PATHOGENETIC ASPECTS OF MYELODISPLASTIC SYNDROMES

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The patogenesis of MDS is complex and remain elusive. Figure 3 shows a hypothetical model. The proposed models agree that a multistep process occurs through which a hematopoietic stem cell is mutated and attains a growth advantage. This may occur as a result of environmental damage or inherited predisposition. The mutated clone is associated with morphological dysplasia, impaired differentiation and genomic instability. Cytokine secretion and apoptotic pathways are altered and as well as may be impairment of immune responses. Presumably, in the early stages, increased production of proapoptotic cytokines leads to excessive apoptosis, correlating clinically with cytopenias and a cellular bone marrow. As the disease progresses, further genetic and epigenetic events occur, resulting in decreased apoptosis, clonal expansion and progression to AML. Clinical testing of a number of molecules that affect these myriad molecular mechanisms is currently being done, characterization of genomic expression patterns will inform both diagnosis and prognostication. Further insight into the molecular mechanisms of MDS will provide an avenue for more tailored and effective therapy in the future.

INTRODUCTION

The myelodysplastic syndromes (MDS) comprise a heterogeneous group of clonal hematopoietic stem cell disorders characterized by ineffective hematopoiesis and a variable risk of transformation to acute myeloid leukemia (AML) (1).

It has been suggested that MDS arise from a hematopoietic stem cell that has suffered irreversible DNA damage. In a early phase of disease, impaired differentiation and increased apoptosis predominate, but as disease progression occurs, increased proliferation and accumulation of immature cells result.

Diagnosis and current classification systems (e.g. FAB or WHO) are based on findings in peripheral blood and expecially in the bone marrow as well as on cytogenetic abnormalities (2,3). In particular, diagnosis of MDS should be suspected in any patient with unexplained cytopenia(s) or monocytosis. A careful examination of peripheral blood as well as bone marrow smears show evident morphological abnormalities in one or more lineages. Fig. 1 and 2 depict some of the morphological patterns frequently seen in these diseases. This chapter will summarize some of the observations on the numerous biologic mechanisms implicated in the pathophysiology of MDS, including intrinsic progenitor abnormalities, epigenetic changes, abnormal apoptosis machinery, immunologic influences, abnormal signal transduction pathways and the role of the bone marrow microenvironment.

GENOMIC INSTABILITY

MDS is thought to derive from the somatic mutation of hematopoietic progenitor cell. Confirmation of the clonality in these patients has been demonstrated by analysis of cytogenetic and X-chromosome inactivation studies (4,5). However, scientific evidence has grown in support of the concept that the cytogenetic abnormalities, seen so frequently in MDS, may be acquired during disease progression rather than reflecting the initial clonal event (5,6). Whether a primary or secondary event, genomic instability, as evidenced by karyotypic changes common in MDS, is thought to play an important role in disease pathogenesis.

Cytogenetic abnormalities in MDS result from the accumulation of genomic damage, failure to repair such damage, or both. Although the etiology of most cases of MDS is unknown, exposure to genotoxic agents such as benzene, radiation, or prior treatment with chemotherapeutic agents is known to increase the risk of developing MDS (7). Other environmental agents that may increase the risk include smoking, heavy metals, pesticides, fertilizers, petroleum products, and organic chemicals (8,9,10).

Another possible mechanism underlying genomic instability involves telomere dynamics, and the enzyme telomerase. Telomere erosion may result in chromosome end fusion and subsequent chromosome instability. Shortened telomere length has been reported to be associated with poor prognosis in patients with MDS (11,12).

EPIGENETIC MODIFICATIONS

While genetic alterations are critical in the pathogenesis of MDS, epigenetic changes also contribute significantly to the disease phenotype. A modern definition of epigenetics refers to " modifications in gene expression that are brought about by heritable, but potentially reversible changes in chromatin structure and/or DNA methylation" (13). Epigenetic changes include methylation of cytosine residues followed by a guanine base (DNA methylation) and post-translational modifications of histones that lead to alteration in chromatin structure at specific gene loci, which in turn determine the transcriptional output of gene.

Methylation of CpG dinucleotides concentrated in the promoter regions of some genes (so-called 'CpG island') results in the functional inactivation of those genes without alteration in the primary sequence. Both hypomethylation and hypermethylation of genome have been observed in hematological malignancies. Hypomethylation of Multi Drug Resistance 1 (MDR1) gene has been described in AML samples and was shown to correlate with increased expression as measured by reverse transcripion polymerase chain reaction (RT-PCR), possibly contributing to multidrug resistance in these patients (14,15). Hypomethylation of c-myc and myeloperoxidase have also been described in sample from patients with primary AML and AML arising from MDS (16).

