

Iron Overload, Oxidative Damage and Ineffective Erythropoiesis in Myelodysplastic Syndromes

Rosangela Invernizzi

Associate Professor of Internal Medicine, and Chief, Haematological Cytology Laboratory, University of Pavia

DOI: 10.17925/EOH.2010.04.0.34

Abstract

A high level of apoptosis may be responsible for the ineffective haematopoiesis in myelodysplastic syndromes (MDS). Recently, it has been demonstrated that the erythroid apoptosis of low-risk MDS is initiated at a very early stage in stem cells and is associated with mitochondrial dysfunction. However, the underlying pathogenetic mechanisms causing malfunctioning of mitochondria and initiation of the intrinsic apoptotic cascade are not completely clear. Recent studies suggest a close relationship between impaired iron metabolism and pathogenesis of myelodysplasia. In fact, iron overload, which is apparent in refractory anaemia with and without ring sideroblasts, may lead to the generation of intracellular free radicals, thereby causing oxidative damage and inducing apoptosis in haematopoietic progenitors. This review summarises current knowledge supporting the role of iron-related oxidative damage in the pathogenesis of MDS. The relationship between mitochondrial iron homeostasis impairment and ineffective erythropoiesis in refractory anaemia with ring sideroblasts as well as the various functions of the cytosolic and mitochondrial ferritins are also discussed.

Keywords

Myelodysplastic syndromes (MDS), acquired sideroblastic anaemia, erythropoiesis, iron overload, oxidative stress, reactive oxygen species, apoptosis, cytosolic ferritin, mitochondrial ferritin

Disclosure: The author has no conflicts of interest to declare.

Acknowledgements: Rosangela Invernizzi is supported by grants from the Ministry for Education, University and Research, Italy, and from Fondazione IRCCS Policlinico S Matteo, Pavia, Italy.

Received: 17 September 2009 **Accepted:** 27 January 2010 **Citation:** *European Haematology*, 2010;4:34–8

Correspondence: Rosangela Invernizzi, Clinica Medica III Piazzale Golgi 27100 Pavia, Italy. E: r.invernizzi@smatteo.pv.it

Myelodysplastic syndromes (MDS) are clonal diseases of haematopoiesis, i.e. they are conditions where haematopoiesis is maintained by one or a few abnormal clones of haematopoietic cells. During the evolution of the disease normal stem cells tend to decrease in number and to disappear progressively. Disease progression is multistep, with a series of genetic events that reduce the ability of the proliferating clone to differentiate and mature. It has been hypothesised that a high level of apoptosis may be responsible for the ineffective haematopoiesis in MDS, with the apparent paradox of peripheral cytopenia associated with hypercellular bone marrow.^{1–4} This phenomenon is also well documented *in vitro*, where haematopoietic progenitors do not form colonies in spite of the presence of haematopoietic growth factors. This appears particularly relevant in low-risk MDS, i.e. refractory anaemia (RA) with and without ring sideroblasts,^{5–7} where the pathological phenotype is predominantly restricted to impairment of the erythroid cell lineage. By contrast, in the MDS subgroup at high risk of leukaemic evolution (RA with excess of blasts), the percentages of apoptotic cells are lower than in normal subjects and similar to those observed in acute leukaemia.

Apoptosis, triggered by the bone marrow microenvironment and/or intrinsic cellular defects, is regulated at different levels by numerous factors, such as oncogenes and their protein products, haematopoietic growth factors, immunological factors, cell–cell and cell–stromal

interactions, critical adhesion receptors and various cytokines. These cytokines – tumour necrosis factor (TNF)-alpha, interferon-gamma and transforming growth factor-beta – may also be involved through the liberation of intracellular free radicals, with consequent oxidative damage of DNA.^{8,9}

Oxidative Stress and Myelodysplastic Syndrome Pathogenesis

Oxidative stress is the result of an imbalance between the production, through aerobic respiration, of highly reactive oxygen species (ROS) and antioxidant defences (see *Figure 1* and *Table 1*). It can cause cell damage resulting in cell death or altered cell function, carcinogenesis included, and is associated with ageing and various disease states. Many sources of evidence suggest a role for oxidative damage in MDS pathogenesis. Indeed, several studies have revealed the markers of oxidative stress in some MDS patients. These markers include:

- an increased plasma concentration of the lipid peroxidation product malondialdehyde;¹⁰
- the presence of oxidised DNA bases in CD34⁺ bone marrow cells,⁸ but also in the more differentiated CD34[–] bone marrow cell population;¹¹
- the presence of 7,8-dihydro-8-oxoguanine, the most common oxidative DNA damage product, in the urine¹² or in the DNA of peripheral leukocytes;¹³

