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Biophysical differences between chronic myelogenous leukemic quiescent and proliferating stem/progenitor cells

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Abstract

The treatment of chronic myeloid leukemia (CML), a clonal myeloproliferative disorder has improved recently, but most patients have not yet been cured. Some patients develop resistance to the available tyrosine kinase treatments. Persistence of residual *quiescent* CML *stem cells* (LSCs) that later resume proliferation is another common cause of recurrence or relapse of CML. Eradication of quiescent LSCs is a promising approach to prevent recurrence of CML. Here we report on new biophysical differences between quiescent and proliferating CD34+ LSCs, and speculate how this information could be of use to eradicate quiescent LSCs. Using AFM measurements on cells collected from four untreated CML patients, substantial differences are observed between quiescent and proliferating cells in the elastic modulus, pericellular brush length and its grafting density at the single cell level. The higher pericellular brush densities of quiescent LSCs are common for all samples. The significance of these observations is discussed.

Key words: Chronic myeloid leukemia; Atomic force microscopy; Cell mechanics; Personalized medicine; Single cell analysis

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder[1,2] accounting for 1-2 cases per 100,000 in adults. Recent development of tyrosine kinase inhibitors (TKIs) has changed the prognosis of chronic phase CML from a life threatening disease into a treatable chronic disease with substantially increased survival.[3,4] However, some patients develop resistant mutant clones,[5,6] and there are a substantial number of cases of recurrence of CML upon TKI withdrawal.[7,8,9] Various mechanisms have been proposed to better understand leukemia stem cell (LSC) populations that are responsible for recurrence or relapse. Persistence of residual leukemic quiescent stem cells and development of resistant clones are the most probable causes.[2,10,11] It is still not clear how to eradicate surviving quiescent LSC which are resistant to many types of treatment.[2]

Because LSCs are quite rare, it is hard to apply standard biochemical methods such as western blotting to study these cells. Thus, it is important to develop new methods that allow reliable measuring of the cell properties at the single cell level. Atomic force microscopy (AFM) is a biophysical technique capable of measuring physical properties of single cells.[12,13] Typically, AFM can function as a microscope for imaging viable cells[14,15] and even single molecules.[16] The AFM technique is also one of the most convenient methods for studying the mechanics of soft materials in general,[17] and cell mechanics in particular[18,19,20,21] because it can operate with a large range of load forces at small scales with very high accuracy.[22]

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Over the last decade, many studies have demonstrated the link between cell mechanics and various diseases, such as cancer,[20,21,23,24] arthritis,[25] malaria,[26] ischemia,[27] and even aging.[19,28,29] The careful analysis of cell mechanics may enable researchers to obtain new fundamental insights of disease states as well as properties of different cells within normal or diseased cell populations, and help to develop improved methods of diagnosis, prognosis, and treatment.

It is important to develop methods that allow accurate measuring of mechanical properties of cells independently of the specifics of the used methods and instruments. It has been shown that the elastic modulus (*aka* the effective Young's modulus) and the parameters of the pericellular coat (*aka* the pericellular brush layer) can be such objective characteristics of cells.[30] These characteristics can be experimentally measured with the help of AFM.

To extract the instrument and material independent biophysical characteristics of cells, the force indentation data have to be analyzed with the help of a mechanical model. The Hertz model[31] and its various modifications[23,32] have been widely used to determine the elastic modulus of cells. In these models, the cell is considered as a homogenous and isotropic material, and the cell shape is not often taken into account. However, the cell surface is typically non-flat even at the nanoscale. Various membrane protrusions, which can be seen in optical confocal microscopy (see, e.g.,[20]) can be detected with AFM.[33] It has recently been found that the pericellular brush interferes with indentation measurements of elastic properties of the cell body, and a new model must be used[34] which separates contribution of the pericellular brush layer and deformation of the cell body in the AFM indentation experiments. Interestingly, cancer cells may look artificially softer if the cellular brush is not taken into account as was shown for the case of human cervical epithelial cells.[20]

