Leukemia Research 40 (2016) 54–59

Contents lists available at ScienceDirect

Leukemia Research

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

Isolation of a novel chronic lymphocytic leukemic (CLL) cell line and development of an *in vivo* mouse model of CLL

Joshua Kellner[a], William Wierda[b], Elizabeth Shpall[a], Michael Keating[b], Ian McNiece[a,*]

[a] Department of Stem Cell Transplantation and Cellular Therapy, The University of Texas, MD Anderson Cancer Centre, Houston, TX, United States
[b] Department of Leukemia, The University of Texas, MD Anderson Cancer Center, Houston, TX, United States

ARTICLE INFO

Article history:
Received 10 August 2015
Received in revised form
28 September 2015
Accepted 14 October 2015
Available online 19 October 2015

Keywords:
CLL
Cell line
Stem cell

ABSTRACT

Leukemic cell lines have become important tools for studies of disease providing a monoclonal cell population that can be extensively expanded *in vitro* while preserving leukemic cellular characteristics. However, studies of chronic lymphocytic leukemia (CLL) have been impeded in part by the lack of continuous human cell lines. CLL cells have a high spontaneous apoptosis rate *in vitro* and exhibit minimal proliferation in xenograft models. Therefore, there is a need for development of primary CLL cell lines and we describe the isolation of such a line from the bone marrow of a CLL patient (17p deletion and TP53 mutation) which has been in long term culture for more than 12 months with continuous proliferation. The CLL cell line (termed MDA-BM5) which was generated *in vitro* with continuous co-culture on autologous stromal cells is CD19+CD5+ and shows an identical pattern of somatic hypermutation as determined in the patient's bone marrow (BM), confirming the origin of the cells from the original CLL clone. MDA-BM5 cells were readily transplantable in NOD/SCID gamma null mice (NSG) with disease developing in the BM, liver and spleen. BM cells from quaternary serial transplantation in NSG mice demonstrated the presence of CD19+CD5+ cells with Ig restricted to lambda which is consistent with the original patient cells. These studies describe a new CLL cell line from a patient with del(17p) that provides a unique model for *in vitro* and *in vivo* studies.

1. Introduction

CLL is one of the most common types of adult leukemia and presents as an abnormal population of B lymphocytes proliferating in the blood, bone marrow (BM), or tissues. All the CLL cells within an individual are clonal and are characterized phenotypically by expression of CD19, CD20 and CD23 with co-expression of the pan T-cell marker CD5 [1]. *In vivo*, CLL cells display characteristics consistent with defects in apoptosis and prolonged survival, however *in vitro* CLL cells from peripheral blood or lymphoid tissues undergo rapid spontaneous apoptosis [2]. These data have led to the current view that survival and proliferation of CLL cells depends on the microenvironment [3–5]. Bidirectional interactions between malignant lymphocytes and non-transformed cells may lead to the establishment of an abnormal microenvironment which is able to inhibit apoptosis of neoplastic B cells [2]. While many cell lines have been isolated for various hematological malignancies, a lack of cell lines representative of CLL disease has hampered a full understanding of disease pathogenesis and development of new treatments.

Attempts to isolate CLL lines include transformation by Epstein-Barr virus or other B-cell activation stimuli. However, over time in culture these cells exhibit diminished CD5 expression [6,7]. Further, *in vivo* models have been described, however, many of these CLL xenograft models use cell lines with an atypical CLL phenotype (*i.e.* lacking CD5 expression), and exhibit minimal or no peripheral disease [8–9]. Alternate approaches focused on adoptive transfer using cotransfer of autologous T cells however, the T cells can induce graft-versus-host disease resulting in death of the animals [10].

The studies described herein were initiated to isolate stromal cell lines from the BM of CLL patients to evaluate the relationship between the microenvironment and CLL cells. Stromal cells have been isolated from 4CLL BM samples and we observed that maintenance of the non-adherent (NA) cells with the adherent stromal cells resulted in persistence of CLL cells. Herein, we describe the isolation of a novel CLL cell line from one of the BM samples. These cells have now been in continuous culture for more than 12 months and have been passaged through 5 serial transplants in immune compromised mice.

* Corresponding author. Fax: +1 713 563 4851.
E-mail address: imcniece@mdanderson.org (I. McNiece).

