Constitutively Activated Rho Guanosine Triphosphatases Regulate the Growth and Morphology of Hairy Cell Leukemia Cells

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Abstract
Hairy cell leukemia (HCL) is a rare type of chronic B-cell leukemia characterized by the hairy morphology of the leukemia cells. All of 5 HCL samples and an HCL-derived cell line, BNBH-I, showed serrated edges and hairlike projections in May-Grünwald Giemsa stain and protruding actin spikes and lamellipodia in phalloidin stain. These structures were hardly detected on B-cell chronic lymphocytic leukemia (B-CLL) and precursor B-cell acute lymphocytic leukemia (B-ALL) cells. Because Rho guanosine triphosphatases (GTPases) regulate the formation of these structures, we examined the expression levels and activation states of Rho GTPases in HCL cells. RhoA, Rac1, and Cdc42 were overexpressed and constitutively activated in HCL samples and BNBH-I cells but not in B-CLL or precursor B-ALL cells. Next we overexpressed dominant-negative (DN)-RhoA, DN-Rac1, and DN-Cdc42 in BNBH-I. As a result, each DN mutant repressed the growth of BNBH-I cells by more than 50% and inhibited actin spike formation, but only DN-Rac1 suppressed lamellipodia formation. We also found that enforced expression of constitutively active-RhoA, Rac, or Cdc42 in the proB-cell line Ba/F3 was sufficient to induce actin spike formation, whereas none of these molecules produced lamellipodia. These results indicated that constitutively activated Rho GTPases regulate the growth and unique morphology of HCL cells. Int J Hematol. 2003;77:263-273.

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1. Introduction

Hairy cell leukemia (HCL) is a rare chronic lymphoproliferative disorder that affects middle-aged adults [1]. Most patients manifest isolated splenomegaly and pancytopenia associated with circulating HCL cells. HCL cells possess hairy cytoplasmic projections on their surfaces and strong tartrate-resistant acid phosphatase (TRAP) activity [2,3], both of which have been regarded as pathognomonic of HCL. HCL cells show surface immunoglobulin (sIg) and rearranged Ig genes [4]. The cells are negative for CD5 and intensely positive for CD11c, CD22, CD25, and sIg [5,6]. On the basis of these clinical and cytological features, HCL is now considered to be a distinct type of chronic (mature) B-cell leukemia.

However, most Japanese patients with HCL have features that differ considerably from those of the typical HCL (tHCL) seen in Western countries [7,8]. Thus we previously defined this distinct HCL subtype as HCL Japanese variant, which we denote here as HCL-J. HCL-J has common features with tHCL but differs in several aspects [7]. Patients with HCL-J have splenomegaly and minimal lymphadenopathy. The abnormal cells of HCL-J have long microvilli and ruffles; the surface morphology is indistinguishable from that of tHCL. On the other hand, whereas leukopenia is common in tHCL, the majority of HCL-J patients have a moderate degree of leukocytosis. In May-Giemsa stained films, HCL-J cells have intermediate features between tHCL cells and B-CLL cells but can be distinguished from these cells. In addition, HCL-J cells possess weak TRAP activity and reveal the CD5−, CD11c+, CD25− phenotype, differing from the phenotypes described for tHCL and B-CLL [1,8].
Most studies on HCL have been performed to discriminate HCL from other types of chronic B-cell leukemia. Therefore these analyses have focused on the profile of expression of cell surface molecules on HCL cells, as described above. In addition, because of the unique morphology of HCL cells and their predominant localization to the spleen, the functional roles of adhesion molecules such as CD44, CD11c, and CD54 have been the major targets of research into HCL cells [9,10]. By contrast, only a few analyses have been performed on the function of intracellular signaling molecules in HCL cells. Moreover, the molecular mechanisms governing the growth and morphology of HCL cells remain unknown. Although recent progress in the treatment of HCL is remarkable and newly introduced interferon-α and purine analogues (deoxycoformycin and 2-chlorodeoxyadenosine) have dramatically improved the prognosis of HCL patients [11], the precise mechanism of action of these drugs on HCL cells remains unknown.

Rho guanosine triphosphatases (GTPases) form a subfamily of the Ras superfamily of GTP-binding proteins [12-14]. Mammalian Rho GTPases are grouped into 6 subclasses: Rho (RhoA, RhoB, RhoC), Rac (Rac1, Rac2, Rac3, RhoG), Cdc42 (Cdc42Hs, G25K, TC10),Rnd (Rnd3, Rnd1/Rho6, Rnd2/Rho7), RhoD, and TTF (translocation three four). As do other members of the Ras superfamily, Rho GTPases function as molecular switches by cycling between an inactive guanosine diphosphate (GDP)-bound form and an active GTP-bound form. The activity of Rho GTPases is positively regulated by guanine nucleotide exchange factors (GEFs), which promote the exchange of bound GDP for GTP, although they are negatively controlled by GTPase-activating proteins (GAPs), which increase the intrinsic hydrolysis rate of bound GTP. In addition, Rho GTPase activity is regulated by guanine nucleotide dissociation inhibitors, which inhibit both the change to GTP and the hydrolysis of bound GTP.

