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A mechanistic pathogenetic model of myelodysplastic syndrome (MDS), separating MDS into 2 groups of disease

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Abstract

Myelodysplastic Syndrome (MDS) remains a set of enigmatic diseases difficult to diagnose and treat, with little progress in understanding disease biology and only empirical and imprecise classification. The current prevalent model defines MDS as a hyperproliferative state of marrow progenitor cells with excessive intramedullary programmed cell death (PCD) leading to failure of production of peripheral blood elements. The failure of this model to generate progress led to a review of the data on which the model is based. From that review, a new model of MDS pathogenesis is presented. First, MDS is separated into 2 sets of disease, one aggressive and usually fatal, often with progression to AML, the second more indolent, with infrequent to absent leukemic progression. The model for aggressive MDS incorporates unrepaired DNA damage and impaired PCD to generate clonal, hypercellular, but ineffective and hypoproliferative marrow, with inherent genetic instability (a mutator phenotype) leading to frequent progression to AML. In contrast, low grade MDS appears to be a set of possibly unrelated diseases that lack the mutator phenotype intrinsic to the biology of aggressive MDS. The proposal is concordant with available data, and provides a rational model for evaluating MDS biology and for development of new diagnostic methods and treatment approaches for these diseases.

Keywords: MDS, pathogenetic model, myelodysplastic syndrome

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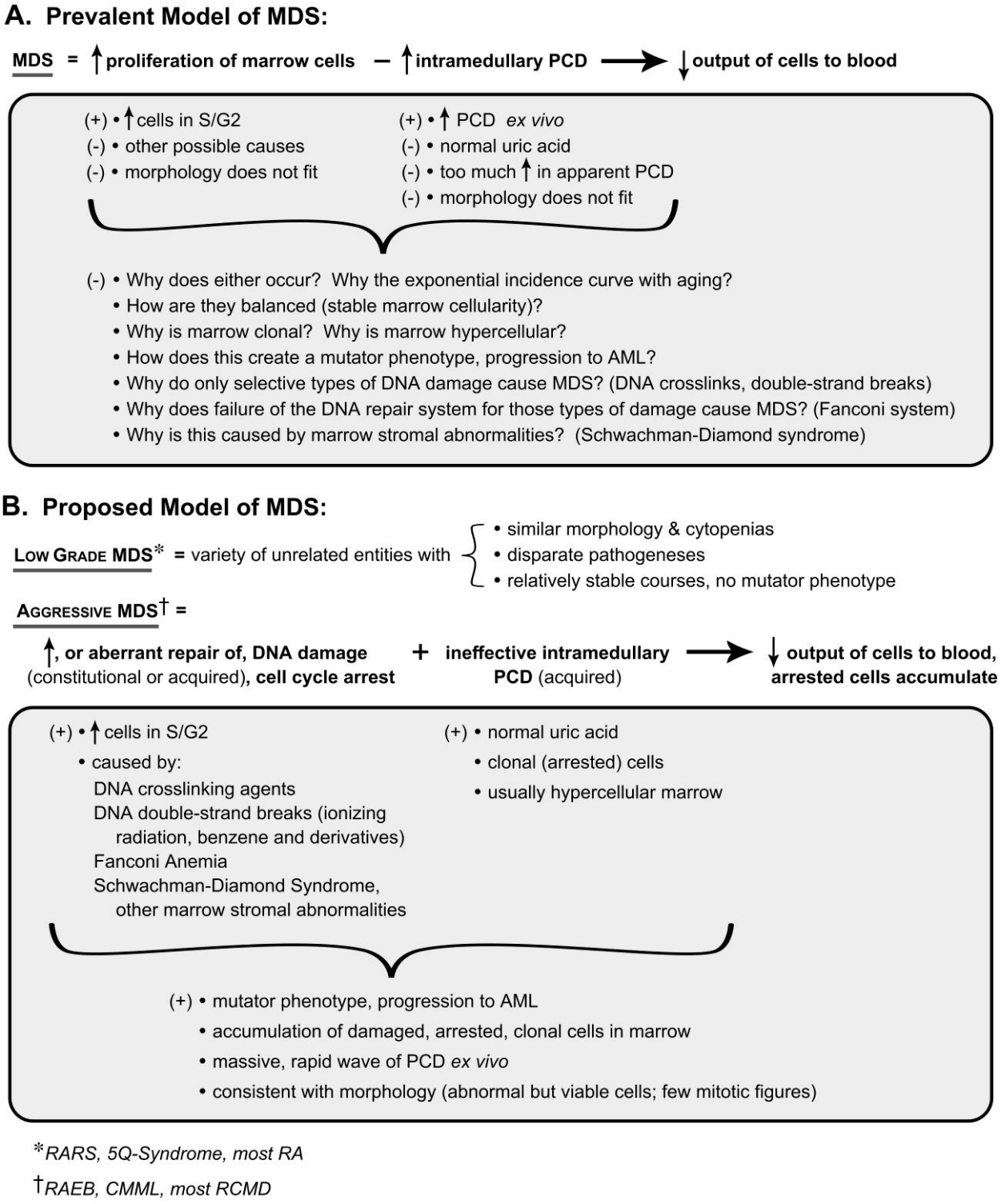
Introduction

