Angiogenic Growth Factors: Autocrine and Paracrine Regulation of Survival in Hematologic Malignancies

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ABSTRACT
Recent research has focused on the role of angiogenic growth factors and their ability to mediate tumor growth and metastases, both in solid tumors and in hematologic malignancies. The bone marrow microenvironment is the setting for a wealth of complex interactions that include cell-to-cell contacts as well as secretion of and response to soluble factors. Abundant evidence supports the role of basic fibroblast growth factor (bFGF) in contributing to the dysregulation of apoptosis that is the hallmark of chronic lymphocytic leukemia (CLL). In fact, CLL cells themselves express bFGF; intracellular levels of this cytokine correlate with clinical CLL stage. Other stromal factors mediate the inhibition of apoptosis in CLL as well, suggesting that strategies to block the responses of CLL cells to these factors may represent effective therapies. More broadly, the class of agents known as angiogenesis inhibitors may offer important advantages with respect to the treatment of numerous types of malignancies. Currently, a number of clinical trials are under way to evaluate the clinical potential of several different angiogenesis inhibitors in several hematologic neoplasms. The Oncologist 2001;6(suppl 5):4-7

INTRODUCTION
The bone marrow microenvironment plays a critical role in regulating the growth and differentiation of hematopoietic cells. Both secreted growth factors and cytokines as well as direct cell-to-cell contacts affect these processes. Figure 1 highlights some of the key components of the diverse interactions between different cell types and the many soluble factors leading to hematopoietic cell stimulation [1]. Cell growth and proliferation, expression of characteristic cell surface markers, and production and secretion of specific growth factors are all mediated by such complex interactions.

One type of growth factor is angiogenic growth factor, which stimulates the development of new blood vessels. Two types that have been identified are vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). Both are especially important in promoting new vessel growth in response to inflammatory cytokines and other growth factors. The actions of VEGF are specific to endothelial cells, the site of the VEGF high-affinity tyrosine kinase receptors. The actions of bFGF, in contrast, are pleiotropic, targeting numerous cell types [2].

Certain cytokines induce the expression of VEGF and bFGF by vascular smooth muscle cells, thereby indirectly, rather than directly, stimulating endothelial cell proliferation [3]. “Indirect” angiogenic cytokines include platelet derived growth factor BB and transforming growth factor-β [3]. Both cause upregulation of VEGF and bFGF transcription in vascular smooth muscle cells. Hypoxia is another highly angiogenic stimulus; it has been found to increase the expression of VEGF but not bFGF [3].

It has long been appreciated that new vessel growth is essential for the expansion of solid tumors greater than 2 mm³ in size [4]. The number and density of neoangiogenic vessels seem to have prognostic significance for some solid tumors. The importance of angiogenic activity in the progression of hematopoietic malignancies, however, is less clear. There is some evidence that angiogenic growth factors figure prominently in the pathogenesis of a number of hematologic cancers [4-6]. Bellamy and colleagues studied a variety of human hematopoietic cell lines to determine whether VEGF mRNA and protein were expressed [4]. They found VEGF mRNA transcripts in all 12 human hematopoietic...
cell lines examined, and they noted VEGF protein expression as well. Flt-1, a VEGF receptor, was present in five of these lines, suggesting that autocrine mechanisms may be involved in VEGF-mediated growth stimulation of hematopoietic cancer cells.

**GROWTH FACTOR MECHANISMS: ESCAPE FROM APOPTOSIS (CLL)**

Although many malignancies result from abnormal cell growth, others develop as a consequence of impaired apoptosis. One example is chronic lymphocytic leukemia (CLL). CLL is the most common leukemia occurring in adults and is characterized by an accumulation of small B-lymphocytes. Despite its prolonged, indolent clinical course, most patients eventually die of their disease [7].

