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Eur J Cancer, Vol. 26, No. 11/12, pp. 1113–1115, 1990.
Printed in Great Britain
0277-5379/90 $3.00 + 0.00

The Myelodysplastic Syndrome

THE MYELODYSPLASTIC syndrome (MDS) includes a range of haematological abnormalities in which a clonal population of haemopoietic stem cells arising from an initial genetic insult progresses to a preleukaemic state and ultimately to overt acute myeloblastic leukaemia (AML) [1, 2]. This probably develops as a sequence of events, over many years, in which the earliest stages may be difficult to detect by conventional techniques. It is likely that the mechanism of progression from a trivial haematological aberration to overt leukaemia involves successive genetic changes resulting in abnormal control of cell proliferation and differentiation. Expansion of an abnormal clone may be related to independence from normal growth factor control, insensitivity to normal inhibitory factors and suppression of normal haemopoiesis. In many cases clonal evolution is accompanied by increasing chromosome abnormalities, increasingly malignant characteristics in the bone marrow and eventually AML. Usually neither the nature of the genetic insult nor the lesion produced is known, though MDS may follow either chemical or radiation attack on the marrow. We are now beginning to define the molecular lesions in the genome that are associated with myelodysplasia and the development of leukaemia.

CLINICAL PICTURE

MDS has been well defined since the study published by the French–American–British (FAB) group in 1982 [3]. The clinical manifestations vary from mild anaemia to incipient AML. Five categories of disorder have been defined on the basis of cellular morphology and the percentage of blast cells in the bone marrow [3]. The early stages of MDS may be associated with minimal haematological signs [4] and in practice many cases are discovered by accident after routine blood examination. The haematological picture includes peripheral blood cytopenias associated with a cellular bone marrow in which the cells appear dysplastic and have a high premature death rate. There are numerous functional abnormalities in progenitor cells, immature myeloid cells and in the end cells of all lineages. Although patients usually have a hypercellular bone marrow with a peripheral blood cytopenia, in many cases the marrow is hypoplastic [5] and difficult to differentiate from aplastic anaemia. The occurrence of MDS as a late event in well established cases of aplastic anaemia [6] suggests a common aetiology in some cases. Progenitor cell cultures are usually abnormal. We have found erythroid colonies in peripheral blood cultures to be reduced or absent in 79% of patients at diagnosis and myeloid colonies to be reduced in 45%.

The median age of myelodysplastic patients at diagnosis varies in different reports from about 60 to 75 years, presumably depending on referral patterns and methods of selection. The number of patients below the age of 50 years varies between 3 and 30% in different series. MDS appears to be uncommon in children, but when it occurs there is a high rate of leukaemic transformation to both acute lymphoblastic leukaemia (ALL) and AML. There are no specific symptoms or signs other than those of progressive haemopoietic failure.

GENETIC ABNORMALITIES

It is generally accepted that any genes which code for proteins mediating the cellular response to growth factors may be potential "oncogenes", whether they code for growth factors, receptors, inner membrane or cytoplasmic proteins or nuclear-binding proteins. However, the full range of protein kinases, transcription factors, ribonucleases, cell cycle control proteins, suppressor proteins, matrix and adhesion molecules has hardly begun to be identified. Mutations in any of these could result in disordered proliferation contributing to the malignant process. The identity of the transformed stem cell in MDS is not known but it is often assumed that this is a totipotential cell. In a few cases all the haemopoietic lineages, including lymphoid cells, derive from the same clonal origin [7]; in others the origin is not so clear [8]. The sequential accumulation of genetic damage during leukaemogenesis may be located in the same target cell or in stem cells of varying lineage potential. The role of stromal cell damage is still undefined.

The progress of MDS, and therefore the patient's prognosis, is often measured in terms of haematological indices, such as the degree of anaemia or neutropenia or blast cell count. As the genetic lesions occurring in MDS become better understood it may be more appropriate to measure progression in terms of the burden of these lesions. While some lesions may occur early in the process and others, such as complex chromosome abnormalities, occur late, the total accumulation of lesions indicates the amount of genetic damage, which determines the severity of the haematological disorder.

