Surface morphology of leukemic cells from chronic myeloid leukemia under atomic force microscopy

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ABSTRACT: Atomic force microscopy (AFM) represents an important instrument for measuring mechanical properties of biological materials ranging from single molecules to normal or malignant cells. AFM provides a 3D profile of the surface on a nanoscale, by measuring forces between a sharp probe (<10 nm), supported on a flexible cantilever, and surface at very short distance (0.2-10 nm probe-sample separation). The AFM tip “gently” touches the surface and records the small force between the probe and the surface. The patients were three normal human subjects and nine patients with chronic myeloid leukemia in different phases of disease. With atomic force microscopy, numerous spicules were observed on the surface of leukemic cells, especially in blastic phase of CML.

KEYWORDS: atomic force microscopy, 3D profile, leukemic cells

Introduction:

Chronic myeloid leukemia (CML) is a clonal disorder of hematopoietic stem cell characterized by exaggerated proliferation of granulocytic lineage with leukocytosis, basophilia, anaemia, splenomegaly, characteristic Philadelphia chromosome results from the t(9;22)(q34;q11) reciprocal translocation between chromosomes 9 and 22, presence of the BCR-ABL1 gene fusion [1,2].

Atomic force microscopy (AFM) represents an important instrument for measuring mechanical properties of biological materials ranging from single molecules to normal or malignant cells.

In the case of AFM (atomic force microscopy) technique, the surface of the sample is scanned with a sharp tip having few microns length and a diameter of less than 100 Å. The tip is at the free end of a cantilever which has a length of 100-200 µm. Forces developed between tip and the surface, induce deformation of the console which is measured by a detector yielding a topographical image of the surface. Most often the forces associated with AFM technique are van der Waals interatomic force.

Two different regimes are possible: contact and non-contact. In contact mode the tip is held a few angstroms of the surface and the force is repulsive. In non-contact mode the tip is held at a distance of several tens, even hundreds of angstroms of the surface (the force is attractive; mostly van der Waals interactions of long-distance) so contact between the tip and the surface is very weak or even nonexistent. In this mode AFM console runs a forced oscillation motion quite near the sample surface. The average distance between the tip and the surface is tens to hundreds of angstroms, so images of less smooth surfaces are possible. Force between tip and surface in non-contact mode is very low, around 10-12 N. This is an advantage when studying very soft or elastic samples because the studied area is affected to a much lesser extent. If stiffer samples are used, images obtained with the contact and non-contact modes are similar.

Aim: to observe the difference in surface morphology between leukocytes from normal human subjects and from patients with chronic myeloid leukemia (CML).

Patients and Method:

Bone marrow was collected from three normal human subjects and nine patients with chronic myeloid leukemia in different phases of disease: three patients in chronic phase, three patients in accelerated phase and three patients in blastic phase. Bone marrow smears were coloured MGG. The morphology of the cells were than observed with optical microscope and atomic force microscope (AFM). For measurement a XE-100 from Park Systems was used with the following features: True non-contact mode; Z scan range: 25 µm; Resonant frequency: 1.7 kHz • Laser type: LD (630 nm) Noise floor: 0.03 nm (typical), 0.05 nm (maximum)
Results and discussion:

In chronic phase of CML the bone marrow was hypercellular with myeloid hyperplasia and left shift, basophilia, increased erythroid ratio and megakaryocytes. Percentage of blasts in bone marrow was lower than 10%, the myeloid maturation was morphologically normal with little or no dysplasia and a near-normal life span [3].

In accelerated phase of CML blasts was between 10 -19% of white blood count in peripheral blood or nucleated bone marrow cells, peripheral basophils was more than 20% and a moderate or severe myelofibrosis was present.

In blast crisis percentage of blasts was more than 20% in the bone marrow or peripheral blood [4]. Abnormal cytoadhesion or anchorage properties of malignant progenitors were present. In vitro culture studies had identified abnormalities in the proliferation and differentiation of CML progenitors, their interactions with bone marrow stroma and their requirements for and responsiveness to growth factors and negative growth regulators [5-8].

No significant difference was identified between the morphology of the cells from normal subjects and patients with chronic myeloid leukemia in chronic phase under optical microscope (fig,1,2,3).

With atomic force microscope, numerous spicules were observed on the surface of leukemic cells, especially in blastic phase of CML, showing a significant difference of roughness between normal cells and leukemic cells (fig.4,5,6,7).
Fig. 7. CML blastic phase/LAM - AFM phase

Conclusion

AFM had advantages in analyzing cell membrane in the nanometer level and revealed significant difference in terms of roughness of the cell surface between normal cells and leukemic cells from the patients with CML.

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