Myelodysplastic syndrome (MDS) comprises a poorly understood set of marrow failure diseases characterized by clonal hematopoiesis, hypercellular marrow, dysplastic morphology of marrow precursors, acquired cytogenetic abnormalities in marrow precursors, and an exponential rise in incidence with progressive age.[1-3] Some subtypes of MDS (most refractory cytopenia with multilineage dysplasia, refractory anemia with excess blasts, and the related entity chronic myelomonocytic leukemia) follow an aggressive course with short survival (median 8-30 months) and frequent (20-40%) progression to a subset of acute myeloid leukemia (MDS-related or MDR-AML).[4] MDR-AML comprises about half of AML cases, is characteristic of AML in the elderly, and is refractory to most treatment. Other MDS subtypes (most refractory anemia, refractory anemia with ringed sideroblasts, 5q- syndrome) follow a relatively stable course with survival approaching that of age-matched peers and infrequent or absent progression to AML. These disparate clinical diseases have been grouped historically under the rubric MDS because of shared clinical, morphologic and cytogenetic features. However, these features appear to be secondary manifestations of disease caused by, rather than intrinsic to, the underlying pathogenetic processes of MDS; none of the features are always present and all may arise after onset of disease.[2, 3] The pathogenesis of MDS, as a group or of individual subtypes, remains unknown. The drastically differing clinical course of MDS subtypes suggests the possibility that despite their common features, subtypes may differ fundamentally in pathogenesis. The frequent progression of aggressive MDS to AML suggests aggressive MDS is a mutator phenotype disease with inherent genetic instability.[1-3] Conversely, the stability of low grade MDS subtypes, with infrequent to absent progression to AML, suggests low grade MDS lacks a mutator phenotype.[1-3]



Current Prevalent Model of MDS

MDS patients have increased marrow cells with S or G2 DNA content, in some reports exceeding 50% of marrow cells and interpreted as indicating active proliferation of marrow cells. [5-9] A variety of techniques also document increased PCD *ex vivo* in marrow cells in MDS, up to 50% of cells in some reports.[5, 6, 8, 10-13] These two sets of data have led to the current prevalent model of MDS: apparent marrow hyperproliferation offset by apparent increased PCD resulting in decreased production of blood cells and peripheral blood cytopenias. (See Fig. 1A.)

There are conceptual problems with this model. The model provides no causation for either hyperproliferation or excessive programmed cell death, no explanation for how the combination might

be balanced to provide a steady state of marrow cellularity, or why the marrow is typically hypercellular. It provides no explanation for clonality of hematopoietic precursors, and no explanation for why MDS behaves as a mutator phenotype, with frequent transformation to AML. The model does not fit the observed morphology of MDS. It provides no explanation for the consistent causal relationship of MDS with only very specific types of DNA damage (specific classes of cytotoxic drug, ionizing radiation, benzene exposure). It provides no explanation for consistent causation by defects of only one of the multiple DNA repair systems (the Fanconi system), or by marrow stromal abnormalities such as Schwachman-Diamond Syndrome. Of perhaps most importance, this model has led to little progress in diagnosis, classification, or treatment of MDS in one and one half decades. Given these shortcomings, a review of data on which the model is based, and exploration of other possible interpretations of the data, is warranted.