Chromosome 5 haemopoietic genes

Much of our knowledge of genetic changes in MDS has followed observations of cytogenetic abnormalities [9]. There is a particular concentration of genes relating to growth control and haemopoiesis on the long arm of chromosome 5 and these regions are commonly deleted in MDS [10]. The association of a specific deletion of a critical region of chromosome 5 with the development of an abnormal clone of premalignant haemopoietic stem cells suggests that the lesion is an essential part of the process, though the deletions are inconsistent and only one allele is affected. Growth factor genes can undergo the same pathological processes that result in the activation of proto-oncogenes, and abnormalities in the structure of the GM-CSF gene and its messenger RNA have been described in patients with AML in whom no structural abnormality of chromosome

5 is seen [11]. It is intriguing that the genes coding GTPase activating protein (GAP) that binds to $p21^{ras}$ and the PDGF and CSF-1 receptors are located at 5q [12]. There is of course the possibility that the crucial region of chromosome 5 codes an as yet unidentified repressor gene.

Mutant ras *genes*

The *ras* gene family, H-*ras*, K-*ras* and N-*ras*, code for 21 kD proteins that have GTPase activity and have been implicated in the control of cell proliferation [13]. Mutations in these genes give rise to abnormal protein products that have the capacity to transform certain cells to a malignant phenotype. *ras* mutations have been found in a wide range of human malignancies and N-*ras* has been particularly implicated in AML and chronic myeloid leukaemia (CML) [14, 15]. Activation of these genes is associated with mutations in codons 12/13 or codon 61. In our original study [16] mutational *ras* activation, assessed by polymerase chain reaction (PCR) and hybridisation with synthetic oligonucleotide probes together with the use of a nude mouse tumorigenicity (NMT) assay in some cases, revealed point mutations in 21 out of 50 cases of MDS, including 3 with primary acquired sideroblastic anaemia. N-*ras* mutations were found most commonly. In 2 cases double mutations were found. Recent findings with more extensive use of the NMT assay revealed 40 out of 75 patients with mutant *ras* genes in nucleated blood cells.

The presence of mutations in patients with the most benign forms of MDS, presumably at an early stage in the preleukaemic process, suggests that it may be an early event. However, there appears to be a high rate of leukaemic transformation in patients with mutant *ras* genes. In 39 of our patients with a *ras* mutation, 14 have progressed to acute leukaemia while only 3 out of the 36 with no mutation have transformed.

The evidence suggests that *ras* mutations are found both in early and in late stages of haemopoietic malignant progression. Among 70 patients treated for lymphoma by standard chemotherapy regimens between 1 and 13 years previously and with no residual disease or haematological abnormality, 9 had mutant *ras* genes revealed by the polymerase chain reaction and hybridisation, again suggesting that this can be an early lesion in the preleukaemic process [17]. *ras* mutations have also been found in DNA from 3 out of 19 samples of peripheral blood leucocytes from healthy, haematologically normal subjects. 2 had H12 valine mutations and 1 had coincidental N12 alanine and H12 aspartate mutations [18]. These results suggest a high incidence of *ras* mutations in a normal population, the inability of mutant *ras* genes alone to produce observable preleukaemic changes and the existence of subjects predisposed to future preleukaemic change.

Mutant fms *genes*

The c-*fms* proto-oncogene encodes the functional receptor for CSF-1. Mutations at codon 301 mimic a ligand-induced conformational change, resulting in constitutive tyrosine kinase activity and transforming activity in NIH3T3 cells [19]. Mutations at codon 969 further enhance the transforming activity of 301 mutants, but are insufficient to transform by themselves. The tyrosine residue at codon 969 is thought to serve a negative regulatory function, loss of which releases the receptor from its control. Two mutations at codon 301 and 14 mutations at codon 969 have been detected in MDS and AML patients. They appear to occur in about 20% of patients with CML and M4 AML but with a significantly lower frequency in other types of MDS. In 2 cases *fms* mutations were found in AML patients that were not present in an earlier MDS stage. In 1 case of refractory anaemia with excess blasts a *fms* mutation disappeared after transformation to AML [20].

In haematologically normal patients studied following cytotoxic therapy, 11 out of 70 had a mutation at codon 969, 3 of whom also had a *ras* mutation [21]. This supports the suggestion that point mutations are an early manifestation of the leukaemogenic process, associated with clonal haemopoiesis at an earlier stage than the lesions giving rise to overt haematological abnormality.