While mutations in cell cycle control genes such as p15,

p16, and p19 have rarely been described in MDS, hypermethylation of p15 is common. Hypermethylation of the p15INK4B gene promoter has also been observed in 30-50% of MDS cases and a correlation with the percentage of bone marrow blasts has been shown (17,18). p15INK4B is a cyclin-dependent kinase inhibitor that is critical in regulating the G1 phase of the cell cycle. Its activation is downstream of the TGF- /SMAD pathway. This suggests that one mechanism of proliferation of leukemic cells is escape of regulation of G1 phase of cell cycle. Further evidence for the importance of this event in MDS pathogenesis derives from the observation that the degree of methylation correlates with the risk of evolution to AML and clinical prognosis (17). Other genes frequently methylated and silenced in myeloid malignancies include E-Cadeherin, RAR β, and SOCS-1 (19,20).

ABNORMALITIES IN SIGNALING PATHWAY

GROWTH FACTORS

The ineffective hematopoiesis that is characteristic of MDS has led to the investigation of pathways involved in transducing signals from erythropoietin (EPO) and other growth factors. When bone marrow cells from MDS patients are cultured in colony forming assay in the presence of EPO, erythroid colony formation is reduced in comparison to normal controls (21,22). EPO signaling involves a complex cascade of events beginning with the binding of EPO to the erythropoietin receptor (EPO -R). Upon binding EPO, the Janus Kinase, JAK2, is activated (23,24). JAK2 activation leads to downstream tyrosine phosphorylation of a number of proteins. Activation of signal transducer and activator of transcription 5 (STAT5), a downstream protein of JAK2 that is thought to be important in EPO signaling, is impaired in MDS (25). This observation, combined with the other reports of normal presence of EPO-R in MDS patients, indicates that alteration of EPO signaling pathway may have an important role in MDS (26).

Alterations in other growth factor pathways have also been reported in MDS patients samples. GM-CSF and G-CSF priming of reactive oxygen species (ROS) production in neutrophils of patients with MDS is impaired (27). Thrombopoietin signaling has been investigated in the dysmegakaryocytopoiesis seen in MDS, but its role in unclear (28,29).

VASCULAR ENDOTHELIAL GROWTH FACTOR

Vascular endothelial growth factor (VEGF) is a key regulator of angiogenesis. Angiogenesis is increasingly thought to have an important role in MDS and is regulated by multiple signals, including hypoxia inducible factor-1 (HIF-1) and Ras (30). Myelomonocytic precursors of patients with MDS and AML overexpress both VEGF and its high affinity receptor, Flt-1 (31). Inhibition of VEGF reduces leukemia colony formation in clinical samples from MDS patients (32). The importance of VEGF has led to the clinical investigation of VEGF receptor antibodies and VEGF tyrosine kinase inhibitors for the treatment of MDS.

APOPTOSIS

Apoptosis is an ordered cellular process that regulates cell population size in a variety of conditions. First described by Wyllie and coll. (33), apoptosis is an energy-dependent process characterized morphologically by cytoplasmic and nuclear condensation, fragmentation of nuclei into "apoptotic bodies", preservation of plasma membrane integrity and phagocytosis of cellular debris by macrophages in the absence of an inflammatory response (34,35,36). This death mechanism is crucial in maintaining a precise number of cells in a given organism. Alterations in apoptosis have been implicated in a variety of medical disorders including myelodysplasia. A variety of stimuli or insults serve as initiators of the apoptotic pathway. These include chemotherapy drugs, ultraviolet and gamma irradiation, chemical exposure, viral infection, steroid hormones, and various cytokines (e.g.,TNF-α, Fas ligand, TGF-β) (34).

The process of apoptosis may be conceptually divided into extrinsic and intrinsic pathways. Extrinsic triggers include death ligands (e.g., Fas ligand, TNF-α, TNF-related apoptosis-inducing ligand [TRAIL]) which bind to cell surface receptors and activate downstream signal trasduction pathway. Intrinsic signals that activate the apoptotic pathway result from cellular stress, including exposure to radiation, chemicals or infectious processes. Removal of cellular survival signals (e.g., growth factors) may also trigger intrinsic activations of cell death. Both estrinsic and intrinsic activation of a family of cytosolic aspartate-specific cysteine proteases (caspases). Caspases are the final effector molecules of apoptosis, responsible for the cleavage of both cytosolic and nuclear proteins that result in the stereotypic destruction of the cell.

