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Oxidative Stress and the Myelodysplastic Syndromes

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Abstract

The evolution of higher organisms from anaerobic to aerobic living has promoted an elaborate mechanism of defense against potentially toxic oxidants. Many environmental toxicants implicated in the pathogenesis of myelodysplastic syndromes (MDS), including benzene and ionizing radiation, exert toxicity via pro-oxidant mechanisms. The emerging data suggest a probable genetic susceptibility to environmental carcinogenesis through functional polymorphic variants in enzymes that metabolize toxicants and/or protect against oxidative stress. The most studied enzyme is NAD(P)H:quinone oxidoreductase (NQO1). $CD34^+$ cells from individuals homozygous for the *NQO1 C609T* nonfunctional allelic variant are incapable of enzyme induction following exposure to benzene, thus potentially increasing the hematotoxicity of benzene metabolites. Serologic and molecular markers of oxidative stress are present in many patients with MDS and include an increased concentration of the lipid peroxidation product malondialdehyde and the presence of oxidized bases in $CD34^+$ cells. Potential mechanisms of oxidative stress include mitochondrial dysfunction via iron overload and mitochondrial DNA mutation, systemic inflammation, and bone marrow stromal defects. The biological activity of the antioxidant aminothiol amifostine in vivo suggests that these pathways may be meaningful targets for future therapy in MDS patients. *Int J Hematol.* 2003;77:342-350.

Key words: Myelodysplastic syndrome; Oxidative stress; Apoptosis; Cytokine

1. Introduction

1.1. Myelodysplastic Syndromes

The myelodysplastic syndromes (MDS) are characterized by ineffective hematopoiesis and constitute a heterogeneous group of progressive, irreversible multipotent stem cell disorders characterized by cytopenias, abnormalities of erythroid, granulocytic, and megakaryocytic maturation associated with an increased propensity to transform into acute myeloid leukemia (AML). These disorders are typically diseases of the elderly with a median age at diagnosis of greater than 65 years [1]. The French-American-British (FAB) classification of MDS based on bone marrow and peripheral blood morphology [2] has recently been replaced by the World Health Organization (WHO) classification, which now incorporates morphologic, cytogenetic, and immunologic features to establish the lineage and degree of maturation of the neoplastic cells [3]. The different morphologic features and lengths of survival times among patients reflect the heterogeneity of MDS, even within FAB/WHO subgroups. Low-risk MDS is characterized by refractory cytopenias and low leukemic burden, coupled with a marked increase in the apoptotic index and proliferative fraction. The erythroid cell lineage is usually most prominently affected, and blast cells account for less than 10% of the total bone marrow nucleated cell count. In contrast, high-risk MDS is accompanied by a decrease in the apoptotic index with a corresponding elevation in blast percentage. High-risk MDS has an increased frequency of cytogenetic abnormalities, with the most common karyotypic lesions involving chromosomes 8 (gain/trisomy), 5 (loss/deletion), and 7 (loss/deletion) [4]. The heterogeneity of clinical outcomes within individual FAB subtypes has led to the development of prognostic scoring systems incorporating biological variables such as karyotype [1].

1.2. Oxidative Stress: Mechanisms and Defenses

The evolutionary transition from anaerobic to aerobic life has led to a requirement that higher organisms develop an elaborate set of defense mechanisms to prevent intracellular damage by highly toxic molecular oxygen. Humans are regularly exposed to exogenous oxidants, occupational and environmental toxicants, as well as the endogenous byproducts of many intracellular metabolic processes. Despite antioxidant defense mechanisms, cell damage from oxygen free radicals

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(OFR) is ubiquitous, and the consequences of excess OFR production depend on the intrinsic antioxidant defense and DNA repair capacities of the cell. Oxidative stress may therefore result from an increased exposure to oxidants or from a reduced level of protection against oxidants, with the possibility of both of these stress factors occurring simultaneously. Oxidative stress may damage DNA and/or proteins and result in cell death or altered cell function, including carcinogenesis.