Apoptosis is a regulated process of gradual disruption of the nucleus, followed by fragmentation of the cytoplasm leading to cell death. It is a normal physiologic process that occurs in many settings: it is necessary for tissue remodeling and repair, and it occurs when essential growth factors or cytokines are withdrawn [8]. The fundamental problem in B-cell malignancies such as B-CLL is that the malignant lymphocytes are arrested in G0 because of disturbance of the normal mechanisms regulating apoptosis [9, 10]. Several molecular defects may be responsible. Specifically, the anti-apoptosis bcl-2 oncogene and its family members are very often overexpressed in CLL cells; an imbalance between proapoptotic bax and bcl-2 appears to be responsible for the failure of apoptosis induction in many CLL cases [11]. Other molecular defects include p53 gene inactivation, impaired B-cell differentiation, and aberrant expression of adhesion molecules [11].

Numerous cytokines or growth factors, by inhibiting apoptosis, have been shown to promote cell survival and influence the longevity of CLL cells. Several of these key cytokines, including VEGF, bFGF, and stromal cell-derived factor (SDF)-1, derive from bone marrow stromal cells (Fig. 2). In particular, bFGF plays a critical role in the pathogenesis of CLL and has been studied quite extensively [12].

Intracellular bFGF levels in the lymphocytes of CLL patients have been measured using an enzyme-linked immunoassay [12]. In a study of 36 patients with CLL and 15 normal blood donors, in which the CLL patients were staged according to the modified Rai staging system (low-, intermediate-, or high-risk), significant differences were observed in bFGF levels in CLL cells among the different patient groups. In high-risk CLL patients, the median intracellular bFGF was 381.5 pg/2 × 10^5 cells, compared with 90.5 pg/2 × 10^5 cells in intermediate-risk patients (p = 0.0001) and 4.9 pg/2 × 10^5 cells in low-risk patients (p < 0.0001 for low- versus high-risk; p = 0.00119 for low- versus intermediate-risk).

Fludarabine is a proapoptotic agent that is often used to treat CLL. CLL cells have been cultured in the presence of
fludarabine to determine whether the level of bFGF influences CLL cell susceptibility to apoptosis induction [12]. After 4 days in culture with 3 µmol/l fludarabine, CLL cells expressing low, intermediate, and high levels of bFGF showed viability rates of 46%, 47%, and 64%, respectively [12]. Prolonged culture sustained these differences, with viability rates of 16%, 24%, and 46% for CLL cells expressing low, intermediate, and high levels of bFGF, respectively. These differences in cell viability according to bFGF levels were significant (p = 0.0063 for high versus low bFGF levels; p = 0.0114 for high versus intermediate bFGF levels) [12].

Addition of exogenous bFGF (100 ng/ml) has also prolonged survival of CLL cells cultured in the presence of fludarabine. This escape from fludarabine-induced cell death occurred regardless of the endogenous levels of bFGF in the cells. Morphologic analysis confirmed fludarabine-induced apoptosis in CLL cells and abrogation of these changes by the addition of bFGF [12]. CLL cells from a patient with low-risk disease showed virtually no apoptosis prior to the addition of fludarabine to the medium. Incubation with fludarabine for 36 hours resulted in apoptosis in 70% of the cells, but addition of bFGF to fludarabine decreased the proportion of apoptotic cells to 22.5% [12].

Investigations seeking to elucidate the mechanisms by which bFGF prolongs survival in CLL cells have been done using established cell lines [13]. The results showed that bFGF upregulates bcl-2 expression in a time-dependent fashion. Enhanced levels of bcl-2 apparently underlie the resistance to apoptosis conferred by exogenous bFGF, prolonging CLL cell survival [13]. Upregulation of bcl-2 and delayed apoptosis was also observed when NIH 3T3 cells were induced to express bFGF [14].

In addition to bFGF, other cytokines have the potential to cause similar effects on CLL cells. One example is SDF-1. Stromal cells in the bone marrow normally produce high levels of SDF-1, contributing to early B-cell differentiation. B-CLL cells possess SDF-1 receptors and can home to stromal cells that produce this chemokine. When peripheral blood mononuclear cells from CLL patients are cultured in vitro, a subset undergo morphologic changes and spontaneously display stromal cell markers and express SDF-1 [15]. Co-culture of CLL cells with these cells protects the CLL cells from spontaneous apoptosis, prolonging their survival. These nurse-like cells (NLCs) confer their anti-apoptotic actions to B-CLL cells via SDF-1. NLCs express stromal cell markers in vitro, providing further inferential evidence for the participation of stromal cells in the bone marrow microenvironment and as an influence on the biologic behavior of CLL cells. Furthermore, addition of synthetic SDF-1 to CLL cells cultured without NLCs has been shown to partially protect cells from initiating apoptosis, although not as effectively as does culturing with NLCs. Addition of anti-SDF-1 antibodies to NLC-CLL co-cultures markedly reduced viability of CLL cells, abrogating the protective effects of the NLC [15].