- elevated levels of ROS in red blood cells and platelets¹⁴ with a consequent decrease in cellular antioxidants such as glutathione (GSH); and
- higher peroxide levels in CD34⁺ bone marrow cells.¹⁵

In addition, an association between reactive oxygen metabolites and karyotypic abnormalities was demonstrated in a panel of MDS patients.¹⁶ The finding of higher antioxidant enzyme expression in MDS bone marrow granulocytes supported a model for the role of oxidative stress in the ineffective haematopoiesis of MDS.¹⁷ On the other hand, a dysfunction of the intrinsic antioxidant and DNA repair capacity due to genetic lesions proved to be responsible for inadequate compensatory feedback in some MDS patients. This led to oxidative DNA damage and favoured genomic instability and the propensity to chromosomal breaks.¹³

Very interestingly, an animal model for genomic instability in MDS progression was recently provided, showing that the combination of increased DNA damage and ineffective repair could create an environment for the acquisition of genetic alterations. Using a two-step mouse model with overexpression of human mutant *NRAS* and *BCL2* genes, Rassool et al. observed a progressive increase in the frequency of DNA damage. This damage led to an increased frequency of error-prone repair of DNA double-strand breaks during myeloid leukaemic disease progression. There was a concomitant increase in ROS, in part dependent on the RAS–RAC pathway. DNA damage and error-prone repair could be decreased or reversed *in vivo* by N-acetyl cysteine antioxidant treatment.¹⁸ Another experimental model confirmed that inadequate control of intracellular levels of ROS may contribute to leukaemia development. A protein interacting with the antioxidant protein thioredoxin was found to be overexpressed in human leukaemic blasts, and the encoding gene was a target for proviral integration in virus-induced mouse leukaemia.¹⁹

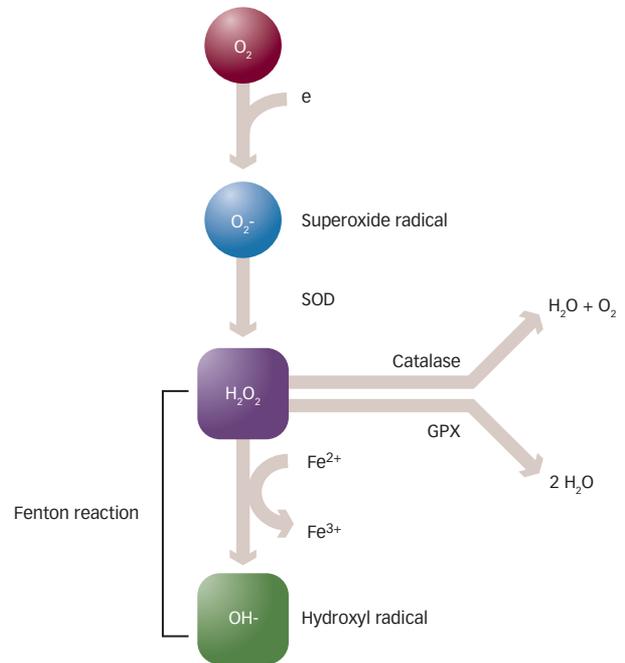
Various signalling pathways involving the FoxO transcription factors, the p38 mitogen-activated protein kinase (MAPK) and the ataxia telangiectasia mutated (ATM) protein,^{20–22} as reviewed by Ghaffari²³ and Nimer,²⁴ were implicated in the loss of haematopoietic stem cells due to ROS generation and oxidative stress in a murine model. Through these pathways, ROS signalling may participate in the regulation of proliferation and apoptosis of normal and neoplastic haematopoietic progenitor cells. Interestingly, pharmacological inhibition of MAPK phosphorylation decreases apoptosis in MDS CD34⁺ progenitors and leads to a dose-dependent increase in erythroid and myeloid colony formation *in vitro*.²²

Thus, although the source of ROS and metabolism of haematopoietic stem cells is not fully clear, inflammation, overexpression of proapoptotic cytokines and oncogene activation, as well as bone marrow stromal defects, may contribute to cause oxidative damage in MDS patients. However, other factors probably play the crucial role.