Free radicals are molecules with 1 or more unpaired electrons. Electron acceptors such as molecular oxygen (O_2) react readily with free radicals to become radicals themselves. Although oxygen is not the only oxidizing agent that cells or organisms are exposed to, it is the most widespread. Molecular oxygen contains 2 unpaired electrons. The first single-electron reduction of molecular oxygen (the univalent pathway of O_2 reduction) produces the superoxide radical ($^{\cdot}O_2^-$), which has low reactivity and toxicity but may function as an important second messenger in the cell [5]. $^{\cdot}O_2^-$ is then reduced to hydrogen peroxide (H_2O_2), either spontaneously or via superoxide dismutase catalysis. Hydrogen peroxide is highly reactive and reacts with partially reduced metal ions such as Fe^{3+} and Cu^{2+} (with water elimination) to produce the extremely powerful oxidant, the hydroxyl radical ($OH^{\cdot}$), via the Fenton reaction. Because copper plays a role in the attachment of DNA to the nuclear matrix [6], it is likely that $OH^{\cdot}$ is formed in close proximity to the DNA target. This univalent pathway ends with the production of water following a fourth electron reduction. Ground state O_2 and $^{\cdot}O_2^-$ are mild oxidizing agents, with H_2O_2 and $OH^{\cdot}$ being the stronger oxidants [7]. $OH^{\cdot}$ is considered the most important radical in OFR-related cell damage, with other intermediates playing a role in reactions that are attributed to $OH^{\cdot}$ [8]. Peroxidation of membrane lipids to organic peroxyl radicals initiates a chain reaction that may explain various membrane-mediated effects of OFR [9]. A role for nitric oxide (NO) in cellular redox reactions is acknowledged, with NO reacting with $^{\cdot}O_2^-$ to form the reactive peroxynitrite anion ($ONOO^-$) [9], although, in contrast, NO has also been reported to act as a chain-breaking antioxidant in lipid peroxidation [10].

Oxidative DNA damage generates DNA base modifications and DNA helix alterations. In the case of the latter, single-strand and double-strand DNA breaks may result from OFR-related stimulation of enzymatic DNA cleavage. $OH^{\cdot}$ reacts with the deoxyribose backbone on purine and pyrimidine bases to generate abasic sites, which are mutagenic in vivo. Some DNA base modifications can also induce point mutations following base misreading at replication [9]. The most common base modification is 8-oxo-2′-deoxyguanosine (8-OHdG), which can produce AC to TA transversions. Such transversions have been observed in the TP53 tumor suppressor gene [11] and the RAS oncogene [12].

The initial cellular response to oxidative stress is to enlist antioxidant defense and repair systems to minimize the damage accrued or to repair the damaged components. This repair is achieved through antioxidant enzymes, nonenzymatic antioxidants, and repair enzymes. The antioxidant enzymes make up the backbone of the cellular defense system, and include manganese superoxide dismutase (Sod2), catalase, and glutathione peroxidase (GPX), which act catalytically to convert harmful oxidants to less reactive molecules (Figure 1). Sod2 catalyzes the dismutation of $^{\cdot}O_2^-$ to H_2O_2, which is detoxified by catalase to yield water and O_2. GPX reduces H_2O_2 with reduced glutathione (GSH) as the electron donor. The lineage specificities of these individual enzymes reflect varying defense requirements. Examples include a limited role for GPX1 in red cells [13] and the lack of a hematopoietic phenotype in the human disease acatalasemia (congenital absence of functional catalase). Although the detailed functional analysis of antioxidant enzymes in hematopoietic cells is awaited, preliminary data from our laboratory indicate marked differences in the expression of antioxidant enzymes among lineages and maturity stages [14]. Defense against extracellular and intracellular OFR is also provided by nonenzymatic antioxidants such as lipophilic (eg, bilirubin) and hydrophilic (eg, GSH) molecules or protein components, which act as free radical scavengers. GSH with its reactive sulfhydryl group has 3 antioxidant mechanisms of action: (1) as scavenger of free radicals such as $^{\cdot}O_2^-$, $OH^{\cdot}$, and lipid hydroperoxides; (2) as a substrate for glutathione peroxidase; and (3) in the direct repair of oxidative DNA lesions [9]. Oxidatively modified DNA is subject to removal or excision repair by a variety of DNA repair enzymes, which include endonucleases, glycosylases, polymerases, and ligases [7]. If DNA base modifications induced by OFR are not removed, they may lead to mutagenesis.