Cell Cycle Data and DNA Damage in MDS

The inference of hyperproliferation as a fundamental characteristic of MDS is based on the finding of increased cells in S and G2 of the cell cycle in MDS patients. This interpretation is inconsistent with the morphologic appearance of MDS marrow, which generally shows significant numbers of post-mitotic cells (metamyelocytes, bands/segs, orthochromatic normoblasts), little mitotic activity, and only modest numbers of cells with dispersed chromatin. While it is true that proliferation of cells results in an increase in S/G2 versus G0/G1 cells, this is only a surrogate marker for proliferation (actual mitotic division being definitive), and there is an alternative interpretation for the data. Some types of DNA damage (interstrand crosslinks, double strand breaks) cause cell cycle arrest at the S/G2 interface, with accumulation of cells in apparent S or G2 by DNA content. [14-19] The increased cells in apparent S or G2 mimic findings associated with proliferation, but indicate the opposite, a stalled cell cycle until DNA repair is effected. Agents that cause these types of DNA damage (DNA crosslinking drugs [alkylating agents, platinum derivatives, nitrosoureas], ionizing radiation) are recognized causes of aggressive subtypes of MDS. The drugs or their metabolites have 2 active alkyl groups, bind directly to DNA, and physically create interstrand crosslinks. Ionizing radiation creates double strand breaks through creation of reactive oxygen species. Another set of agents that cause MDS and MDR-AML, benzene and its derivatives, also cause oxidative damage of DNA with creation of double strand breaks.[20]

The Fanconi DNA repair system (FS) is integral to repair of specific types of DNA damage, namely DNA interstrand crosslinks and DNA double strand breaks. FS defects, in addition to causing aplastic anemia, also cause aggressive MDS and MDR-AML.[21-24] Occurrence in FA of clinical abnormalities involving bone (skeletal deformities) and marrow cells (aplastic anemia, MDS, MDR-AML) suggests ongoing exposure of marrow cells to DNA damage of a type that is repaired through the Fanconi system. Of interest, the Fanconi system is not found in invertebrates, having evolved in parallel with the endoskeleton, and hence also with the intramedullary marrow compartment, likewise suggesting exposure in the endoskeleton to these types of DNA damage.[24]

Schwachman Diamond Syndrome (SDS) is a constitutional abnormality characterized by marrow failure, pancreatic insufficiency, and frequent development of MDS and MDR-AML. The gene mutated in SDS, *Sbds*, encodes a requisite protein for osteoprogenitor cells to generate the marrow stromal stem cell niche.[25] In transgenic mice, mutations of *Sbds* disrupt hematopoiesis, with subsequent evolution of MDS and MDR-AML, even when the deletion is restricted to marrow stromal (osteoprogenitor) cells.[26] Loss of function of another gene, *Dicer1*, results in a similar pattern of findings mediated through decreased expression of *Sbds*, even when *Dicer1* loss of function is restricted to osteoprogenitor cells.[26] How an abnormal stromal cell generates MDS and MDR-AML is speculative, but it is of interest that the normal marrow stem cell niche is characterized by very low oxygen tension.[27-29] As noted, oxidative damage of DNA causes MDS and MDR-AML. A plausible pathogenetic model for MDS evolution in SDS is that loss of function of Sbds disrupts the stem cell niche, exposing hematopoietic progenitors to higher oxygen tensions, with oxidative damage of DNA similar to that caused by radiation and benzene derivatives, with subsequent evolution of marrow failure, MDS, and MDR-AML.

To summarize, multiple lines of evidence link specific types of DNA damage to the pathogenesis of aggressive subtypes of MDS. These kinds of DNA damage cause cell cycle arrest with increased cells with S/G2 DNA content. This provides an alternative explanation for this finding in MDS, also providing a potential explanation for the mutator phenotype of aggressive MDS. If this analysis is correct, MDS marrow is hypo- (not hyper-) proliferative. The data also suggest that the Fanconi system evolved to protect hematopoietic progenitors by repair of

oxidative and crosslink DNA damage, that the hypoxic stem cell niche evolved for a similar purpose, and that failure of the niche hypoxic state, or failure or overload of the repair system, lead to marrow failure, MDS and MDR-AML.