Oxidative Damage and Intracellular Ferritins

Iron overload may also lead to ROS generation, impairment of biological macromolecules and abnormal haematopoiesis.²⁵ In fact, it contributes to the generation of non-transferrin-bound iron, which is promptly taken up by cells. Excess free iron in cells, identified as the labile iron pool (LIP), catalyses the generation of ROS as it is a substrate of the Fenton reaction. This reaction leads to a consequent

Figure 1: Reactive Oxygen Species



Reactive oxygen species (ROS) are oxygen derivatives produced by aerobic respiration. The acceptance of a single electron (e) by an O₂ molecule generates the free radical O₂⁻ (superoxide radical). This radical is then reduced to hydrogen peroxide (H₂O₂) via superoxide dismutase (SOD). H₂O₂ is then converted to water (H₂O) by catalase or glutathione peroxidase (GPX) or reacts with partially reduced iron (Fe²⁺) to produce the hydroxyl radical OH⁻ via the Fenton reaction. While O₂ and O₂⁻ are mild oxidants, H₂O₂ is a strong oxidant and OH⁻ is considered the most powerful oxidant.

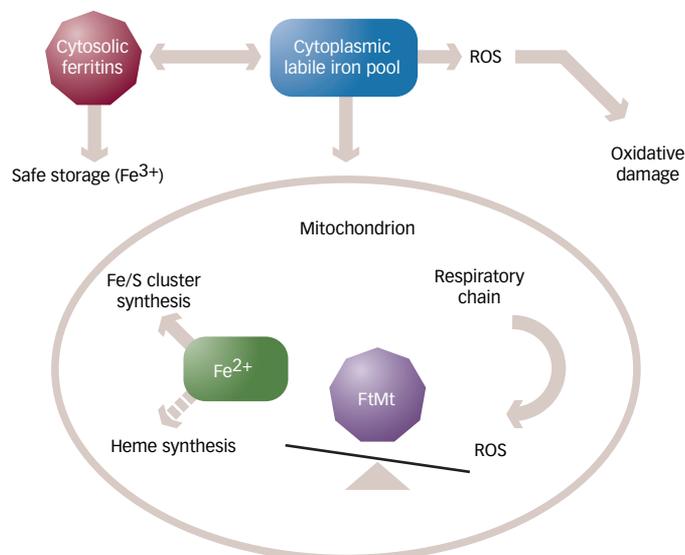
Table 1: Antioxidant Agents

Antioxidant Enzymes
Manganese superoxide dismutase (MnSOD2)
Catalase
Glutathione peroxidase (GPX)
Non-enzymatic Antioxidants
Reduced glutathione (GSH)
Peroxiredoxin
Ascorbic acid
Carotenoids
Repair Enzymes
Endonucleases
Glycosylases
Polymerases
Ligases

decrease in cellular antioxidants, such as GSH, and oxidation of lipids, proteins and DNA, thereby causing cell and tissue damage.^{26,27}

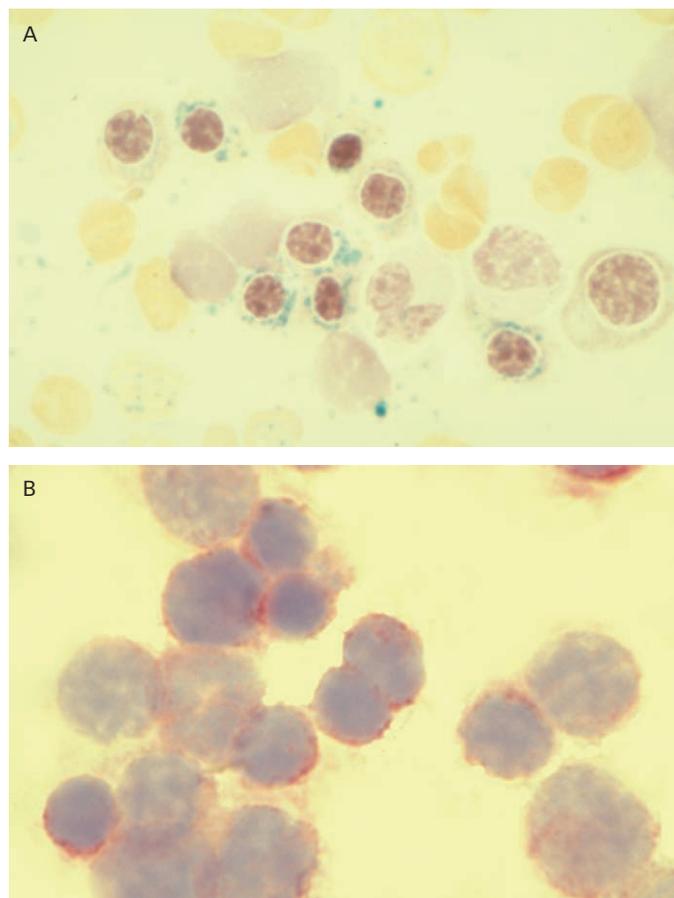
It is well known that cytosolic ferritin is used for the purpose of iron storage inside the cell and that it has the important role of maintaining the equilibrium between the amount of cytoplasmic LIP needed for cytosolic and mitochondrial enzyme function and excess iron²⁸ (see Figure 2). Cytosolic ferritin plays an important role in converting potentially harmful free iron to less soluble ferric iron by virtue of the ferroxidase activity of the H subunit. Its expression is regulated translationally through regulatory elements, and tissue distribution is variable. In cellular models, the upregulation of the ferritin heavy chain, induced by the NF-κB transcription factor, suppresses ROS accumulation, JNK signalling and TNF-alpha-induced apoptosis, suggesting that the