1.3. Physiological Aging, Oxidative Stress, and Hematopoietic Cells

The basis of the oxidative stress hypothesis of aging is that age-related loss of physiological function results from the progressive and irreversible accumulation of oxidative damage [15]. Various studies in the last decade have shown a strong correlation between increasing age and the accumulation of oxidative damage. Age-related increases in the levels of the oxidized base 8-OHdG, a known oxidative DNA damage product, have been reported in various cells and tissues [16,17]. In mice, the observed age-related increase in 8-OHdG was not a consequence of the inability to remove 8-OHdG but rather arose from an increased sensitivity of the tissues to oxidative stress [17]. An age-associated decline in DNA repair mechanisms was shown following treatment with ultraviolet radiation or H_2O_2 [18]. A complete return of 8-OHdG to pretreatment levels was seen in young but not old fibroblasts [16].

Oxidative damage and repair has not been systematically studied in hematopoietic cells in the context of aging or resistance to pro-oxidant compounds. In vitro resistance of $CD34^+$ cells to alkylating agents can be conferred, however, by transfection of the glutathione *S*-transferase P1 gene, which may mediate protection by conjugating glutathione to toxic peroxide products of alkylating agent drugs [19].

2. Initiation of MDS

The etiologic insults leading to the development of MDS and the latency period between the initial genomic insult and first clinical manifestation of the disease are largely unknown. Men are reported to have a slightly increased risk

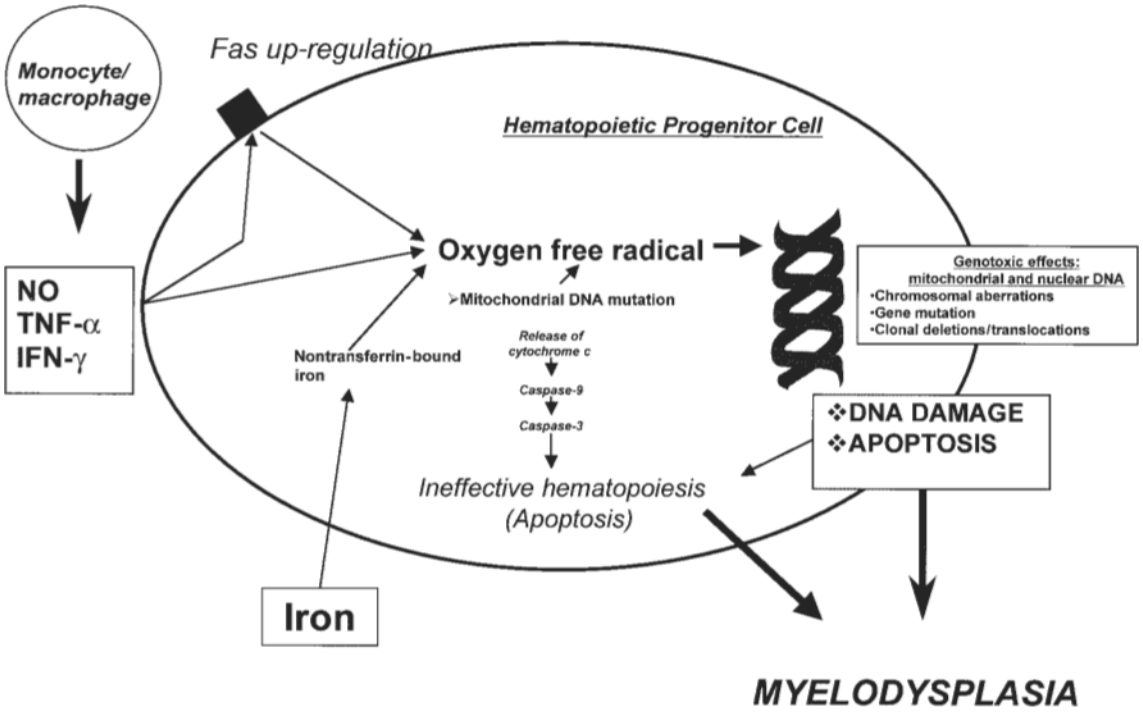

Figure 1. The mechanisms of oxygen free radical generation, the consequences of oxidative damage, and the cellular antioxidant defense systems. NO indicates nitric oxide; $ONOO^-$, peroxynitrite anion; H_2O, water; O_2, molecular oxygen; H_2O_2, hydrogen peroxide; GSH, reduced glutathione; GSSG, oxidized glutathione; $OH^·$, hydroxyl radical; Fe^{3+}, ferric iron, Cu^{2+}, cupric copper.