Adhesion receptors also mediate prolonged survival of CLL cells. As one example, α4β1 integrin mediates an interaction between CLL cells and extracellular matrix fibronectin. In vitro, when this interaction causes B-CLL cells to attach to fibronectin, apoptosis is prevented and cell survival increases. One consequence of the integrin-CLL cell interaction is an increase in the bcl-2/bax ratio, thereby protecting CLL cells from apoptosis [16]. Taken together, these results suggest that strategies to block the response to stromal cell-derived cytokines may prove effective in the treatment of CLL and other hematologic malignancies (Table 1).

### Table 1. Evidence defining bFGF as a survival factor for CLL

- CLL cells express elevated levels of intracellular bFGF
- Intracellular bFGF levels in CLL cells correlate with stage
- bFGF contributes to the cells’ resistance to an apoptotic stimulus

### Angiogenesis Inhibitors as a New Anticancer Treatment Strategy

Recognition of the significance of stromal cell-tumor cell interactions has spurred an intensive research effort to develop targeted molecular therapies that can disrupt these interactions, thereby decreasing tumor cell viability. Clinical trials have already begun with inhibition of the VEGF receptor and c-kit. The c-kit receptor tyrosine kinase is expressed by hematopoietic progenitor cells, mast cells, germ cells, and some human tumors [17]. The ligand that binds to c-kit plays a pivotal role in the stimulation of hematopoietic stem cells, often in synergy with other cytokines. Luens and colleagues have shown that c-kit ligand acts together with thrombopoietin and flk2/flt-3 ligand to enhance the number of primitive hematopoietic progenitor cells capable of engraftment [18]. This holds potential benefit for patients undergoing stem cell transplants. An alternative application of the inhibition of c-kit receptor tyrosine kinase activity may be in the treatment of tumors whose growth is stimulated by c-kit. Treatment with STI571, which inhibits c-kit kinase activity, decreases downstream activation of target proteins critical to cellular proliferation and survival, thus promoting apoptosis [17].

Considering the importance of angiogenesis-promoting factors in supporting growth and inhibiting apoptosis in hematologic malignancies as well as solid tumors, the angiogenesis inhibitors represent a novel class of agents for...
cancer treatment (see articles by List and Giles in this supplement [19, 20]). Unlike typical cytotoxic therapies, which target tumor cells specifically, these agents influence the function of endothelial cells and other normal cells interacting with the malignant cells. Consequently, a number of different therapeutic strategies can be pursued. It is thought that antiangiogenic therapy will not generate drug-resistant cell populations, given that malignant cells are not the direct target. Antiangiogenic agents could be given concurrently with conventional chemotherapy, combining two different modes of action to affect tumor growth. In this application, they may confer additional advantage; for instance, they may show an immunity (since they do not directly target tumor cells) to factors such as hypoxia or inefficient drug delivery kinetics, which would otherwise be a significant barrier to therapy. Alternatively, angiogenesis inhibitors could be administered for a prolonged period after completion of cytotoxic chemotherapy, theoretically preventing residual disease populations from achieving significant size and minimizing development of new genetic aberrations. Agents with antiangiogenic properties could possibly even be given prophylactically to individuals at high risk for developing cancer [21].

For all of these reasons, antiangiogenesis as a novel therapeutic strategy holds great promise as a means of mediating tumor growth and metastatic progression [22]. The results of numerous ongoing clinical trials evaluating the clinical utility of angiogenesis inhibitors in patients with a variety of hematologic neoplasms will be received with great interest.

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