Leukemia Research 61 (2017) 77–83

Contents lists available at ScienceDirect

Leukemia Research

journal homepage: www.elsevier.com/locate/leukres

Research paper

Establishment of cell line with NK/NKT phenotype from myeloid NK cell acute leukemia

A. Darji[a,*], N. Desai[a], R. Modi[b], B. Khamar[b], S. Rajkumar[c,*]

[a] Cadila Pharmaceuticals Ltd, 1389, Trasad Road, Dholka, Ahmedabad – 382225, Gujarat, India
[b] Cadila Pharmaceuticals Ltd, Cadila Corporate Campus, Sarkhej-Dholka Road, Bhat, Ahmedabad – 382210, Gujarat, India
[c] Institute of Science, Nirma University, Ahmedabad – 382481, Gujarat, India

ARTICLE INFO

Keywords:
Acute myeloid leukemia (AML)
Cell line
Karyotyping
NK/NKT phenotype
Cytoxicity

ABSTRACT

Acute Myeloid Leukemia (AML) is the most common malignancy in adults with a 5-year survival rate of 27% of the total affected population. For effective treatment and new drug discovery, cell lines are considered as a very important tool. Here we report an establishment of a continuous human cell line AML-004 with a hypo-diploid chromosome 44 and presence of both NK/NKT phenotypes. The cell line was isolated from the blood sample of myeloid NK cell acute leukemia patients and extensively characterized by flow cytometery, morphology, and cytogentic analysis. Cytotoxicity by standard chemotherapeutic drugs was also examined. As characterized by Giemsa staining, the predominant cell type in the culture had high nuclear/cytoplasmic ratio. Cytogenetic analysis revealed high chromosome instability and structural abnormalities confirming the source of cell line from a patient with AML. The karyotype of the isolated cells did not alter up to around 40 passages. These AML-004 cells lacked specific markers for B and T lymphoid cells, but expressed surface receptors for lymphoid/NK cells. Cells also lacked the presence of early progenitors. The proliferation of the isolated cells was inversely proportional to the IL-2 concentration confirming presence of NK phenotype. AML-004 was resistant against standard chemotherapeutic drugs excluding cisplatin. Thus, AML-004 cells provide a continuous source of human cells for designing novel therapies for patients with T-lymphoblastic leukemia/lymphoma.

1. Introduction

Acute Myeloid Leukemia (AML) is a neoplastic disorder. According to SEER 2017 [1], approximately 21,380 patients will be diagnosed with acute myeloid leukemia (AML) with greater than 10,590 deaths only in the United States by 2017. For better management & prognosis of AML, it is required to introduce new drugs either as standalone or as a part of combination therapy [2]. The establishment of cell line is required to fuel new drug development and it's testing *in vitro* and *in vivo*. There are > 1500 different human leukemia-lymphoma cell lines developed in the past five decades [3,4]. The majority of these developed cell lines belong to lymphoid origin. NK cell acute leukemia is a rare subtype with distinct morphological and immuno-phenotypical features which is categorized as provisional entity of T-lymphoblastic leukemia/lymphoma [5]. To understand and design the drugs for AML of ambiguous lineages, cells lines derived from the patients serve as an important resource. It also provides indefinite storability in liquid nitrogen, monoclonal origin, sustained proliferation [6], differentiation arrest at discrete mutation stage, stability of features in long term culture and a platform for designing the drug therapy [6–8]. Isolation of myeloid origin primary NK cells with CD56, cytoplasmic CD3 and CD16 antigen has been reported earlier but there are no reports of continuous myeloid NK cell line [9–11].

In this study, we report a new leukemia cell line named AML-004, which was established from peripheral blood samples of a patient with Myeloid NK cell acute leukaemia as per the guidelines for characterization and publication of human malignant hematopoietic cell lines [6]. The cell line has unique immunophenotype along with cytogenetic properties. The proliferation of the cell line is inhibited by IL-2 in a dose dependent manner which indicates the presence of CD56 Dim phenotype of NK cells. This cell line may help in the evaluation and identification of treatment path for patients suffering from NK cell lymphoblastic leukemia and would significantly aid exploratory studies for effective drug-design. To the best of our knowledge, this case represents a previously unrecognized form of acute leukemia.

* Corresponding authors.
E-mail addresses: avani.mehta@cplbio.com (A. Darji), ndesai@cplbio.com (N. Desai), rimodi@cadilapharma.co.in (R. Modi), bmk@cadilapharma.co.in (B. Khamar), shalini.rjk@nirmauni.ac.in (S. Rajkumar).

http://dx.doi.org/10.1016/j.leukres.2017.09.007
Received 5 August 2017; Received in revised form 11 September 2017; Accepted 12 September 2017
Available online 13 September 2017

2. Materials and methods

2.1. Chemicals and reagents

Gemcitabine – Tabicad (1000 mg), 5-Floro Uracil (5-FU) – Florac (50 mg/ml), doxorubicin – Cadria 50 (50 mg) and cisplatin – Platin 10 (0.5 mg/ml) were sourced from Cadila Pharmaceuticals Ltd. DMEM (Dulbecco's modified Eagle's Medium) and RPMI 1640 (Roswell Park Memorial Institute); Sigma Aldrich supplemented with 10% (v/v) FBS (Fetal bovine serum); Gibco. CellTiter 96® AQueous One Solution was procured from Promega, UK.