of MDS compared with women, with studies yielding a male-female ratio of 2:1 in the incidence of the disease [20]. However, the 5q– syndrome is found to be more frequent in women than in men [21]. It is assumed that the male predominance of MDS reflects a greater exposure of males to environmental toxins in the workplace, but this is clearly not the case for selected subgroups such as the 5q– syndrome. The vast majority (>80%) of MDS cases occur de novo without a history of definitive exposure to known mutagens. Secondary MDS follows exposure to chemotherapeutic drugs or radiotherapy. A higher frequency of clonal chromosomal abnormalities is found in secondary MDS, with a greater tendency to early leukemic transformation [22]. More than 80% of patients presenting with MDS are older than 60 years. Throughout their lives these patients may gradually accumulate genomic damage in hematopoietic cells through exposure to endogenous and exogenous carcinogens, and this damage may be reflected in the complex genomic abnormalities seen in many MDS patients. Although most MDS patients do not present with complex genomic damage, it remains likely that the etiology of MDS is linked to the processes associated with the aging of bone marrow stem cells.

Those etiologic agents most tightly linked to MDS are alkylating agent chemotherapeutic drugs, ionizing radiation, and benzene. Other weak associations have been noted for exposure to dark hair dye [23], tobacco smoke (including current and ex-smokers) [24], alcohol [25], pesticides [26], organic solvents [27], and a variety of occupations, including agricultural and health care workers [26,27]. Several studies have reported a higher frequency of exposure to environmental toxicants for MDS patients with karyotypic abnormalities [28,29]. The most detailed and largest of these studies demonstrated a higher frequency of exposure to specific compounds, including copper, arsenic, asbestos, and silica, in the karyotypically abnormal group. Furthermore, an association was found between specific karyotypic abnormalities and exposure to selected toxicants, such as inorganic dusts (eg, asbestos) with chromosome 7 abnormalities and radiation with chromosome 8 abnormalities [28].

If these weak associations represent a genuine biological causative role and not simply statistical chance, then plausible hypotheses are required to test these hypotheses. Oxidative stress is one possible mechanism of cellular damage for many but not all of these toxicants. The targets of this putative oxidative stress are yet to be well defined (inflammatory cells including macrophages, bone marrow stroma, or direct hematopoietic stem cell toxicity), and the routes by which environmental toxicants might stress bone marrow hematopoietic stem cells also remain unclear.

2.1. Benzene

Epidemiologic data support a role for benzene in the etiology of MDS and AML [30]. The carcinogenic properties of benzene are mediated via genotoxic benzene metabolites that form DNA adducts and also generate OFR [31]. Benzene hematotoxicity is likely to depend on the balance of

peroxidases, which is essential for the generation of toxic reactive intermediates of benzene metabolism, and detoxification enzymes such as NAD(P)H:quinone oxidoreductase (NQO1) within hematopoietic stem cells [32] and/or marrow stromal cells [33]. Overexpression of the antioxidant thiol, thioredoxin, attenuates benzene-induced leukemia in a murine model [34] and also protects against hematotoxicity from UV-C and dioxin [35].

2.2. Radiation

The epidemiologic data supporting a role for ionizing radiation exposure in the etiology of MDS and AML are also strong [36]. An early increase in the incidence of high-risk MDS and AML cases followed the atomic bomb radiation exposures [37]. An increased incidence of low-risk MDS is now also emerging with a long latency period (>30 years) [36]. It is provocative to speculate that this long latency may be linked to the persistent inflammation in survivors of these radiation exposures [38], raising the possibility that chronic inflammation may contribute to hematotoxicity and the development of low-risk MDS in susceptible individuals. Transmissible genomic instability (chromosome aberrations/base oxidation) has been documented in vitro and in vivo following exposure to ionizing radiation, and this instability is mediated at least in part by oxidative stress [39,40]. Transmissible genomic damage through generations is potentially an important mechanism for both the initiation and progression of MDS.

