CONCISE REPORT

HL-60 Cell Line Was Derived From a Patient With FAB-M2 and Not FAB-M3

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The leukemia from which the human cell line HL-60 was derived was classified in 1976 as acute progranulocytic leukemia (APL), although it was recognized to show a number of atypical features. In the ensuing 10 years, the concept of APL and its integral association with t(15;17) has evolved, and the concept of APL as a morphologically recognizable entity has become embodied in the term French-American-British classification M3 (FAB-M3). It is now recognized that not every case of leukemia with a high proportion of progranulocytes can be classified as FAB-M3. We reviewed the light and ultrastructural morphology of the original diagnostic material from this case, and we report that the leukemia from which HL-60 was derived does not conform to the currently recognized entity of FAB-M3 and is more appropriately classified as an acute myeloblastic leukemia with maturation, FAB-M2.

A HUMAN ACUTE myeloid leukemia cell line, HL-60, was derived from a 35-year-old woman diagnosed and treated at M.D. Anderson Hospital. The patient was considered to have acute progranulocytic leukemia (APL) but had atypical clinical features, demonstrated cytogenetic abnormalities other than the typical t(15;17), and lacked several of the morphologic findings expected in APL. The original cell cultures were established in the Laboratory of Tumor Cell Biology of the National Cancer Institute in 1976, and the unique nature of HL-60 soon became apparent: it was able to grow in suspension culture without added conditioned media, and it was a demonstrably malignant human cell line that not only proliferated but also differentiated along myeloid lines without exogenous inducers. HL-60 has proven to be a productive model for investigating the process of myeloid differentiation, and has also been a convenient source of various mRNAs and proteins (such as tumor necrosis factor and myeloperoxidase) found in hematopoietic cells. Recent studies have characterized HL-60 at the level of genes and gene expression, focusing in particular on the role oncogenes play in this leukemic cell line. It has been shown, for example, that both the N-ras and the c-neu genes are altered in HL-60, that c-myc is stably amplified, that the p53 gene, which has been localized to 17p13, is largely deleted, and that one allele of the GM-CSF gene, which has been localized to 5q21-q23, is truncated. Several investigators have pointedly compared molecular features of APL (French-American-British classification M3, FAB-M3) to those of HL-60, possibly hoping that this cell line would prove to be a valid in vitro model for FAB-M3. Because of the continued importance and frequent study of this cell line (the original description has been cited more than 700 times in the scientific literature), and because no other case of FAB-M3 has ever been established as a continuous cell line, we were prompted to review the original diagnostic material from this case. We now report that the leukemia from which HL-60 was derived does not conform to the currently recognized entity of FAB-M3 and is more appropriately classified as an acute myeloblastic leukemia with maturation, FAB-M2.

MATERIALS AND METHODS

The Wright-Giemsa stained bone marrow smears and the myeloperoxidase stain from the patient’s initial diagnostic bone marrow aspirate were well preserved and were reviewed. Electron micrographs from the initial aspirate were also reviewed, as were photographs from the initial cytogenetic studies.

RESULTS

Light-microscopic findings. Review of the bone marrow smears (by Dalton and Stass) showed 37% myeloblasts, 44% progranulocytes, and 8% cells at the myelocyte stage or beyond in contrast to the originally reported differential of 4% myeloblasts, 85% progranulocytes, and 4% cells at the myelocyte stage or beyond. This discrepancy is related to the fact that the leukemic cells showed marked nuclear/cytoplasmic asynchrony, as previously noted (Fig 1A). Whether most of the leukemic cells are classified as blasts or progranulocytes can be debated. The FAB group has published guidelines for making the distinction between blasts and progranulocytes, but the guidelines are less ambiguous when applied to normal myeloid maturation than to cases like this one, in which maturation is highly asynchronous. We classified a cell as a blast if the nucleus was centrally located and the chromatin was finely dispersed, even when there was prominent cytoplasmic granulation. In any event, this problem does not have a major bearing on our contention that this case of leukemia was not FAB-M3, as will be discussed below. The leukemic cells had blastic, rounded, centrally located nuclei and showed none of the lobulated nuclear configurations seen in the variant form of FAB-M3 (Fig 2A). Most of the leukemic cells had conspicuous cytoplasmic granulation, but they did not have the enlarged, angular granules that frequently obscure the nuclear outline in hypergranular FAB-M3 (Fig 2B). No faggot cells or Auer rods were identified. Most of the mature neutrophils