Figure 2: Physiological Role of Ferritin



Cytosolic ferritin is used for iron storage purposes inside the cell and acts as an iron buffer: it maintains the equilibrium between the amount of the cytoplasmic labile iron pool (LIP) useful for cytosolic and mitochondrial enzyme requirement and the excess iron. In mitochondria, iron is utilised by Fe/S and heme synthesis. Iron storage in mitochondrial ferritin (FtMt) reduces the Fenton reaction and protects mitochondria from iron-dependent oxidative damage. ROS = reactive oxygen species.

Figure 3: Bone Marrow Aspirate from a Patient with Refractory Anaemia with Ring Sideroblasts



A: Erythroblasts showing peculiar iron stain due to mitochondrial iron overload: Perls positive granules tend to form a ring surrounding the nucleus (Perls' reaction, x1,250).
B: Mitochondrial ferritin (FtMt) is detected in granules ringing the nucleus (immunoalkaline phosphatase reaction, x1,250).

antioxidant activity of ferritin involves iron sequestration.⁹ The biological role of the ferritin L chain is little known, except for its ability to facilitate iron core formation inside the protein shell.

Although excess iron is stored primarily in the cytoplasm, the major iron-consuming organelle is the mitochondrion, where iron is utilised by Fe/S clusters and heme synthesis (see *Figure 2*). Since the organelle is also the major site of ROS production, iron must be tightly regulated to reduce the Fenton reaction. During the past decade some progress has been made in the elucidation of how mitochondria regulate iron homeostasis and toxicity.^{29,30} A mitochondrial protein with similar functional properties to ferritin has been identified.^{31,32} The protein, named mitochondrial ferritin (FtMt), presents some peculiarities:

- it is encoded by an intronless gene;
- it has been found in the genome of various mammals and is also present in plants and in drosophila; and
- it is an homopolymer and shows ferroxidase activity like H-ferritin.

Its messenger RNA (mRNA) does not contain any functional iron-responsive elements suggesting that the protein expression is not iron dependent and it is not ubiquitous but cell-specific. Its expression in cells with high metabolic activity and oxygen consumption suggests a role in protecting mitochondria from iron-dependent oxidative damage (see *Figure 2*). Recent studies have in effect demonstrated that its major functional role is to act as an antioxidant and, depending on its level of expression, it can induce cytosolic iron starvation³³ with a consequent reduction in cell proliferation and heme synthesis.^{34,35}

Iron Overload in Myelodysplastic Syndromes

Little is known about iron regulation in MDS. However, there is evidence that although due to ineffective erythropoiesis low-risk MDS patients are transfusion-dependent and transfusion therapy is the main cause of iron overload, it is not the only contributing factor. Iron starts to accumulate even without transfusion therapy.^{36,37} According to the pathophysiological model of iron-loading anaemias (thalassaemia and congenital sideroblastic anaemia), this could be due to increased intestinal iron absorption, which is caused by ineffective erythropoiesis, hypoxia and, to some extent, suppression of hepcidin production.^{36,38} RA with ring sideroblasts (RARS) is a subgroup of MDS with unknown abnormalities of iron metabolism characterised by ineffective erythropoiesis associated with iron accumulation in the mitochondria of immature red cells.³⁹ In a study of the natural history of RARS, Cazzola et al. found that mild iron overload was commonly found at presentation, but only caused clinical manifestations of haemochromatosis in patients who subsequently had a regular need for blood transfusion. Iron-overload-related complications were the most common causes of death.⁴⁰