2.3. Other Environmental Toxicants

Other environmental toxicants that are implicated by epidemiologic data in the etiology of MDS and have mechanisms of cytotoxicity and/or mutagenesis that may be mediated directly through oxidative stress include H_2O_2 (hair dyes) [23], arsenic, asbestos, and copper (via the Fenton reaction) [28].

2.4. Genetic Susceptibility to Oxidative Stress?

Functional polymorphic variants have now been identified in the genes encoding many enzymes, including those responsible for the metabolism of environmental carcinogens and antioxidant enzymes. Several of these enzymatic variants have now been implicated in the etiology of both de novo and therapy-related AML, and these enzymes include those that also function within antioxidant defense pathways, such as NQO1 [41] and glutathione *S*-transferase P1 [42]. An overrepresentation of the *NQO1 C609T* nonfunctional polymorphic variant (plus high-activity of cytochrome P450 2E1) was noted in Chinese workers exposed to high concentrations of benzene and who subsequently developed bone marrow suppression [43]. It is unknown how many of these patients subsequently developed MDS or AML. Benzene metabolites are unable to induce NQO1 expression in subjects homozygous for the *C609T* nonfunctional allelic variant, and this fact may provide an explanation for hematopoietic cell susceptibility to benzene poisoning [44].

Other genes encoding antioxidant enzymes with polymorphic variants include *GPX1*, *Sod2*, and *HO-1* (encoding the enzyme heme oxygenase–1), and each of these is potentially associated with an increased susceptibility to oxidative stress. Functional polymorphisms also exist in genes encoding enzymes that repair oxidative DNA damage, and preliminary data suggest that these enzymes may also be relevant in protecting against the development of subtypes of AML, although numbers in the study were small [45]. No large systematic case-control studies of polymorphic frequencies in MDS patients for any of these allelic variants have been published to date.

3. Maintenance and Progression of Established MDS

Once established, MDS may progress via worsening cytopenias because of less effective hematopoiesis or via transformation to acute myeloid leukemia. The relationship between ineffective hematopoiesis and leukemic transformation has yet to be established, but a clear difference in apoptotic rate distinguishes the two processes [46]. It is, however, unclear if a leukemic stem cell is already present at MDS diagnosis and develops a progressive growth advantage or if a clonogenic leukemic cell transforms from an apoptosis-prone ineffective clone. There is substantial circumstantial evidence for the role of oxidative stress in the pathogenesis of MDS (Figure 2). For example, an increased serum concentration of free malondialdehyde is indicative of excess lipid peroxidation [47]. Whether oxidative stress is a fundamental causative process or merely a consequence of other pathogenetic mechanisms remains to be elucidated.

Bone marrow and blood $CD34^+$ cells from many MDS patients harbor oxidized pyrimidine bases, and these bases are not found in more mature cells from MDS bone marrow or in healthy individuals. Base oxidation was associated in this study with an elevated serum concentration of tumor necrosis factor α (TNF-α) and a low intracellular GSH concentration [48]. These findings suggest that the progenitor compartment in MDS patients is subject to oxidative stress and that perhaps only clones capable of surviving and resisting that stress will give rise to mature progeny. Growth of bone marrow progenitors from MDS patients can be stimulated by in vitro incubation with antioxidant thiols, including N-acetylcysteine [49] and amifostine [50]. N-acetylcysteine is a glutathione precursor and increases the intracellular concentration of GSH. N-acetylcysteine stimulated growth of colony-forming units–granulocyte-macrophage in patients with both low-risk and high-risk MDS, reduced apoptosis in short-term culture, and reduced secretion of TNF-α into the short-term culture supernatant [49]. Amifostine is a phosphorylated aminothiol that protects primitive hematopoietic progenitors in vivo from chemotherapy-induced toxicity [51] and stimulates hematopoiesis by promoting the formation of hematopoietic progenitors [52]. Preincubation with amifostine augments growth of primitive progenitors from MDS bone marrow in a dose-dependent manner [50], with multipotent progenitors and erythroid bursts being more potently stimulated than myeloid progenitors. No clonality assays were available from these studies, and it thus remains uncertain if the augmented growth in vitro was within the MDS clone or occurred in residual normal progenitor cells.