Programmed Cell Death in MDS

A variety of techniques document increased *ex vivo* PCD (50% or more in some reports) in marrow cells in MDS, with early appearance of a DNA digestion ladder (at 4 hours) after aspiration. [5, 6, 8, 10, 11] Analytical methods include in situ end labeling/tunel assays, annexin 5 analysis, demonstration of caspase activation, and appearance of a DNA digestion ladder after marrow aspiration. These results have been validated repeatedly. Of note, the assays are all *ex vivo* assays, but results have been extrapolated to indicate increased *in vivo* (intramedullary) PCD in MDS.

A simple observation gives pause to this interpretation. Uric acid is an obligate byproduct of *in vivo* cell degradation. Hypercellular marrow (the MDS norm) constitutes a large organ. If excessive PCD is occurring in this large organ (and the model additionally posits active proliferation of cells in this organ), increased production of uric acid is inescapable. Yet, to the contrary, serum uric acid levels are not increased in MDS patients.[2, 3] Also of note, PCD has a morphologic equivalent, pyknotic and fragmented nuclei in degenerating cells, with macrophages degrading cellular debris; these microscopic features are lacking in MDS marrow.

Of additional interest is the appearance of a ladder of digested DNA fragments at 4 hours after marrow aspiration in MDS. As the name implies, PCD is a program. The time for completion of the PCD program, signaled in part by appearance of a DNA digestion ladder, is approximately 4 hours.[5] Thus, rather than substantiating excessive *in vivo* PCD in MDS, the appearance of a DNA digestion ladder at 4 hours after marrow aspiration suggests the PCD is an *ex vivo* event initiated by marrow aspiration. While marrow cells must be abnormal to be triggered rapidly to PCD by aspiration, such data have no bearing on *in vivo* levels of PCD.

Finally, to maintain stable marrow cellularity, cell production must equal cell loss (through egress of cells to blood or intramedullary PCD). While PCD takes approximately 4 hours, the cell cycle takes 24 hours, a ratio of 1:6. Since dying cells cannot proliferate, the maximum PCD rate allowable for steady state marrow cellularity is 14.3% (1/7) (see appendix), and this only if every non-dying cell is proliferating, no cells are exiting to blood, and all marrow cells are hematopoietic cells (i.e., no lymphocytes, plasma cells, etc.). Any reduction in proliferating cells, egress of cells to blood, or lymphocytes/plasma cells in marrow will reduce this 14.3% rate accordingly. Thus, it is mathematically impossible that reported high PCD rates in marrow in MDS actually indicate *in vivo* PCD. There must be another explanation for the PCD data, and if so there must also be another explanation than proliferation for cell cycle data in MDS.

Separation of Low Grade MDS from Aggressive MDS

As reviewed, available data suggest that MDS is divisible into 2 groups, one characterized by progressive, usually fatal disease in the absence of successful intervention, often complicated by progression to AML, the other a more indolent set of diseases requiring supportive care and lacking such risk of leukemic progression.[1-3] (Fig. 1b) Because the two sets of disease differ fundamentally in clinical behavior, reflecting apparent differences in underlying pathogenesis, i.e. presence or absence of a mutator phenotype, we propose separation of MDS into these two groups (low grade MDS, aggressive MDS) for both clinical purposes and for analyzing MDS biology. At present we have no definitive testing to separate these 2 groups, and must rely on the WHO MDS classification and the International Prognostic Scoring System (IPSS) to classify patients.[30, 31] A better understanding of MDS pathogenesis may lead to improved diagnostic testing for this purpose.

Pathogenetic Model of Aggressive MDS

The preceding review suggests a different model for aggressive MDS. (Fig. 1b) The model requires disruption of 2 intracellular pathways: failure or overload of a repair system for DNA damage to which marrow cells are exposed (such as DNA crosslink or double strand break damage; there may be other possibilities), and failure of a PCD pathway normally activated by that damage. Progeny of a stem cell with this set of defects acquiring appropriate DNA damage would arrest the cell cycle, but being unable to repair or die would accumulate. To produce observed cell cycle data in MDS, the arrest should be at the S/G2 interface. Accumulating arrested cells would be clonal, if any of the causative defects were genetically acquired, and would generate hypercellular but ineffective marrow.