More recently, Malcovati et al. studied the effect of transfusion dependency and secondary iron overload, as assessed by serum ferritin, on the survival of patients with MDS. Interestingly, transfusion dependency was found to significantly worsen the probability of surviving and to increase the risk of progressing to leukaemia. The effect of iron overload was mainly seen among patients with RA and RARS.^{41,42} Also, in MDS patients undergoing allogeneic stem cell transplantation, iron overload may be associated with adverse outcome.⁴³ Therefore, in MDS patients with severe anaemia in whom iron overload due to ineffective erythropoiesis and multiple blood transfusions will eventually

result in damage to vital organs there is a rationale for iron chelation therapy. Oxidative stress mediated by iron overload may induce apoptosis in haematopoietic progenitors.⁴⁴ Interestingly, increased serum non-transferrin-bound iron levels were observed in MDS,¹⁰ and a correlation was found between serum ferritin levels of low-risk MDS patients and ROS in their erythrocytes and platelets.¹⁴

The contribution of iron overload to ineffective haematopoiesis is evident also by the reduction in transfusion requirement and the increase in platelet and neutrophil counts following iron chelation therapy.³⁷ Iron chelation therapy was also able to reduce toxic free iron species and parameters of oxidative stress as well as ROS-mediated hypoxic suppression of hepcidin in low-risk iron-overloaded patients with MDS.^{45,46}

Iron Overload and Mitochondrial Dysfunction in Refractory Anaemia with Ring Sideroblasts

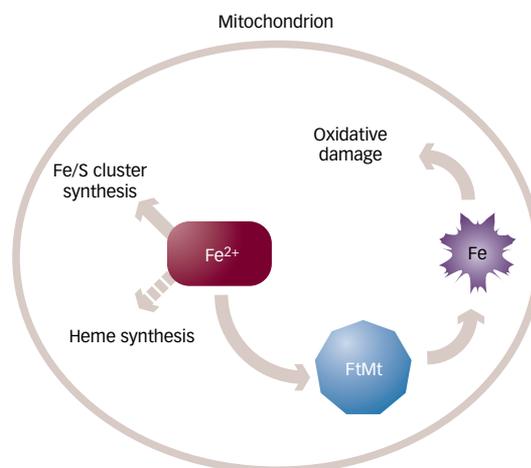
As regards RARS pathogenesis, several studies highlight the association between impaired iron homeostasis and mitochondrial dysfunction. It is well known that mitochondrial DNA lesions in genes encoding enzymes of the electron transport chain are involved in both acquired and inherited sideroblastic anaemia and pathways, including mitochondrial oxidative phosphorylation. Thiamine metabolism and iron–sulphur biosynthesis have been identified as primary defects in the hereditary forms.⁴⁷ These defects result in pathological iron deposition within mitochondria.

Interestingly, in RARS it was observed that the iron deposited in the perinuclear mitochondria of ringed sideroblasts is present in the form of FtMt⁴⁸ (see *Figure 3*). Initial data suggested that the expression of FtMt in these cells was a response to mitochondrial iron accumulation. However, the finding that bone marrow cells from patients with RARS start to express FtMt in the early stages of erythroid differentiation, before evident iron accumulation,⁴⁹ indicated a more complex mechanism of gene regulation.

FtMt overexpression in RARS erythroid progenitors was paralleled by an upregulation of genes involved in the process of erythroid differentiation and was associated with cytochrome C release, with subsequent initiation of the intrinsic apoptotic pathway and increased sensitivity to death ligands triggering the extrinsic pathway.⁴⁹ Even if further studies are needed to clarify whether FtMt overexpression is the cause or the result of mitochondrial iron deposition and whether FtMt is expressed in all conditions of mitochondrial iron excess, findings suggest that, initially in RARS, the iron accumulation in FtMt may be protective. It may preserve mitochondrial DNA integrity and increase cell resistance to oxidative damage. In the absence of iron utilisation for heme synthesis, FtMt may potentially degrade to a haemosiderin-like molecule that is redox-active, leading to subsequent mitochondrial damage, impairing heme synthesis and altering the balance between cell growth and death (see *Figure 4*). The recent finding that FtMt overexpression in a neoplastic cell line sensitises cells to oxidative stress via an iron-mediated mechanism and increases apoptosis levels supports this suggestion.⁵⁰

Other recent studies have demonstrated that CD34+ cells from patients with RARS have a peculiar gene expression profile characterised by upregulation of mitochondria-related genes, in particular those of heme synthesis.⁵¹ On the other hand, some genes are downregulated. An example of this is *ABC7*, which encodes a transporter of iron from the