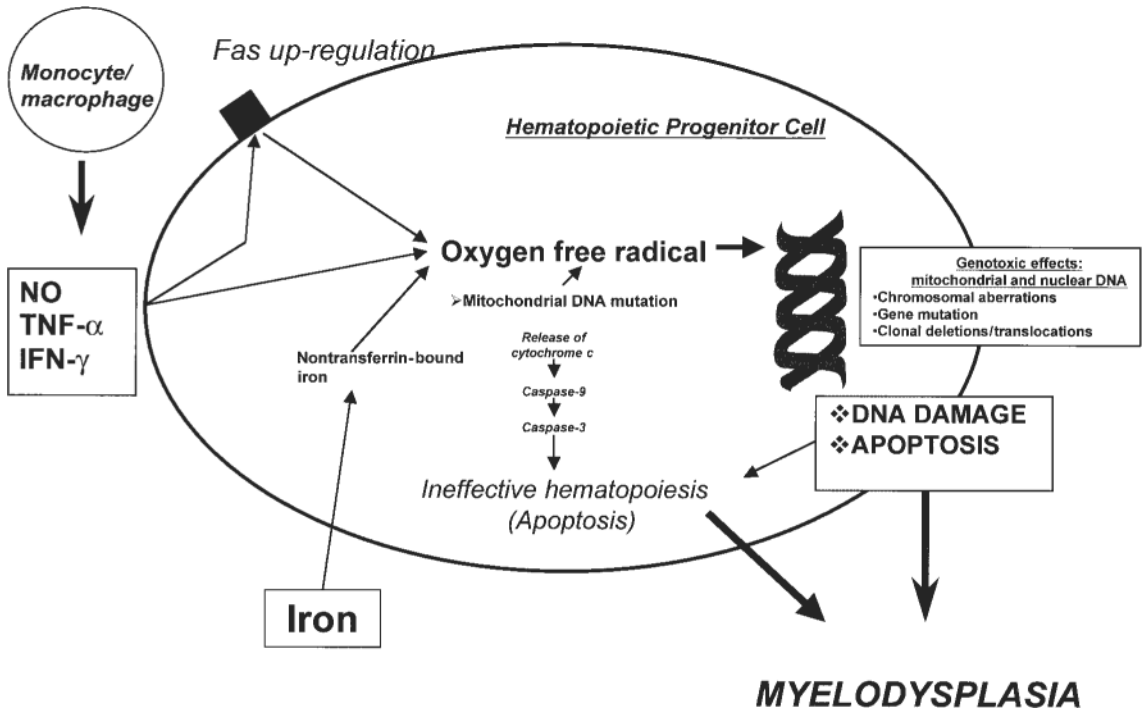

Figure 2. Oxidative stress mechanisms in the maintenance and progression of MDS. NO indicates nitric oxide; TNF-α, tumor necrosis factor α; IFN-γ, interferon γ.

3.1. Mitochondrial Dysfunction: Iron Overload and Mitochondrial DNA Mutation

Free iron plays a key role in the generation of highly reactive oxygen free radicals via the Fenton reaction. Excess body iron due to ineffective erythropoiesis is often observed in MDS patients at presentation, particularly in refractory anemia with ring sideroblasts (RARS). Iron overload is thought to contribute to the generation of low molecular weight iron complexes or nontransferrin-bound iron, which is taken up more readily by the cell than the transferrin-bound form. Uptake of nontransferrin-bound iron favors oxidative damage and consequent apoptosis. Serum nontransferrin-bound iron levels have been reported to be elevated, particularly in the low-risk MDS group, compared with healthy control subjects [47]. Iron uptake into erythroblasts is normal in patients with RARS, but iron accumulates in the cytoplasm and its incorporation into mitochondria is paradoxically reduced (at least in short-term experiments in vitro) [53]. The most persuasive evidence for a major contribution of iron overload to ineffective hematopoiesis is the reduction in red cell transfusions and the increase in platelet and neutrophil counts observed in up to two thirds of patients treated with desferrioxamine in long-term iron chelation therapy [54]. Maximal improvement of erythropoiesis was seen after at least 18 months of iron chelation therapy.