Evidence for alterations in intramedullary apoptosis in early MDS was first suggested through morphological examination of bone marrow hematopoietic cells. Increased apoptosis of bone marrow progenitors was postulated to account for the clinical observation in early MDS of peripheral blood cytopenias in the presence of a hypercellular bone marrow. It was further postulated that decreased apoptosis may explain the later clinical disease progression and accumulation of immature progenitor cells.

Increased apoptosis in MDS has been shown by morphology, immunohistochemestry, flow cytometry and molecular detection of activated apoptosis-related proteins (37-39). Upregulation of Fas in MDS bone marrow samples has also been reported (40-42). Flow cytometry studies have been utilized to better characterized which specific MDS marrow cells are involved in apoptosis. Using flow cytometric of annexin V on the surface of apoptotic cells, Parker and coll. (43) demonstrated increased apoptosis in CD34+ cells from early MDS patients compared with late MDS patients. Li et al. (44) showed that apoptosis occurred predominantly (but not exclusively) in non-clonal cells as determined by concurrent FISH in patients with a suitable clonal marker. Rajapaksa and coll. (45) analysed CD34+ and CD34- subfractions of bone marrow from MDS patients and evaluated the sub-diploid (sub-G1) DNA peak after staining with propidium iodide. They observed that the proportion of CD34+cells with sub-G1 DNA (apoptotic) was increased in comparison to normal bone marrow and bone marrow from AML patients. Bcl-2 and c-Myc oncoprotein levels were also evaluated. C-Myc:Bcl-2 oncoprotein ratios were highest in early MDS sample and lower in late MDS and AML samples. The ratio of the pro-apoptotic BAX to anti-apoptotic BCL-2 was increased in early-stage

MDS but decreased in more advanced disease (37,46). This observation supports the hypothesis that the relative balance between cell-death and cell-survival signals is associated with the increased apoptosis in MDS progenitors. The cause of abnormal apoptosis in MDS is unknown. Both intrinsic cellular defects and extrinsic factors are being investigated in ongoing research. Alterations in the immune-mediated signals, citokine release, and other aspects of bone marrow microenvironment have been implicated.

IMMUNE DYSREGULATION

There is growing evidence that immune dysregulation plays a role in MDS pathophysiology. The relationship between MDS and autoimmunity stimulated the investigation into the immune system in MDS. The incidence of autoimmune disorders appears to be increased in patients with MDS (47). Autologus cytotoxic T lymphocytes have been observed to exert inhibitory effects on MDS myelopoiesis in vitro. Moreover, the feactures of MDS may overlap with aplastic anemia (AA) and large granular lymphocyte (LGL) disease, two diseases thought to be related to autoreactive T lymphocytes (48,49). Clinical studies indicated activity of antithymocyte globulin (ATG) and cyclosporine in the treatment of select groups of patients with MDS (50-52). Given the added observation that tumor necrosis factor alpha (TNFα) mRNA and protein levels are elevated in both bone marrow and plasma samples of patients with MDS , recent clinical trials have evaluated the efficacy of treatment with immunosuppression and anti-TNF therapy (53-57).

Single agent ATG has resulted in complete hematologic responses in up to 10-15% of patients (51). Predictors of response to immunosuppressive therapy include younger age, presence of a paroxysmal nocturnal hemoglobinuria (PNH) clone, human leukocyte antigen (HLA) DR15, hypocellularity, and normal karyotype (58). Responses to ATG have been associated with disappearance of T-cell clones that demonstrate V beta clonality and which suppress hematopoiesis ex vivo (59). Deeg et al. (53) treated 14 tranfusion-requiring patients with MDS with the combination of ATG and the soluble TNF receptor etanercept. Forty-six percent of patients responded, with five patients achieving periods of red blood cell and platelet independence that exceeded 2 years. These impressive results lend further evidence to the premise that immunomodulation may be effective in select patients with MDS.

Fundamental questions remain unanswered about the precise mechanisms underlying autoimmunity in MDS. The hypothesis that T lymphocyes attack specific antigens on MDS clonal progenitors remains unproven. Likewise, it is unclear why some patients respond to immunosuppression and others do not. Important future investigations will include confirmation of the efficacy of immunosuppressive and anti-TNF therapies in phase III clinical trials and in identifying subsets of patients who will most benefit from these therapies.