Figure 4: Possible Pathogenetic Mechanism



In refractory anemia with ring sideroblasts (RARS), the aberrant expression of mitochondrial ferritin (FtMt) may reflect a defence mechanism of the cell against the oxidative damage caused by excess iron. In the absence of iron utilisation for heme synthesis, further excessive iron accumulation may cause FtMt degradation, damage mitochondria and trigger apoptosis.

mitochondria to the cytoplasm.⁵² All these findings suggest that mitochondrial dysfunction, in particular excessive ROS production and excess iron accumulation, plays a critical role in the physiopathological mechanism of anaemia in RARS.⁵³ Experimental data support this suggestion. In a crimsonless (crs) zebrafish mutant, the mutation of a mitochondrial heat shock protein (HSPA9B) produces oxidative stress and apoptosis in blood cells.⁵⁴ Superoxide dismutase (SOD2) deficiency induces the phenotype of sideroblastic anaemia in chimaeric mice, as the mitochondrial enzyme SOD2 is the principal defence against the toxicity of superoxide anion radicals.⁵⁵

Future Directions

Over the past decade substantial progress has been made in the elucidation of the mechanisms by which mitochondria protect themselves from iron excess and associated oxidative damage. However, further studies are needed to fully clarify the possible pathogenetic role of iron-induced oxidative damage in the ineffective erythropoiesis of MDS. Further studies should also identify the signalling pathways implicated in the loss of haematopoietic progenitors due to ROS generation and oxidative stress. The results of these studies might also be important from a clinical point of view, as they might provide the rationale for therapeutic strategies based on the combination of iron chelators and antioxidants for MDS patients. A prolonged high-dose use of these agents may inhibit apoptosis and promote cell differentiation by reducing abnormal cytosolic or mitochondrial iron accumulation and, consequently, oxidative damage. ■



Rosangela Invernizzi is an Associate Professor of Internal Medicine and Chief of the Haematological Cytology Laboratory at the University of Pavia. She is a member of the Italian Societies of Haematology, Experimental Haematology, Histochemistry and Internal Medicine, and the European Haematology Association. Dr Invernizzi's main field of interest is myelodysplastic syndromes. She recently investigated the role of bone marrow cell apoptosis in the molecular pathogenesis of these disorders and the role of a novel mitochondrial ferritin in regulating mitochondrial iron homeostasis and heme synthesis. She graduated in medicine and has specialised in haematology, internal medicine and oncology at Pavia University.