An association between mitochondrial dysfunction and the presence of ring sideroblasts has been established with the identification of mutations in genes encoding mitochondrial heme synthesis proteins as causative in congenital sideroblastic anemia [55]. Several studies have demonstrated functional mitochondrial abnormalities in MDS patients [56-58], but they were unable to distinguish between a primary mitochondrial abnormality and secondary events signaling through the mitochondrion. One putative primary mitochondrial defect is mitochondrial DNA mutation. Mitochondrial DNA is more susceptible to DNA damage (lacking histones for protection) and also lacks effective DNA repair mechanisms. Several mutations have been described in mitochondrial genes encoding enzymes of the electron transport chain [59], although whether these mutations are features of MDS per se remains contentious. Abnormalities of electron transport may lead to the accumulation of intramitochondrial iron as ring sideroblasts with consequent local free radical–induced mitochondrial DNA toxicity. An excess of point mutations scattered throughout the mitochondrial genome in MDS patients would be compatible with oxidative mitochondrial DNA damage or other mutagenic mechanisms [60]. Irrespective of whether the defect is primary or secondary, mitochondrial electron transport abnormalities produce cellular oxidative stress with oxidative damage to both mitochondrial and nuclear DNA. In animal models, older animals are more susceptible than younger animals to oxidative mitochondrial DNA damage [17], but whether repair of this damage diminishes with age remains unclear.

3.2. Systemic Abnormalities: Autoimmunity and Inflammation

A high prevalence of immunologic abnormalities in patients with MDS has been described [61,62], and these

reports provided early evidence that bone marrow failure in MDS may be mediated by the immune system, at least in part. Immunosuppression with antithymocyte globulin [63] or cyclosporine [64] can abrogate transfusion dependence and restore hematopoiesis in patients with low-risk MDS. Therapeutic response to antithymocyte globulin is associated with the release from the inhibitory effects of autoreactive $CD8^+$ T-cells on the growth of colony-forming unit–granulocyte-macrophage progenitors [65]. This therapeutic observation supports a pathogenetic role for autoreactive cytotoxic T-cells in some patients, particularly in hypocellular MDS. This event may not necessarily be disease initiating, however, because accentuated apoptosis is known to expose and present neoantigens to the immune system and has the potential to induce autoimmunity as a secondary phenomenon.

The serum and bone marrow concentrations of several inflammatory cytokines, including TNF-α and transforming growth factor β, are elevated in some MDS patients [66,67]. TNF-α induces an enhancement of reactive oxygen intermediate leakage from the mitochondrial respiratory chain, and this leakage may lead to oxidative DNA damage. Exposure of $CD34^+$ cells to TNF-α and interferon γ up-regulates the surface receptor Fas [68]. Although the functional role of Fas up-regulation in MDS is still debatable, Fas expression is associated with autoreactive T-cells in patients with trisomy 8 but not those with monosomy 7. This finding in turn implies a greater pathogenetic role for autoreactive T-cells in patients with trisomy 8 [69].

Induction of nitric oxide synthase is enhanced on Fas receptor stimulation in normal bone marrow $CD34^+$ cells, which leads to an increased production of the toxic metabolite NO [70]. NO has various roles within the hematopoietic and immune systems, including a role as a mediator of oxidative reactions [71]. Normal bone marrow cells or $CD34^+$ cells exposed to NO produced a dose-dependent inhibition of progenitor colony formation [72]. An increase in the number of bone marrow macrophages has been reported in patients presenting with MDS [73], and this finding appears to indicate the major source of the increased nitric oxide synthase protein observed in bone marrow of MDS patients [74].

3.3. Stroma and the Bone Marrow Microenvironment

In vitro studies of the functional integrity of the hematopoietic microenvironment in MDS have been controversial. Although there are suggestions that such a microenvironment is functionally normal [75], increasing evidence indicates the presence in MDS marrow of alterations in the function of adherent cell layers. Abnormalities of both the macrophage and fibroblast stromal components include an increased apoptotic index and high production of TNF-α and interleukin 6 [76-78]. MDS stromal supernatant contains a higher superoxide concentration than normal stromal supernatant [77]. It is likely that most pro-oxidant stimuli in MDS bone marrow are therefore generated by stromal cells. Similarly, genetic susceptibility to MDS may in fact be mediated by the metabolism of potential carcinogens within stromal cells to produce indirect hematopoietic cell toxicity [33,79].