TREATMENT

Conventional therapy for patients with MDS relies largely on the administration of blood components to combat cytopenias and antibiotics to counter infections. Most patients with MDS die from haemorrhage or sepsis resulting from the poor production of leucocytes and platelets, whose failure to mature normally renders them functionally inadequate. Attempts at more specific therapy have taken several directions. Differentiation agents, including retinoic acid and 1,25 dihydroxyvitamin D_3, have been used with varying degrees of success [22]. Alpha-interferon has not proved clinically useful [23]. High-dose conventional chemotherapy has had limited success in some younger patients [22] and for a few, bone marrow transplantation may offer the prospect of cure [24].

More recently there have been energetic attempts to evaluate the therapeutic role of recombinant human growth factors, either alone or in combination with cytotoxic agents. Clinical remissions can be obtained with granulocyte colony-stimulating factor, granulocyte–macrophage colony-stimulating factor, or interleukin-3 alone in some cases [25–27] but there are no data on survival.

Treatment schedules are evolving rapidly and it will be impossible to define a standard approach to therapy until we have acquired far more experience. In many patients with MDS the clinical signs are so minimal as to warrant no action, except long-term follow-up and careful scrutiny for signs of progression.

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Eur J Cancer, Vol. 26, No. 11/12, pp. 1115–1118, 1990.
Printed in Great Britain

0277–5379/90 $3.00 + 0.00

Chemotherapy Alone for the Treatment of Early-stage Hodgkin's Disease

INTRODUCTION

THE INTRODUCTION of modern radiotherapy with megavoltage equipment has greatly improved the prognosis of early-stage Hodgkin's disease (ESHD). In 1966 the eradication capacity of radiotherapy was first demonstrated by Kaplan who showed that the risk of recurrence was a function of dose: the proportion of in-field recurrences dropped from 60%–80% with 10 Gy or less to 1.3% at 40 Gy [1]. Thus the possibility of cure of ESHD became a reality, with a change from palliative to radical treatment policy with high-dose radiation and extended-field techniques. Successive improvements of technical and staging procedures, including the use of parallel opposed anterior-posterior portals, linear accelerators, lymphography and surgical laparotomy, resulted in complete response (CR) rates of more than 90% and a high cure rate of more than 85% [2–7]. Nevertheless, differences in technical procedures and radiation delivery equipment can affect the final results, particularly disease-free survival (DFS). Thus results from the many studies of extended-field radiotherapy in ESHD show considerable variation in DFS ranging from 65% to 82.5% at 10 years [2–10]. In addition, infield recurrence rate may vary in relation to the experience of the radiotherapist [11]. Despite this wide variation of DFS, the same studies have shown similar high overall survival (OS), with more than 85% of ESHD patients alive 10 years after initial treatment [2–9]. The differences between DFS and OS are essentially due to the effectiveness of modern chemotherapy in the salvage of patients who relapse after radiotherapy. In the late 1960s, De Vita *et al.* introduced the MOPP regimen (mustine, vincristine, procarbazine and prednisone) into the management of advanced stage Hodgkin's disease [12]. The superiority of MOPP or its derivatives in improving the long-term outlook for relapsing ESHD patients compared with palliative radiotherapy or single-agent chemotherapy was clearly demonstrated by Zagars and Rubin in an historical comparison between two similar ESHD series initially treated with radiotherapy alone, before and after 1969 [13]. On the basis of these observations, chemotherapy was combined with radiotherapy for the treatment of ESHD, especially to improve DFS. Several randomised studies in which combined chemo-radiotherapy was compared with radiotherapy alone demonstrated that DFS could be improved, but again OS was similar with both options [2,14–16]. Moreover, in these studies, an increased frequency of side-effects, including secondary leukaemia, was usually observed.

Nevertheless, chemotherapy compared with radiotherapy has several advantages: (1) it is less expensive and available worldwide; (2) its use is associated with less variable results between institutions; (3) it does not induce abnormal muscle and bone growth; and (4) it does not require accurate staging procedures. Based on these considerations, isolated studies in paediatric patients were started to determine whether chemotherapy alone would be a useful option in ESHD. These studies, although in