BONE MARROW MICROENVIRONMENT

CYTOKINE MILIEU

The observation of increased bone marrow apoptosis in patients with early MDS stimulated the evaluation of the bone marrow microenvironment as a mediator of MDS pathophysiology. Relative deficiency or overproduction of numerous cytokines, including interleukin 1β (IL-1β), IL-6, IL-8, stem cell factor, erythropoietin, transforming growth factor beta (TGF-β), GM-CSF and TNF-α have been measured in the bone marrow and serum of patients with MDS with unclear and sometimes conflicting results (60-63). Of all of these cytokines, increased TNF-α has been consistently associated with elevated Fas antigen expression on CD34+ cells. Fas is a membrane protein that

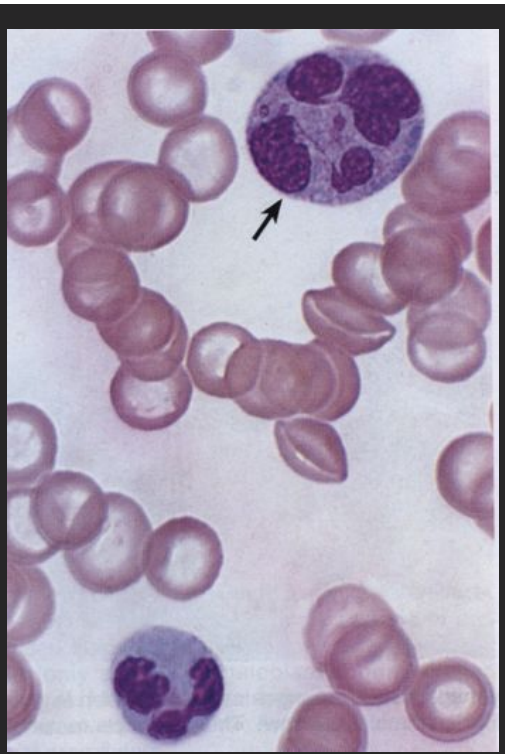
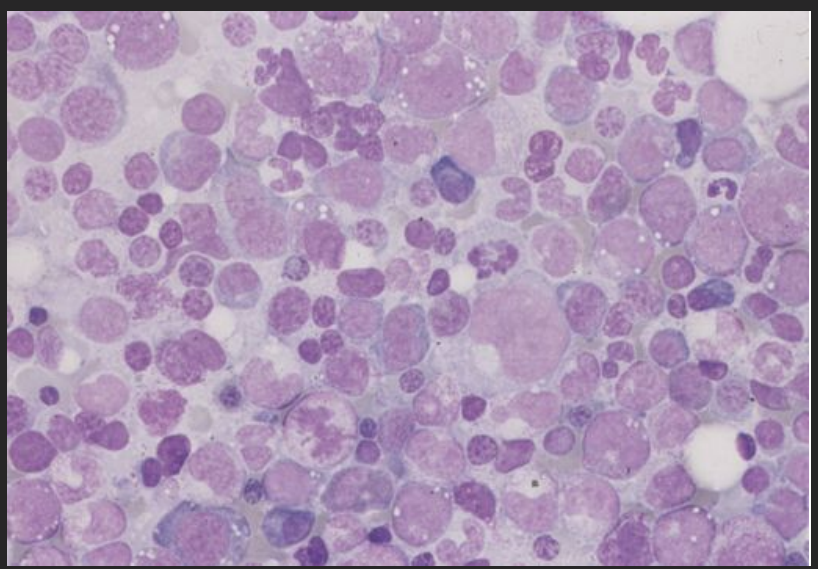


Fig.2 - Neutrophil abnormalities in MDS

Fig.1-Bone marrow picture of a patient with MDS in leukemic trasformation



can initiate apoptotic signals in response to crosslinking by the Fas ligand (41). The resultant downstream activation of caspases, which are the critical proteases that result in apoptotic cell death, indicated the importance of elevated TNF-α levels in promoting apoptosis in MDS (64). The source of increased TNF-α is likely marrow macrophages and T-lymhocytes.