3.4. Endogenous Stress Defense Mechanisms in MDS Cells

Recent gene expression profiling data indicate a down-regulation of many stress-response proteins in $CD34^+$ cells, and although not suggestive of oxidative stress per se, these data indicate a relative failure of stress defense in this cellular compartment [80]. Preliminary data from our laboratory show markedly up-regulated expression of the antioxidant defense enzymes Sod2 (3- to 6-fold) and GPX1 (3- to 9-fold) in MDS neutrophils, but only modest up-regulation of GPX1 (and normal Sod2 levels) in the primitive cellular compartment ($CD34^+$ cells) [14].

3.5. Therapeutic Studies: Antioxidant and Anti–TNF-α

Intravenous treatment of MDS patients with the aminothiol amifostine produces transient multilineage stimulatory effects in up to 70% of patients across all FAB subtypes, although these responses are not durable or clinically meaningful [81]. Although treatment with amifostine improved blood counts, the persistence of abnormal karyotypes was noted, suggesting that hematopoietic stimulation occurred within the MDS clonogenic cells. Therapeutic strategies targeted to the inhibition of TNF-α activity have thus far been disappointing [82,83], with the exception of thalidomide [84]. Thalidomide, however, has many potential mechanisms of action, including immunosuppression and anti-inflammatory properties. Therapeutic agents with the potential to interfere with the inflammatory component of MDS and currently in clinical trial include infliximab and CC-5013 (also known as Revimid).

4. Putative Mechanistic Link between Oxidative Stress and Genomic Aberrations: Fanconi Anemia

Fanconi anemia (FA) is an inherited chromosome fragility syndrome with multisystem defects that include a high frequency of MDS/AML. Karyotypic abnormalities in the MDS/AML clones are typically deletional with a spectrum similar to that of de novo and secondary MDS [85]. FA is genetically heterogeneous, and 7 genes have thus far been implicated in the pathogenesis as a result of cell fusion complementation genetic analysis [86]. Although the most prominent phenotypic feature is the high cellular sensitivity to chromosome breaks on cell exposure to DNA cross-linking agents, other phenotypic features include the overproduction of OFR [87] and an increased production of TNF-α. Thus, the bone marrow defect in FA appears to be a model for de novo MDS. A model for selective clonal outgrowth of cells resistant to the hematopoietic inhibition of TNF-α and perhaps oxidative stress has been proposed [85]. Further mutations that inactivate these inhibitory signaling pathways may generate a cell with a profound proliferative advantage over its apoptosis-prone counterparts.

Exposure of untreated FA cells to concentrations of the pro-oxidant H_2O_2 damages normal cells but exerts little effect on FA red blood cells and hematopoietic progenitors, suggesting that circulating FA cells are resistant to additional oxidative insult [88]. One potential explanation is that only

hematopoietic cells capable of resisting oxidative stress can give rise to mature circulating progeny in both FA and de novo MDS. In support of this hypothesis are the demonstration MDS neutrophils (and to a lesser extent $CD34^+$ cells) resistant to spontaneous apoptosis [89] and our preliminary observations of augmented antioxidant enzyme expression in MDS neutrophils [14].

Recent evidence suggests that the breast cancer susceptibility gene BRCA2 is a common target of the known FA proteins [86]. In addition to its role in DNA repair by homologous recombination, BRCA2 is also required for the transcription-coupled repair of oxidized purine nucleotides (8-OHdG) [90]. In the absence of a functional BRCA2 gene, persisting oxidized nucleotides may lead to cellular apoptosis and/or mutation of critical growth regulatory genes such as RAS and to homologous recombination DNA repair defects producing genomic instability and chromosomal deletion.

5. Conclusion

The presence of markers for oxidative stress in patients with established MDS indicate a potential role for pro-oxidant pathways in the maintenance/progression of the MDS phenotype. This stress may be mediated by a variety of mechanisms, many of which are broadly inflammatory and could contribute to both apoptosis and DNA mutation. Furthermore, several pro-oxidant toxicants are implicated as causative for the initiating events that lead to the development of a transformed myelodysplastic clone. Unraveling these complex pathways should lead to novel therapeutic avenues and potentially to strategies aimed at preventing MDS.

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