and for other RP and bone marrow failure disorders.