Rho GTPases have been shown to play a crucial role in the regulation of actin organization, thereby mediating various cellular functions and processes, such as maintenance of cell form, motility, contraction, adhesion, cytokinesis, and phagocytosis. The actin cytoskeleton is composed of filamentous actin (F-actin) and many actin-binding proteins. F-actin is organized into several discrete structures in response to external stimuli as follows: stress fibers, long bundles of actin filaments that traverse the cell and are linked to the extracellular matrix through integrins and focal adhesion complexes; lamellipodia, a thin protrusive meshwork of actin filaments that dominates the leading edge of motile cells; and filopodia, fingerlike protrusions that contain a tight bundle of long actin filaments in the direction of the protrusion. Although the construction of these structures is thought to be orchestrated by Rho GTPase family members in a strict sense, the molecule playing the most critical role in their construction has been determined. For example, RhoA is a major regulator of growth factor- or lysophosphatidic acid (LPA)-induced stress fiber formation in Swiss 3T3 fibroblasts [15,16]. Rac1 is responsible for platelet-derived growth factor (PDGF)- or insulin-induced lamellipodia formation [15,17], and Cdc42 regulates filopodia formation in response to bradykinin [18]. Rho GTPases previously were considered exclusively involved in regulation of cytoskeletal organization. However, recent accumulated evidence has shown that Rho GTPases also play crucial roles in diverse cellular events, such as membrane trafficking, transcriptional regulation, cell growth control, and cell survival and development [12-14].

Because Rho GTPases play a central role in the regulation of cell shape, we speculated that the dysregulated function of these family members might underlie the unique morphology of HCL cells. In this study, we examined the expression levels and activation states of Rho GTPases in HCL cells. We found that Rho GTPases (RhoA, Rac1, and Cdc42) are overexpressed and constitutively activated in HCL cells but not in B-cell chronic lymphocytic leukemia (B-CLL) or precursor B-cell acute lymphoblastic leukemia (B-ALL) cells, thereby conferring hairy morphology on HCL cells. In addition, we found that the dominant-negative (DN) forms of RhoA, Rac1, and Cdc42 individually inhibit the growth of the HCL-derived cell line. These results are evidence that constitutively activated Rho GTPases regulate the growth and morphology of HCL cells and that, in addition to the difference in morphology and surface phenotypes, the biologic properties of HCL cells are considerably distinct from those of B-CLL cells in terms of regulation of intracellular signaling molecules.

2. Materials and Methods

2.1. Reagents and Antibodies

Y-27632 (Rock inhibitor), was supplied by Yoshitomi Pharmaceutical Industries (Saitama, Japan). Rabbit anti-RhoA antibody (Ab) (sc179) and rabbit anti-Cdc42 Ab (sc87) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA); rabbit anti-Rac1 Ab from Cytoskeleton (Denver, CO, USA); rabbit anti-Myc-tag Ab from Medical Biological Laboratories (Watertown, MA, USA); and horseradish peroxidase (HRP)-conjugated antirabbit IgG from Promega (Madison, WI, USA). Wortmannin, an inhibitor of phosphoinositol 3-kinase (PI3-K), was purchased from Sigma (St Louis, MO, USA) and 4-aminophenyl-7-(t-butyl)pyrazolo[3,4-d]pyrimidine (PP1), an inhibitor of tyrosine kinases, from Alexis (Santiago, CA, USA).

2.2. Leukemia Cells from Patients and Cell Lines

Heparinized peripheral blood was obtained from patients with B-cell leukemia, including HCL, B-CLL, and precursor B-ALL, after informed consent was given. All of the patients had leukocyte counts of more than $3 \times 10^9/\mu L$ with more than 90% leukemia cells. The diagnosis of leukemia was made by morphological, cytochemical, and immunological analyses [7,19]. Mononuclear cells (MNCs) were isolated by density gradient centrifugation with Ficoll-Paque Plus (Amersham Pharmacia Biotech, Uppsala, Sweden). The isolated MNCs were resuspended in medium containing 25% fetal bovine serum (FBS) and 10% dimethylsulfoxide and cryopreserved in liquid nitrogen until use. In the experiments with patient samples, the frozen cells were thawed, washed, incubated in RPMI supplemented with 10% FBS at