1. Invernizzi R, The role of apoptosis in myelodysplastic syndrome, *Haematologica*, 2002;87:337–9.
2. Cazzola M, Malcovati L, Myelodysplastic syndromes-coping with ineffective hematopoiesis, *N Engl J Med*, 2005;352:536–3.
3. Fontenay M, Gyan E, Apoptotic pathways to death in myelodysplastic syndromes, *Haematologica*, 2008;93:1288–92.
4. Invernizzi R, Travaglini E, Increased apoptosis as a mechanism of ineffective erythropoiesis in myelodysplastic syndromes, *Clinical Leukemia*, 2008;2:113–20.
5. Parker JE, Mufti GJ, Rasool F, et al., The role of apoptosis, proliferation, and Bcl-2-related proteins in the myelodysplastic syndromes and acute myeloid leukaemia secondary to MDS, *Blood*, 2000;96:3932–8.
6. Pecci A, Travaglini E, Klersy C, et al., Apoptosis in relation to CD34 antigen expression in normal and myelodysplastic bone marrow, *Acta Haematol*, 2003;109:29–34.
7. Invernizzi R, Travaglini E, Lunghi M, et al., Survivin expression in acute leukemias and myelodysplastic syndromes, *Leuk Lymphoma*, 2004;45:2229–37.
8. Peddie CM, Wolf CR, McLellan LI, et al., Oxidative DNA damage in CD34+ myelodysplastic cells is associated with intracellular redox changes and elevated plasma tumor necrosis factor- α concentration, *Br J Haematol*, 1997;99:625–31.
9. Pham CG, Bubici C, Zazzeroni F, et al., Ferritin heavy chain upregulation by NF- κ B inhibits TNF α -induced apoptosis by suppressing reactive oxygen species, *Cell*, 2004;119:529–42.
10. Cortelezzi A, Cattaneo C, Cristiani S, et al., Non-transferin-bound iron in myelodysplastic syndrome: a marker of ineffective erythropoiesis?, *Hematol J*, 2000;1:153–8.
11. Novotna B, Bagryantseva Y, Siskova M, et al., Oxidative DNA damage in bone marrow cells of patients with low-risk myelodysplastic syndrome, *Leuk Res*, 2009;33:340–43.
12. Honda M, Yamada Y, Tomonaga M, et al., Correlation of urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG), a biomarker of oxidative DNA damage, and clinical features of hematological disorders: a pilot study, *Leuk Res*, 2000;24:461–8.
13. Jankowska AM, Gondek LP, Szpurka H, et al., Base excision repair dysfunction in a subgroup of patients with myelodysplastic syndrome, *Leukemia*, 2008;22:551–8.
14. Ghoti H, Amer J, Winder A, et al., Oxidative stress in red blood cells, platelets and polymorphonuclear leukocytes from patients with myelodysplastic syndrome, *Eur J Haematol*, 2007;79:463–67.
15. Gonçalves AC, Cortesao E, Alves V, et al., CD34 cells are under oxidative stress - Possible implication in MDS pathogenesis, *Haematologica*, 2009;94(Suppl. 2):323.
16. Fracchiolla NS, Bamonti Catena F, Novembrino C, et al., Possible association between reactive oxygen metabolites and karyotypic abnormalities in myelodysplastic syndromes, *Haematologica*, 2003;88:594–7.
17. Bowen D, Wang L, Frew M, et al., Antioxidant enzyme expression in myelodysplastic and acute myeloid leukemia bone marrow: further evidence of a pathogenetic role for oxidative stress?, *Haematologica*, 2003;88:1070–72.
18. Rassool FV, Gaymes TJ, Omidvar N, et al., Reactive oxygen species, DNA damage and error prone repair: A model for genomic instability with progression in myeloid leukemia?, *Cancer Res*, 2007;67:8762–71.
19. Erkeland SJ, Palande KK, Valkhof M, et al., The gene encoding thioredoxin-interacting protein (TXNIP) is a frequent virus integration site in virus-induced mouse leukemia and is overexpressed in a subset of AML patients, *Leuk Res*, 2009;33:1367–71.
20. Ito K, Hirao A, Arai F, et al., Regulation of oxidative stress by ATM is required for self-renewal of hematopoietic stem cells, *Nature*, 2004;431:997–1002.
21. Ito K, Hirao A, Arai F, et al., Reactive oxygen species act through p38 MAPK to limit the lifespan of hematopoietic stem cells, *Nat Med*, 2006;12:446–51.
22. Navas TA, Mohindru M, Estes M, et al., Inhibition of overactivated p38 MAPK can restore hematopoiesis in myelodysplastic syndrome progenitors, *Blood*, 2006;108:4170–77.
23. Ghaffari S, Oxidative stress in the regulation of normal and neoplastic hematopoiesis, *Antioxid Redox Signal*, 2008;10:1923–40.
24. Nimer SD, MDS: a stem cell disorder-but what exactly is wrong with the primitive hematopoietic cells in this disease? *Hematology Am Soc Hematol Educ Program*, 2008:43–51.
25. Arosio P, Levi S, Ferritin, homeostasis, and oxidative damage, *Free Radic Biol Med*, 2002;33:457–63.
26. Esposito BP, Breuer W, Sirankapracha P, et al., Labile plasma iron in iron overload: redox activity and susceptibility to chelation, *Blood*, 2003;102:2670–77.
27. Hershko C, Link G, Cabantchik I, Pathophysiology of iron overload, *Ann N Y Acad Sci*, 1998;850:191–201.
28. Arosio P, Ingrassia R, Cavadini P, Ferritins: a family of molecule for iron storage, oxidation and more, *Biochim Biophys Acta*, 2009;1790:589–99.