2.2. Patient sample details

The cells were isolated from a blood sample collected at Gujarat Cancer Research Institute after taking the consent of the patient in a heparin coated vacutainer. Blood sample obtained was of an 80 years old Asian origin female diagnosed with Myeloid NK cell acute leukaemia. The bone marrow report of the patient showed an altered ratio of myeloid to erythroid cells, suppression of both the myeloid & erythroid precursors, absence of mega karyocytes and presence of > 90% blast cells. The cytochemical analysis revealed that the sample was weak positive for Sudan Black B stain and negative for PAS (Periodic acid–Schiff stain).

2.3. Cell isolation and culture conditions

Mononuclear cells were separated using Histopaque-119 [12–14] from 10 ml of blood sample collected. The lymphocytes hence separated were distributed into two tubes (tube A and tube B) and were washed twice with HBSS (Hanks's Balanced Salt Solution); HiMedia containing cocktail of antibiotics at 1 X concentration (Penicillin–100 U/ml, Streptomicin–100 µg/ml, Gentamycin–50 µg/ml). After wash, the cells were centrifuged and the cell pellet from each tube was re-suspended in 10 ml of Media A and Media B respectively. The base medium for Media A was DMEM and Media B was of RPMI-1640. Both media were formulated with 10% FBS, 10 ng/ml GCSF; Sigma Aldrich and antibiotics at 1X concentration. The cells were seeded in multiple 75 cm^2 flasks with respective media. The flasks were subsequently incubated at 37 °C, 6% CO_2 and monitored for the cell morphology, cell attachment, media consumption and cell division. Upon complete consumption of media the flasks were replenished with the fresh media and once the flasks were confluent the cells were sub cultured.

2.4. Morphological studies

Cell morphology was determined by May-Grünwald Giemsa (MGG) staining. The slides were air dried and fixed using chilled methanol. Two drops of 5% MGG were added to a fixed slide and left for 2 min. The slides were rinsed briefly with tap water, mounted and covered with a cover slip.

2.5. Cytogenetic analysis

For cytogenetic analysis, the cells undergoing amplification and which were in culture at passage 40 were used. Chromosomes were prepared by a standard method [15,16]. Karyotypic abnormalities were identified according to the International System for Human Cytogenetic Nomenclature (IISCN 1995).

2.6. Doubling time

For doubling time analysis the cells at passage 30 were used. The doubling time was calculated using below equation:

$$DT = T \ln 2 / \ln (Xe/Xb)$$

where T is the incubation time in any units, Xb is the cell number at the beginning of the incubation time, Xe is the cell number at the end of the incubation time.

2.7. Cell surface marker analysis

For identification of the cell type, combination of various markers procured from BD Biosciences like Pan Leukocyte maker (CD45), Myeloid Lineage (CD33 and CD13), Mature Myeloid Lineage (CD11b and CD11c), Lymphoid lineage/B-cell (CD19), Lymphoid lineage/T cell (CD4 and CD8), Lymphoid lineage/T-cell (cCD3), Lymphoid/NK cell (CD56) and Early Progenitor (CD117 and HLA-DR) were used. The cells were stained with respective antibodies using standard protocols and analyzed on BD FACS Canto II. The similar protocol was also followed for the intracellular staining. For cell membrane permeabilization 50 µl of saponin buffer prepared in PBS was added along with antibody.

2.8. Effect of IL-2 on CD56 expression and cell proliferation

To study the effect of IL-2 on CD56 expression, change in the cell surface expression of CD56 was analyzed by incubating the cells with different concentrations of IL-2 (Sigma Aldrich) ranging from 3.125ug/ml to 25ug/ml along with appropriate controls. Post co-incubation the cells were stained with anti-CD56 conjugated to PE-Cy7 Fluorescence dye (BD Biosciences) utilizing the same protocol as used for surface staining and the tubes were read on BD FACS Canto II.

For cell growth analysis the cells were co incubated in same manner as for expression assay and post incubation, cell viability was determined by addition of 20 µl CellTiter 96®. Subsequently, the plates were incubated at 37 °C for 1 h. After incubation, Optical Density (OD) was determined at 490 nm. Percentage cell survival was plotted against IL-2 concentration to determine cell growth.

2.9. Efficacy assessment of gemcitabine, 5-FU, cisplatin and doxorubicin on isolated cells

To determine the effect of gemcitabine, 5-FU, doxorubicin and cisplatin on cell growth of isolated cells; the CellTiter 96® was used. For the assay, cells at both passage 5 and passage 75 were used. Cells were harvested and plated along with respective drugs. Gemcitabine (2 µg to 640 µg), 5-FU (62.5 µg to 2000 µg), doxorubicin (31.25 nM to 1000 nM) and cisplatin (7.81 µg to 250 µg) were added to the wells in triplicates along with respective controls. Plates were incubated in a humidified atmosphere with 5% CO_2 at 37 °C for 48 h. Post incubation 20 µl of CellTiter 96®reagent was added to wells and plates were read at 490 nm. Percentage cell survival was plotted against the drug concentration to determine IC 50 value of drugs.

2.10. Statistical analysis

All tests were performed in triplicates and quantitative data were presented as Standard Deviation of mean. Graphical presentation was accomplished using commercial software Prism 5.0 (GraphPAD Software, CA). Results for cytotoxicity studies are displayed as% of control. IC50 was calculated by an Excel add-in ED50V10 file.

3. Results

3.1. Cell amplification and stock preparation

To the best of our knowledge, we provide the experimental proof that cells isolated from the blood of a patient with AML could give rise to a stable cell line. The procedure has led to the establishment of the first permanent cell which was growing in the culture for > 16 months