NEO-ANGIOGENESIS

Another aspect of the bone marrow microenvironment that has emerged as an essential factor in pathogenesis of MDS is neo-angiogenesis. Angiogenesis plays a critical role in tumor growth and metastasis (65). Increased microvessel density has been demonstrated in the bone marrow of patients with hematologic malignancies, including MDS (66-68). Neovascularization is mediated by a variety of angiogenic molecules that are released by both tumor cells and normal host cells. Abnormal elevation of several angiogenic cytokines and growth factors in AML samples has been reported. Important molecules include vascular endothelial growth factor (VEGF), basic fibroblast growth factor, (bFGF), angiogenenin, TNF-α, and TGF-β (69-71). Soluble VEGF receptor has been reported as prognostic factor in both AML and MDS patients (72).

CYTOGENETIC AND MOLECULAR ABNORMALITIES

Cytogenetic abnormalities occur in up to 70% of patients with primary MDS and to 90% of patients with therapy-related MDS (5). A well-described difference between primary and secondary MDS is the complexity of abnormal karyotypes. Chromosomal delections are common in MDS as opposed to the balanced translocations that are seen in AML. Over the last decade, intensive investigation has focused on identifying potential tumor suppressor genes in the regions of genetic loss in MDS. Here, we will review some of the insights based on particular chromosomal abnormalities.

CHROMOSOME 5 DELETIONS

Approximately 20% of patients with MDS have abnormalities of chromosome 5 (73). These abnormalities include interstitial delections of the long arm (5q-), monosomy, and unbalanced traslocations. A separate clinical entity of 5q-syndrome has been described in multiple studies in the literature and is now recognized as a separate entity in the WHO classification of MDS (Fig. 3). It is characterized by refractory macrocytic anemia with dyserythropoiesis, a striking female to male ratio of 3:1, normal or high platelet counts, 5q- as the sole cytogenetic abnormality and a low propensity for transformation to AML. In the other cases, abnormalities on chromosome 5 have been seen in famial cases of MDS and in therapy-related MDS (74,75).
The most critical region of deletion is presumed to lie between 5q31 and 5q33. Numerous hematopoietic growth factors are encoded on the long arm of chromosome 5 and loss of these genes is presumed to play a role in MDS pathogenesis. The genes for IL-3, Il-4, IL-5, interferon regulator factor 1 (IRF-1), M-CSF, GM-CSF, and the receptor for M-CSF are localized on the long arm of chromosome 5. These cytokine are critical in the proliferation of granulocytes. However, it is unclear how deletion of any one of these growth factor genes alone would result in a 5q- syndrome phenotype.

CHROMOSOME 7 DELECTIONS

Partial or complete deletion of chromosome 7 is a common finding in MDS and AML. It is seen in a variety of settings and is generally associated with poor prognosis. Approximately 10% of 7q-deletions are seen in the setting of "de novo" MDS. The remainder of 7q-delections are seen in cases of MDS arising after environmental or chemotherapeutic exposure and in cases related to familial genetic disorders (e.g. , Fanconi anemia, neurofibromatosis 1 NF1, congenital neutropenia) (75). Analysis from patients with juvenile myelomonocytic leukemia, which often have monosomy 7, has shown that approximately 30% have NF1 gene mutations (76). NF1 functions as a tumor suppressor gene, encoding a GTPase activation protein acts as a negative regulatior of Ras activity (77). RAS activation occurs in a significant proportion of adult patients with MDS. Gene mutations of RAS or inactivation of the NF1 gene are thought to play an important role in the progression of MDS with monosomy 7 (78).
The region at 7q22.1 has been suggested as a critical breakpoint in myeloid malignancies (79). Genes of interest that have been mapped to chromosome 7q include erythropoietin, plasmin activator inhibitor, T-cell receptor β, aspargine synthase gene and PIK3CG.

CHROMOSOME 17

Clinically, deletion of the short arm of chromosome 17 (17p-) is seen primarily in treatment-related MDS and is characterized by dysgranulopoiesis and pseudo-Pelger-Huët anomaly (80). The p53 gene is located at 17p13.1. p53 is a critical tumor suppressor gene that has significant roles in cell cycle control, DNA repair, and apoptosis. Loss of p53 has been documented in a variety of cancers, and it is likely that it plays a role in a subset of MDS cases.

TRISOMY 8

Trisomy 8 occurs commonly in both acute and chronic leukemias (Fig. 4). A curious observation has been the disappearance of trisomy 8 clones in the course of the disease. This phenomenon is independent of the percentage of blasts in the bone marrow or the clinical status of disease (81,82). As such, it is unclear how significant trisomy 8 is in the pathogenesis of MDS, and therefore, it has not attracted as much attention as some of other cytogenetic abnormalities.

