Pergamon

European Journal of Cancer Vol. 30A, No. 10, pp. 1407–1408, 1994

0959-8049/94 $7.00 + 0.00

0959-8049(94)00269-X

Comments and Critique

In vitro Growth Behaviour of Acute Myeloid Leukaemic Cells

E. Vellenga

ACUTE MYELOID leukaemia (AML) is characterised by an accumulation of immature blast cells in the bone marrow, finally resulting in disturbed production of normal haematopoietic cells. In these cases, the proliferation and differentiation of normal haematopoietic progenitor cells are suppressed, but polyclonal haematopoiesis can be restored after successful intensive chemotherapy [1]. These findings indicate that leukaemic cells have properties which release them from normal regulatory pathways, and provide them with a growth advantage over the normal haematopoietic progenitor cell.

In vitro culture studies with cells from AML patients have demonstrated spontaneous proliferation of leukaemic progenitor cells in 25–35% of cases [2]. This is a distinct difference from normal haematopoietic progenitors, which are dependent on haematopoietic growth factors for their proliferation and differentiation. Additional studies have revealed that spontaneous *in vitro* proliferation is caused by the constitutive release of growth factors, especially granulocyte–macrophage colony-stimulating factor (GM–CSF) and interleukin-1 (IL-1) [3]. The release of GM–CSF involved the autocrine production of IL-1, since antibodies against IL-1, or the addition of IL-1 receptor antagonists to the culture, eliminated the secretion of GM–CSF [4]. Moreover, the addition of anti-IL-1 antibodies or IL-1 receptor antagonists did abrogate the spontaneous proliferation of the blast cells, indicating that autocrine secretion of cytokines was responsible for the spontaneous proliferation. The cause of the constitutive expression of IL-1 or additional growth factors has not been elucidated.

It is conceivable that an aberrant expression or regulation of suppressor genes or oncogenes could contribute to growth deregulation. Hypophosphorylation of the retinoblastoma gene has been demonstrated in AML [5]. In addition, point mutations have been demonstrated in the *ras*, *myc* and *myb* oncogenes, which in turn modulate the expression of different cytokine genes. This is also illustrated by the fact that cells transfected with the N-*ras* oncogene did express, constitutively, cytokines such as IL-1, GM-CSF and G-CSF [6]. Moreover, chromosome translocations may cause aberrant expression of transcription factors which can further modulate the proliferation and differentiation of AML cells.

Correspondence to E. Vellenga at the Department of Hematology, University Hospital, 9700 RB Groningen, The Netherlands.
Received 23 Mar. 1994; accepted 20 Apr. 1994.

The biological behaviour of AML cells *in vitro* also predicts, to some degree, the clinical outcome of the patients. This was recently emphasised by two studies in which the spontaneous proliferation of AML cells was an independent prognostic factor for the outcome of the patients. In both studies, spontaneous *in vitro* proliferation was associated with unfavourable prognosis [7, 8]. Patients with cells that showed low autonomous proliferation *in vitro* had the probability of 36% survival at 3 years, compared to 3% survival probability in patients with cells that had a high autonomous proliferation [7]. In the second study, the overall disease-free survival at 5 years was 11% for the autonomous proliferation group and 54% for the non-autonomous proliferation group [8].

In this issue of the *European Journal of Cancer* (pp 1511–1516), a study is described in which *in vitro* proliferation of AML cells, in response to haematopoietic growth factors, has been correlated with different biological properties of AML cells, such as the level of expression of c-*myc*, c-*myb* and IL-1. No correlation between *in vitro* growth behaviour of AML clonogenic cells, measured with primary and secondary plating efficacy, and the studied biological characteristics of the whole leukaemic cell population was observed.

The lack of correlation may, in part, be due to the fact that the frequency of AML progenitor cells is very low and not well defined, as shown recently by Lapidot and colleagues [9]. They used immunodeficient SCID mice to study the biological behaviour of human AML cells. AML progenitors did not propagate in SCID mice, if the mice were not treated with human haematopoietic growth factors. However, a strong expansion of human AML blast cells was noted in SCID mice treated with human mast cell growth factor or PIXY321. In addition, they observed a heterogeneity in AML progenitors by sorting cells into CD34- and CD38-positive and negative cell fractions. CD34 is a cell surface marker normally expressed on a small population of bone marrow cells, including progenitor cells and pluripotent cell. Expression of CD38 on CD34+ cells is an important marker for lineage commitment and, therefore, the CD34+/CD38- phenotype defines an immature human cell in normal bone marrow. By using different sorted cell populations, Lapidot and colleagues showed that expansion of AML cells in the SCID mice was restricted to the CD34+/CD38-sorted population. By using the CD34− or CD34+/CD38+ AML fractions, no engraftment of AML cells was shown. In contrast, by using CD34+/CD38− or CD34+/CD38+ in the *in vitro* colony assay

before injection, no differences in colony numbers were shown. These results suggest that different AML progenitor cells can be defined in the whole AML population, dependent on the test system used. Moreover, the findings indicate a hierarchy in AML progenitors, including cells with short-term and long-term repopulating abilities, as has already been defined in the normal counterpart. This expanding knowledge on the biological behaviour of AML progenitors *in vitro* may be used in future clinical trials to optimise new therapeutic strategies, especially in the possible elimination of AML progenitor cells from the graft.