29. Napier I, Ponka P, Richardson DR, Iron trafficking in the mitochondrion: novel pathways revealed by disease, *Blood*, 2005;105:1867–74.
30. Levi S, Rovida E, The role of iron in mitochondrial function, *Biochim Biophys Acta*, 2009;1790:629–36.
31. Levi S, Corsi B, Bosio M, et al., A human mitochondrial ferritin encoded by an intronless gene, *J Biol Chem*, 2001;276:24437–40.
32. Drysdale J, Arosio P, Invernizzi R, et al., Mitochondrial ferritin: a new player in iron metabolism, *Blood Cells Mol Dis*, 2002;29:376–83.
33. Campanella A, Rovelli E, Santambrogio P, et al., Mitochondrial ferritin limits oxidative damage regulating mitochondrial iron availability: hypothesis for a protective role in Friedreich ataxia, *Hum Mol Genet*, 2009;18:1–11.
34. Nie G, Sheftel AD, Kim SF, et al., Overexpression of mitochondrial ferritin causes cytosolic iron depletion and changes cellular iron homeostasis, *Blood*, 2005;105:2161–7.
35. Nie G, Chen G, Sheftel AD, et al., In vivo tumor growth is inhibited by cytosolic iron deprivation caused by the expression of mitochondrial ferritin, *Blood*, 2006;108:2428–34.
36. Cazzola M, Della Porta MG, Malcovati L, Clinical relevance of anemia and transfusion iron overload in myelodysplastic syndromes, *Hematology Am Soc Hematol Educ Program*, 2008:166–75.
37. Gattermann N, Clinical consequences of iron overload in myelodysplastic syndromes and treatment with chelators, *Hematol Oncol Clin North Am*, 2005;19(Suppl. 1):13–17.
38. Nemeth E, Ganz T, Hepcidin and iron-loading anemias, *Haematologica*, 2006;91:727–32.
39. azzola M, Invernizzi R., Sideroblastic anemias, in: Young NS, Gerson SL, High KA (eds.), *Clinical Hematology*, Philadelphia, Mosby Elsevier, 2005:721–32.
40. Cazzola M, Barosi G, Gobbi PG, et al., Natural history of idiopathic refractory sideroblastic anemia, *Blood*, 1988;71:305–12.
41. Malcovati L, Porta MG, Pascutto C, et al., Prognostic factors and life expectancy in myelodysplastic syndromes classified according to WHO criteria: a basis for clinical decision making, *J Clin Oncol*, 2005;23:7594–7603.
42. Malcovati L, Germing U, Kuendgen A, et al., Time-dependent prognostic scoring system for predicting survival and leukemic evolution in myelodysplastic syndromes, *J Clin Oncol*, 2007;25:3503–10.
43. Armand P, Kim HT, Cutler CS, et al., Prognostic impact of elevated pretransplantation serum ferritin in patients undergoing myeloablative stem cell transplantation, *Blood*, 2007;109:4586–8.
44. Farquhar MJ, Bowen DT, Oxidative stress and the myelodysplastic syndromes, *Int J Hematol*, 2003;77:342–50.
45. Choi SO, Cho YS, Kim HL, et al., ROS mediate the hypoxic repression of the hepcidin gene by inhibiting C/EBP- α and STAT-3, *Biochem Biophys Res Commun*, 2007;356:312–17.
46. Ghoti H, Fibach E, Merkel D, et al., Decrease in intra- and extra-cellular free iron species and oxidative stress parameters and increase in serum and urinary hepcidin during treatment with deferasirox in iron-loaded patients with MDS, *Haematologica*, 2009;94(suppl 2):322.
47. Camaschella C, Recent advances in the understanding of inherited sideroblastic anaemia, *Br J Haematol*, 2008;143:27–38.
48. Cazzola M, Invernizzi R, Bergamaschi G, et al., Mitochondrial ferritin expression in erythroid cells from patients with sideroblastic anemia, *Blood*, 2003;101:1996–2000.
49. Tehrani R, Invernizzi R, Grandien A, et al., Aberrant mitochondrial iron distribution and maturation arrest characterize early erythroid precursors in low-risk myelodysplastic syndromes, *Blood*, 2005;106:247–53.
50. Lu Z, Nie G, Li Y, et al., Overexpression of mitochondrial ferritin sensitizes cells to oxidative stress via an iron-mediated mechanism, *Antioxid Redox Signal*, 2009;11(8):1791–1803.
51. Pellagatti A, Cazzola M, Giagounidis AA, et al., Gene expression profiles of CD34+ cells in myelodysplastic syndromes: involvement of interferon-stimulated genes and correlation to FAB subtype and karyotype, *Blood*, 2006;108:337–45.
52. Boultonwood J, Pellagatti A, Nikpour M, et al., The role of the iron transporter ABCB7 in refractory anemia with ring sideroblasts, *PLoS ONE*, 2008;3: e1970.
53. Invernizzi R, Travaglini E, Della Porta MG, et al., Characterization of a cellular model for the study of mitochondrial ferritin physiopathological role in sideroblastic erythropoiesis, *Haematologica*, 2008;93(Suppl. 2):S17.
54. Craven SE, French D, Ye W, et al., Loss of HSPA9B zebrafish recapitulates the ineffective hematopoiesis of the myelodysplastic syndrome, *Blood*, 2005;105:3528–34.
55. Martin FM, Bydlon G, Friedman JS, SOD2-deficiency sideroblastic anemia and red blood cell oxidative stress, *Antioxid Redox Signal*, 2006;8:1217–25.