http://dx.doi.org/10.1016/j.leukres.2015.10.008
0145-2126/

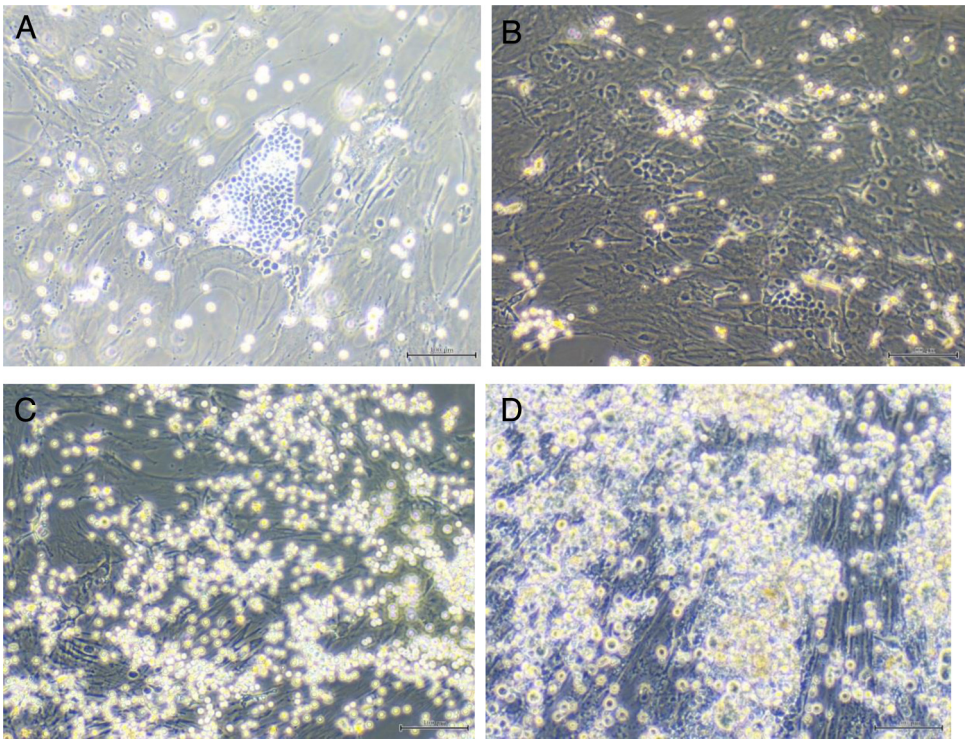

Fig. 1. *In vitro* cell morphology of BM CLL. (A) Culture of BM cells showing the early development of phase-dim cells at 2 weeks of culture. (B) Phase-dim cells with continued expansion. (C) Phase-dim cells distinct to phase-bright cells. (D) 5 months of culture with visible phase-dim and phase-bright cells.

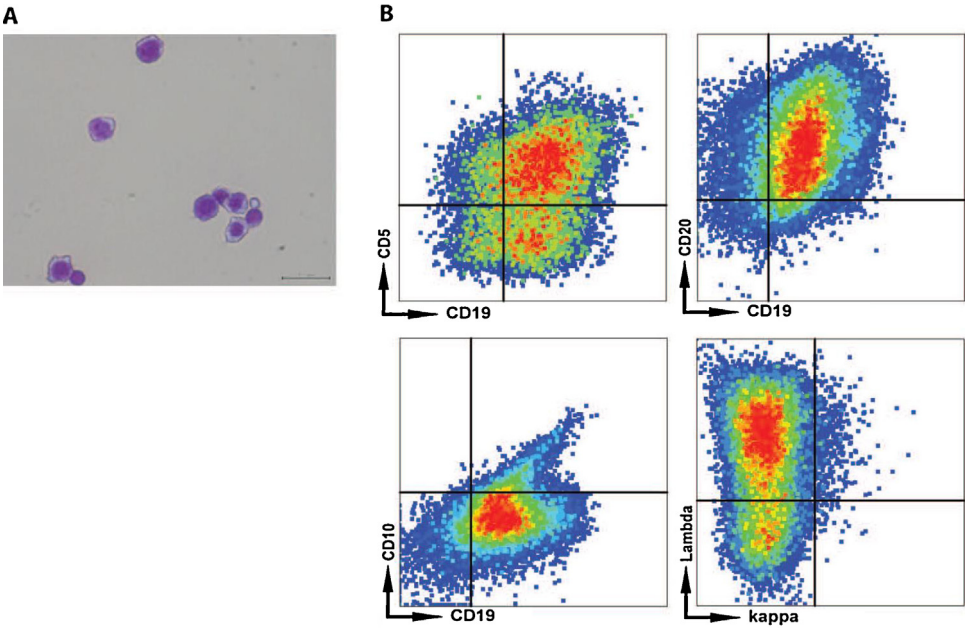

Fig. 2. Phenotypic characterization of CLL cells. (A) Cytospin preparation of MDA-BM5 cells stained with Wright-Giemsa. (B) Flow analysis of MDA-BM5 for CD5/CD19 (upper left), CD19/CD20 (upper right), CD19/CD10 (lower left), and Kappa/lambda (lower right).

2. Materials and methods

2.1. Bone marrow samples

Samples of BM aspirates were obtained from patients with CLL with appropriate informed consent under an Institutional Review Board (IRB) approved protocol. Mononuclear cells (MNCs) were isolated by gradient density separation using Ficoll-paque (GE Healthcare, Pittsburgh, PA) and cultured in T 162cm^2 culture flasks (Corning, Tewksbury, MA) in 25 ml of alpha-minimum essential medium (α-MEM; Mediatech Inc., Herndon, VA) plus 20% fetal bovine serum (FBS; Akron Biotech, Boca Raton, FL) and 1% Penicillin–Streptomycin–Glutamine (GPS, Gibco). The flasks were incubated at 37 °C in 5% CO_2.Each week the media and non-adherent cells were harvested, centrifuged, fresh media used to re-suspend the non-adherent cells and then returned to the original flask. When the adherent stromal layer was confluent, the non-adherent cells were harvested, followed by harvest of the adherent cells using 0.05% Trypsin-EDTA (GIBCO BRL, Grand Island, NY). The adherent and non-adherent cells were combined and split into 4 or 5 new flasks with fresh media. Cryopreservation was performed by concentrating cells in media plus 10% DMSO and freezing in 1 ml ampoules in a Mr Frosty at −80 °C and subsequent transfer to liquid nitrogen. Autologous stromal cells were generated by passaging only adherent stromal cells in media, with weekly removal of media and non-adherent cells.