OTHER CHOMOSOMAL DELETIONS

Loss of portions of chromosomes 3, 11, 12, 13, and the Y chromosome have been described with varying frequency in the literature. Likewise, trisomies involving chromosomes 6,13 and 21 have been documented. Although critical regions on each chromosome have been identified and a number of candidate tumor suppressor genes have been identified, the molecular pathogenesis remains elusive.

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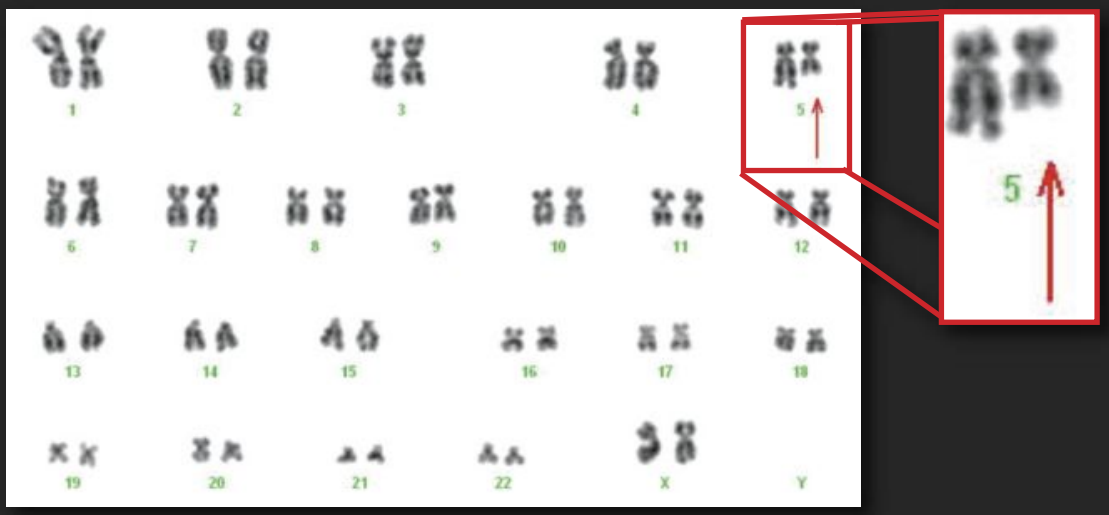

Fig.3 - Deletion of 5q in a patient with MDS.

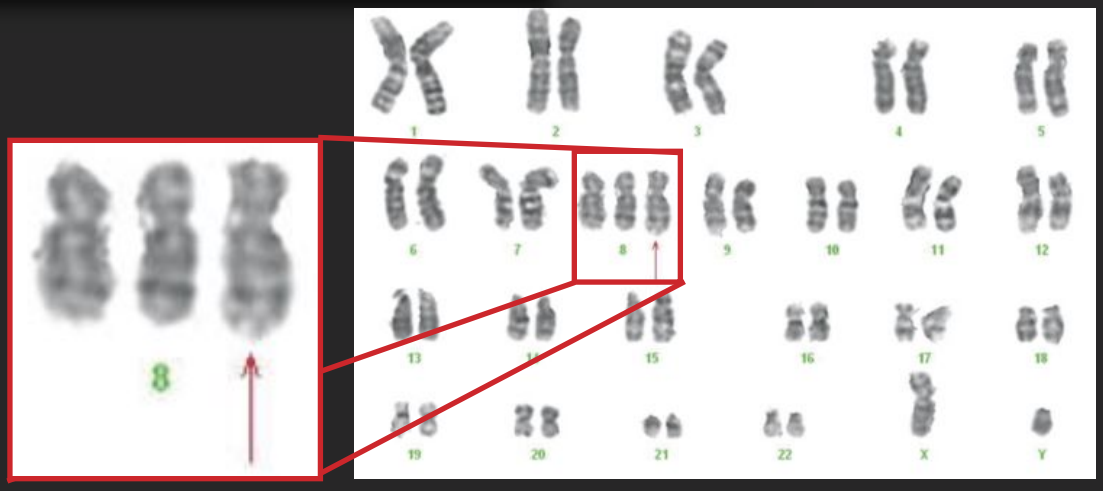

Fig.4 - Trisomy 8 in a patient with MDS.

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