1. Herrmann F, Vellenga E. The role of colony-stimulating factors in acute leukemia. *J Cancer Res Clin Oncol* 1991, **116**, 275–282.
2. Vellenga E, Halie MR. Effects of colony-stimulating factors on myeloid leukaemia cells. In Mertelsmann R, Herrman F, eds. *Hematopoietic Growth Factors in Clinical Applications*. New York, Marcel Dekker, 1991; 363–378.
3. Young DC, Wagner K, Griffin JD. Autocrine secretion of GM-CSF in acute myeloblastic leukaemia. *Blood* 1986, **68**, 11–78.
4. Rambaldi A, Torcia M, Bettoni S, *et al.* Modulation of cell proliferation and cytokine production in acute myeloblastic leukaemia by interleukin-1 receptor antagonist and lack of its expression by leukemic cells. *Blood* 1991, **78**, 3248.
5. Furukawa Y, DeCaprio JA, Belvin M, *et al.* Heterogeneous expression of the product of the retinoblastoma susceptibility gene in primary human leukemia cells. *Oncogene* 1991, **6**, 1343–1346.
6. Demetri GD, Ernst TJ, Pratt ES, *et al.* Expression of *ras* oncogenes in cultured human cells alters the transcriptional and posttranscriptional regulation of cytokine genes. *J Clin Invest* 1990, **86**, 1261–1269.
7. Löwenberg B, Putten van WLJ, Touw IP, *et al.* Autonomous proliferation of leukemic cells *in vitro* as determinant of prognosis in adult acute myeloid leukemia. *N Engl J Med* 1993, **328**, 614–619.
8. Hunter AE, Rogers SY, Roberts IAG, *et al.* Autonomous growth of blast cells is associated with reduced survival in acute myeloblastic leukemia. *Blood* 1993, **83**, 899–903.
9. Lapidot T, Sirard C, Vormoor J, *et al.* A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature* 1994, **367**, 645.

Pergamon

European Journal of Cancer Vol. 30A, No. 10, pp. 1408–1411, 1994

0959-8049/94 $7.00+0.00

0959-8049(94)00218-5

Predictive Value of Thymidylate Synthase and Dihydropyrimidine Dehydrogenase

G.J. Peters, C.L. van der Wilt and C.J. van Groeningen

INTRODUCTION

POTENTIATION OF inhibition of thymidylate synthase (TS) is considered to be the mechanism by which leucovorin modulates the antitumour activity of 5-fluorouracil (5FU) against colorectal cancer [1, 2]. Combination of leucovorin with 5FU has doubled the response rate of single-agent 5FU from approximately 10–15% to 20–40%, depending on schedule and dosing of both 5FU and leucovorin [1, 3]. However, this still means that the majority of patients does not benefit from this treatment in terms of response rate. In most of the studies, no major effect on survival time has been reported.

ROLE OF TS IN THE ACTION OF 5FU

Sensitivity and resistance

Several factors may be responsible for the lack of potentiation of 5FU by leucovorin. These factors are related to an aberration in the metabolism and disposition of either leucovorin or of 5FU. Besides the interpatient variation in pharmacokinetics of both drugs, there are a number of cellular or tumoral factors which determine whether leucovorin can modulate 5FU [1]. In order to be active, leucovorin has to be transported across the cellular membrane, a process mediated by a reduced folate carrier. For the folate antagonist, methotrexate, transport deficiency has been associated with resistance [4]. After transfer of the membrane, leucovorin has to be metabolised to 5,10-methylene-tetrahydrofolate (CH_2-THF), which is the one-carbon donor required for conversion of dUMP to dTMP, the reaction catalysed by TS. CH_2-THF is also essential for the formation of a stable ternary complex between FdUMP (the activated form of 5FU), TS and CH_2-THF. This complex is responsible for inhibition of thymidylate synthase. Although it has been reported that intermediates of the metabolic pathway of leucovorin to CH_2-THF can also support the formation of this ternary complex, CH_2-THF is the best substrate [5]. An even better inhibition is achieved in the presence of polyglutamates of CH_2-THF [6], which are formed by the action of folylpolyglutamate synthetase. A lack of polyglutamylation has been associated with resistance to 5FU [7]. For 5FU, transport deficiency has not been reported to be associated with resistance, but aberrations in its metabolism to FdUMP (the inhibitor of TS) or to FUTP (the metabolite for RNA incorporation) have been associated with resistance [1]. Considering the inhibition of TS, a number of possibilities exist, such as an increased activity of the enzyme (for instance, due to gene amplification) or altered kinetics of the enzyme (e.g. decreased affinity of either FdUMP or CH_2-THF to TS, leading to a diminished inhibition) [1]. Thus, it would be of interest to determine these parameters in patients who are receiving 5FU in combination with leucovorin for anti-cancer treatment. This information would also be

Correspondence to G.J. Peters.
The authors are at the Dept. of Oncology, Free University Hospital, PO Box 7057, 1007 MB Amsterdam, The Netherlands.
Received 10 May 1994; accepted 17 May 1994.