Persistence of unrepaired DNA damage would create a mutator phenotype. Progressive incorporated genetic damage would generate the clinical and laboratory features of MDS, with cumulative damage leading to transformation to MDR-AML in some patients. Conceptually, for random MDS cases this model would require loss of function of 2 pairs of genes consistent with the exponential incidence curve of random MDS. In Fanconi Anemia, with a constitutional impairment of DNA repair and a resultant mutator phenotype, evolution of MDS would require only acquisition of a block in the PCD path initiated by that DNA damage, consistent with the high incidence of MDS in FA at an early age. Schwachman Diamond Syndrome patients, with potential increased production of DNA damage due to a disrupted stem cell niche, could also require only acquisition of a block in PCD for progression to MDS.

Implications of Model for Diagnostic Testing: Ratios of post-G1 cell cycle compartments (S, G2, M) are stable in normal cells, can be quantified (including M, using phosphorylation-sensitive antibodies)), and may be disrupted by cell cycle arrest due to unrepaired DNA damage.[32-34] This pathogenetic model suggests that such detailed analysis of the cell cycle, with demonstration of an aberrant increase of S/G2 cells in the absence of increased mitoses, could provide definitive testing for diagnosis and monitoring of aggressive MDS, and for separation of aggressive and low grade MDS. Similarly, analysis for increased levels of DNA damage in marrow cells could also provide definitive testing for these purposes. A recent report suggests such testing is feasible, and results support the proposed pathogenetic model.[33]

Animal Model of MDS: There is no animal model to facilitate studies of the biology and treatment of MDS. The proposed pathogenetic model suggests an animal model of MDS could be created by knockout of the Fanconi DNA repair system in marrow cells with simultaneous knockout of an associated PCD pathway (e.g., by crossing a FA knockout mouse with a PCD deficient mouse), thus artificially creating the pathogenetic model in a transgenic mouse.

Implications of Model for Treatment of MDS: Finally, this pathogenetic model suggests a novel treatment strategy for aggressive MDS that should be otherwise largely innocuous to patients. The repair system for both crosslink DNA damage and DNA double strand breaks includes BRCA1 and BRCA2.[23, 24] Both genes are also implicated in the pathogenesis of breast and other cancers.[35, 36] Breast cancer with BRCA1 or BRCA2 mutation is sensitive to inhibition of PARP1, a protein involved in repair of DNA single strand breaks (SSB).[37-39] The rationale for this treatment is that human cells spontaneously develop large numbers of SSBs daily. Inhibition of PARP1 blocks repair of these SSBs, forcing their conversion to DSBs, with repair by the DSB repair system.[40] In normal cells this conversion and repair is transparent, but in cells with deficient repair of DSBs, for example breast cancer with BRCA1 or BRCA2 deficiency, DSB repair fails, leading to death of impaired cells.[40] As suggested by this model, aggressive MDS subtypes and MDR-AML may also have defective repair of DSBs, and indeed the Fanconi gene *FACCD1,* implicated in the pathogenesis of MDS, is the same gene as *BRCA2*.[41] The proposed model suggests that ineffective repair of DSBs and interstrand crosslinks is intrinsic and essential to the pathogenesis of aggressive MDS. Thus, if the model is correct, treatment with a PARP1 inhibitor could selectively eliminate the MDS clone without affecting normal hematopoiesis, a remarkable possibility in a disease with so few current treatment options. In support of this concept, Sapacitabine, a cytosine analog, has shown unexpected (and unexplained) effectiveness in treatment of MDS and MDR-AML.[42] Sapacitabine has a novel mode of action; after incorporation into DNA, it creates unrepairable DNA SSBs, which are converted to DSBs and result in cell death. The effectiveness of Sapacitabine in these diseases is consistent with the proposed model of these diseases with defective response to DNA DSBs, and supports possible use of PARP1 inhibition for treatment of these diseases. Indeed, PARP1 inhibition may be a safer approach for treatment than Sapacitabine, as the effectiveness of PARP1 inhibition is dependent on random occurrence of DNA damage, rather than incorporation of a base analog into DNA.