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Fig 1. Initial bone marrow specimen obtained before treatment from the patient from whom HL-60 was derived. (A) The leukemic cells show blastic nuclei, prominent nucleoli, along with conspicuous cytoplasmic granulation, indicating asynchronous development. (B and C) Mononuclear neutrophils are present (arrows). (D) Eosinophil with interspersed basophilic-staining granules. Wright-Giemsa stain. (E) The majority of cells are myeloperoxidase positive, but staining seems less intense than is usually seen in FAB-M3. Washburn's peroxidase stain.
Fig 2. (A) FAB-M3, variant form. Nearly all the leukemic cells show the characteristic nuclear lobulation; the cytoplasm shows a fine “dusting” of granules, and several cells contain Auer rods. (B) FAB-M3, “hypergranular” form. Most of the cells contain many large cytoplasmic granules, obscuring the nuclear outlines in some cells. Wright-Giemsa stain.
were hyposegmented and were frequently mononuclear (Figs 1B and C). Rare abnormal eosinophils with interspersed basophilic-staining granules were identified (Fig 1D). The peroxidase stain was positive in most of the cells (Fig 1E), but staining seemed less intense than is usually seen in FAB-M3.

**Electron-microscopic findings.** As originally reported, the leukemic cells showed marked nuclear/cytoplasmic asynchrony. The nuclei were rounded and very euchromatic, while the cytoplasm showed well-developed myeloperoxidase positive granulation, similar to what is seen at the progranulocyte stage of maturation. Perinuclear cytoplasmic fibrils were seen in a few cells, and rare nuclear blebs were identified. As also noted in the original detailed case report, the leukemic cells lacked some of the distinguishing features of APL: there were no splinterform granules or Auer rods, and the endoplasmic reticulum was not excessively dilated.

**Cytogenetic study.** Review of the karyotype of the patient's first bone marrow sample obtained before treatment was as previously reported: 75% were 44, XX, −5, −8, −17, + mar 3; 20% were 45, XX, −5, −8, and + mar 3; 5% were 46, XX, −5, −8, −17, +18, +mar 2, +mar 3. We believe that the marker chromosome (mar 3) found in all the metaphases may represent a 17p+ as initially reported. No structural abnormalities of the long arms of chromosomes 15 or 17 were identified.

**DISCUSSION**

The leukemia from which HL-60 was derived was originally classified as APL, but it was noted that the patient had a number of atypical features: her leukemic cells did not show morphologically abnormal granules or Auer rods by light microscopy, nor did they show extensive cytoplasmic fibrils, deformation and dilatation of the endoplasmic reticulum or Auer rods by electron microscopy; she did not have the expected t(15;17), nor did she have coagulation abnormalities, except terminally. Some additional atypical features were reported but not commented on: The patient developed leukemic tumor nodules in her scalp, vagina, and at intravenous (IV) sites, which to our knowledge have not been described in FAB-M3; her presenting leukocyte count, 35.7 × 10⁹/L, was higher than found in most cases of hypergranular FAB-M3; and the leukemia was relatively resistant to intensive induction chemotherapy, an unusual feature for FAB-M3.

The original bone marrow aspirate showed a large proportion of cells with considerable cytoplasmic granulation. Most of these cells were classified as progranulocytes at the time, and, therefore, the diagnosis of APL seemed reasonable. Since that time, however, the concept of APL has evolved and narrowed to the point that APL, or FAB-M3, is now considered a distinct clinical, morphologic, and cytogenetic entity. It is important to realize that not every case of acute leukemia with a high proportion of progranulocytes fits within this category. The study of cases with t(15;17) facilitated the recognition of FAB-M3 as an entity. When this translocation is found, the bone marrow uniformly shows FAB-M3. The study of large numbers of cases with t(15;17) thus provided a standard against which other cases of possible FAB-M3 could be measured. The range of appearance of FAB-M3 is very limited, and experienced observers can recognize cases of FAB-M3 confidently even without information about the presence or absence of t(15;17). In the majority of cases of FAB-M3, the “hypergranular” promyelocyte is the predominant cell type; in the less common, “variant” form of FAB-M3, the “microgranular” promyelocyte is predominant. Cells with multiple Auer rods, “faggott” cells, can be found in nearly all cases of FAB-M3. The leukemia from which HL-60 was derived failed to show either the typical hypergranular promyelocytes of FAB-M3 whose enlarged, angular granules obscure the nuclear outlines, or the microgranular promyelocytes of the variant form of FAB-M3, whose nuclei have a distinctive lobulated shape. Auer rods were not identified. The leukemia also showed two other morphologic features not expected in FAB-M3, namely, mononuclear neutrophils and occasional eosinophils with interspersed basophilic-staining granules.

We emphasize, therefore, that HL-60 is morphologically distinct from FAB-M3 and is not an appropriate model for studying the molecular events unique to the t(15;17) in FAB-M3. This does not, of course, diminish the usefulness of HL-60 in studying broader questions of leukemogenesis and differentiation.

**REFERENCES**


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