Furthermore, as was recently shown,[30] cells can be treated in a self-consistent way as an elastic isotropic and homogeneous material (cell body) surrounded by essentially non-elastic pericellular brush layer which cannot be described by the elastic modulus. The non-elastic brush layer demonstrates the exponential force behavior somewhat similar to the classical polymer entropic brush. Therefore, a term "pericellular brush" was introduced to describe this layer. One can characterize the mechanical properties of cells with three parameters, the elastic modulus, the (equilibrium) length and grafting density (effective number of molecular constituents per unit area) of the pericellular coat/brush.

The above approach to characterize cell mechanics is used in the present work to study critical differences between CML quiescent (G_0) and proliferating (G1) stem and progenitor (S/P) cells. Hereafter G1 is used to include cells in all phases of the cell cycle except G_0, including G1, S, G2 and M phases. In CML the primary driving mutation is a fusion of the Abelson murine leukemia (ABL) gene on chromosome 9 with the breakpoint cluster region (BCR) gene on chromosome 22 (Philadelphia chromosome; Ph+),[35] which results in expression of a fused oncoprotein, termed BCR-ABL. The BCR-ABL oncoprotein is a constitutively active tyrosine kinase that promotes growth and replication through downstream pathways.[2] To study differences in quiescent and proliferating CML stem/progenitor cells, we enriched patient samples for CD34+ cells using immunophenotypic selection from CML blood samples.[36] Although the CD34+ cell fractions are heterogeneous, they contain the great majority of S/P cells and have been shown to reconstitute the entire hematopoietic system of immunocompromised irradiated mice.[37,38,39]

Both populations of proliferating and quiescent cells were subjected to AFM analysis to measure the biomechanical properties described above at the single cell level. We observed statistically significant differences between quiescent and proliferating cell populations for both the Young's modulus (cell "stiffness") of the cell body and the parameters of the pericellular brush. Only one basic parameter, the brush grafting density, showed a common behavior for all four patients, the grafting density. It was higher for the quiescent cells compared to the proliferating ones (though this difference was not statistically significant for one of the patients). The same behavior was observed for two derivative parameters, the brush "size" and "volume density". The significance of these results for prognostics and future treatment of this type of cancer is discussed.

Methods

CML patient samples

CML blood samples were obtained from four patients newly diagnosed with chronic phase CML before any treatment. The patient samples were obtained from Human Blood Bank Facility (HBUC) at MSKCC for research purpose with approval number HBUC# HBS2012091. Some of the patient samples were gifts from Dr. David Scheinberg which were obtained with informed consent for research purpose. Some patient samples were from Dr. Clarkson's lab which were obtained between 1986 and 1990 with informed patient consent for research purpose. The CML blood samples were processed to obtain enriched peripheral blood mononuclear cells (PBMCs) and frozen until further use. After informed consent on Memorial Sloan-Kettering Cancer Center (MSKCC) Institutional Review Board-approved protocols, PBMCs from patients were obtained by Ficoll density centrifugation. Defrosted PBMCs from the four CML patient samples were individually used to isolate the CD34+ fraction using the midiMACS immune-magnetic separation Kit from Miltenyi (Miltenyi Biotec, Bergisch Gladbach, Germany, Cat# 130-0460701). The details of CD34+ cell enrichment and Hoechst 33342 and pyronin-Y staining for G_0 cells enrichment[40] are described in the Supplementary information. The cells described above were plated on culture dishes. The AFM study was done on these live cells (the apical part, similar to[41,42]) directly in the growth medium without any modifications; see the Supplementary information for detail.

Deformation of stem cells with the AFM probe: a model for a spherical cell covered with pericellular brush layer

It has been demonstrated that the use of a two-layer model (cell body plus pericellular "brush" layer) is more accurate than