Low Grade MDS

Low grade MDS by contrast is almost certainly a disparate set of diseases. Although excessive DNA damage or defective repair of damage is central to the model of aggressive MDS, low grade MDS subtypes lack evidence of such an abnormality. While low grade cases share cytogenetic abnormalities, cytopenias, and dysplastic morphology with aggressive MDS, low grade cases tend to be stable diseases. Data suggest some cases (refractory anemia with ringed sideroblasts) may be a mitochondrial cytopathy, rather than attributable to nuclear DNA damage, and may be more similar to Pearson syndrome than to aggressive MDS.[43-45] A hypothetical explanation for other low grade subtypes is that their genetic abnormalities are acquired through random error, rather than mediated by a

mutator phenotype, and while the genetic abnormalities provide a survival advantage to a clone and cause clinical and laboratory findings similar to aggressive MDS, the clone lacks a mutator phenotype and is stable. In this model, clinical similarities between low grade and aggressive MDS could be attributable to similar acquired genetic abnormalities, differences to presence or absence of a mutator phenotype with its associated progressive genetic damage.

Conclusion

A revised model of MDS is proposed, dividing MDS into 2 groups of disease (low grade and aggressive) based on clinical behavior and absence or presence of the potential for leukemic progression. The model for aggressive MDS requires dysfunction or overload of a DNA damage repair system and dysfunction of the PCD pathway initiated by that damage. This combination results in accumulation of damaged marrow cells that can neither repair nor die, and creates a mutator phenotype. Accumulating genetic damage with acquisition of transforming genetic damage, resultant from the mutator phenotype, causes progression to AML in some cases. Low grade MDS, despite clinical and laboratory similarities to aggressive MDS, lacks a mutator phenotype, is potentially stable disease and may be unrelated in pathogenesis to aggressive MDS.

This model is testable. A recent report shows that flow cytometric assessment of myeloid nuclear differentiation antigen (MNDA) in myeloid cells facilitates diagnosis of MDS, and may distinguish low grade from aggressive MDS.[46] The cell cycle can be characterized more accurately than with DNA content alone, including the ability to assess mitotic events, the ultimate measure of proliferation, using antibodies sensitive to phosphorylation events during mitosis.[33, 34] Ratios of post-G1 cell cycle compartments (S, G2, M) are stable in normal cells, and may be disrupted by cell cycle arrest.[32, 33] DNA damage activates cell cycle regulatory and DNA repair systems, identifiable through phosphorylation of component proteins which can be assessed with phosphorylation-sensitive antibodies.[32, 33, 47-49] A recent report shows that such detailed cell cycle and DNA damage analyses are feasible, and results support the proposed model of MDS pathogenesis.[33] The proposed MDS model suggests a much-needed animal model of aggressive MDS could be created by dual knockout of the Fanconi system and an associated PCD path. Such investigations will lead to better understanding of MDS pathogenesis, may provide better tools for diagnosis and classification of MDS, and may lead to novel approaches to treatment of this difficult set of diseases, both MDS and MDR-AML. The model should be tested.

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Conflict of Interest

None.

Appendix: Maximal rate of PCD for stable marrow cellularity

p = proliferating cells
d = dying cells
r = proliferation rate; proliferation takes 24 hours
s = PCD rate; PCD takes 4 hours; therefore, s = 6r(24/4 = 6)

With these assumptions: stable marrow cellularity, no cells exit to blood, dying cells can't proliferate, all non-dying cells proliferate (p + d = 100%).

In any unit of time, for stable cellularity dying cells (ds) must equal cell production (pr):

pr = ds = d6r
p = 6d
Stating d as a %:
% d = d/(p+d) x 100 = d/(6d + d) x 100 = 1/7 x 100 = 14.3%,

Thus, with stable marrow cellularity the maximal % of PCD in marrow cells at any time is 14.3%. If any cells are exiting to peripheral blood, the maximal value of "d" is reduced further. Likewise, any reduction in the % of proliferating cells below 100% will further reduce "d", as will presence of any extraneous cells such as plasma cells or lymphocytes. Thus, even the maximal rate of PCD for stable cellularity (14.3%) is unlikely to be achieved in reality.

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