European Journal of Cell Biology 91 (2012) 978–987

Contents lists available at SciVerse ScienceDirect

European Journal of Cell Biology

journal homepage: www.elsevier.de/ejcb

Invadopodia and rolling-type motility are specific features of highly invasive p190$^{bcr\text{-}abl}$ leukemic cells

Thomas Daubon[a,b,*], Tristan Rochelle[c], Nicolas Bourmeyster[c], Elisabeth Génot[a,b,d,**]

[a] Université de Bordeaux, Physiopathologie du cancer du foie, INSERM U1053, F-33000 Bordeaux, France
[b] European Institute of Chemistry and Biology, 2 rue Robert Escarpit, 33 607 Pessac, France
[c] UMR6187 IPBC, CNRS, Poitiers, France
[d] CHU de Bordeaux, F 33076 Bordeaux, France

ARTICLE INFO

Article history:
Received 17 February 2012
Received in revised form 27 April 2012
Accepted 30 April 2012

Keywords:
Cytoskeleton
Invadopodia
Bcr-Abl
Leukemia
Mobility
RhoGTPase
Guanine exchange factor
Invasion
Matrix remodeling

ABSTRACT

Philadelphia chromosome results of a reciprocal translocation between chromosome 9 and 22. The translocation generates a chimeric oncogene, which, depending on the precise location of the fusion causes chronic myelogenous leukemia, CML (p210$^{bcr\text{-}abl}$) or acute lymphoblastic leukemia, ALL (p190$^{bcr\text{-}abl}$). The difference between p190$^{bcr\text{-}abl}$ and p210$^{bcr\text{-}abl}$ resides in the unique presence of the DH/PH domain in p210$^{bcr\text{-}abl}$. Ba/F3 cells are not motile but acquire spontaneous motility upon ectopic expression of either p190$^{bcr\text{-}abl}$ or p210$^{bcr\text{-}abl}$. Whereas p210$^{bcr\text{-}abl}$-expressing cells present typical amoeboid motility, p190$^{bcr\text{-}abl}$-expressing cells motility appears dependent on rolling movements. Both motility types are triggered by Vav1 in complex with Bcr-Abl, and dependent on Rac1 activity. Interestingly, the RhoA specific p210$^{bcr\text{-}abl}$ DH/PH domain regulates the motility mode by shifting motility from a rolling type toward an amoeboid one. In this study, we show that Ba/F3p190$^{bcr\text{-}abl}$-expressing cells assemble invadopodia-like structures visualized as dense F-actin dots containing the actin polymerization machinery and bestowed with matrix degradation activities. The formation of these structures is driven by the reduction of RhoA activity associated with the loss of the DH/PH domain in p190$^{bcr\text{-}abl}$ and correlates with an increase in Cdc42 activity. Such phenotype could also be obtained by impairing p210$^{bcr\text{-}abl}$ RhoA GEF function. Thus, invadopodia formation in association with rolling-type motility characterizes p190$^{bcr\text{-}abl}$ leukemic cells. The description of invadopodia in cells harboring the p190$^{bcr\text{-}abl}$ oncoprotein presents a novel feature of these highly invasive leukemic cells and provides a novel therapeutic drug target to treat the disease.

Introduction

The Bcr-Abl oncogene is responsible for greater than 95% of human chronic myelogenous leukemia (CML) and has been implicated in cases and is associated with a smaller percentage of acute lymphoblastic leukemia (ALL). Oncogenic activation of Abl is most commonly associated with a chromosomal translocation of the *bcr* gene to the N-terminus of *Abl*, resulting in a hydrid gene encoding the Bcr-Abl oncoprotein. Depending on the breakpoint region on the *bcr* gene, two types of chimeras are commonly found: p210$^{bcr\text{-}abl}$ responsible for CML and p190$^{bcr\text{-}abl}$ responsible for ALL. Activated by oligomerization, both proteins exhibit constitutive and enhanced tyrosine kinase activity, which represents the central mechanism of leukemogenesis (Ilaria and Van Etten, 1996; Lugo et al., 1990). Bcr-Abl upregulates both mitogenic and anti-apoptotic pathways. In addition to these effects on cell growth and survival, Bcr-Abl increases cell migration, an effect which cannot be directly attributed to Abl activation. These behavioral changes are associated with dramatic alterations of the actin cytoskeleton including the formation of membrane ruffles and filopodial extensions as well as increased motility and invasion of leukemic cells (Salgia et al., 1997). The effects of Bcr-Abl on cell migration and adhesion are thought to contribute to the premature release of leukemic cells from the bone marrow and subsequent expansion in the blood and infiltration of peripheral organs (Salgia et al., 1999). However, the mechanisms by which Bcr-Abl induces increased leukemic cell spreading remain poorly understood.

Migration and invasion processes are driven by actin cytoskeleton remodeling which depends on GTPases of the Rho family. Rho GTPases cycle between two states: a GDP-bound inactive

* Corresponding author at: IECB/inserm1053, 2 rue Robert Escarpit, 33600 Pessac, France. Tel.: +33 05400 3059; fax: +33 05400 8726.
** Corresponding author at: IECB/inserm1053, 2 rue Robert Escarpit, 33600 Pessac, France. Tel.: +33 05400 3056; fax: +33 05400 8726.
E-mail addresses: t.daubon@iecb.u-bordeaux.fr (T. Daubon), e.genot@iecb.u-bordeaux.fr (E. Génot).

0171-9335/$ – see front matter © 2012 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.ejcb.2012.04.006

conformation and a GTP-bound active one which binds and activates downstream effectors. These states are controlled by guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs). Selective activation of RhoA, Rac1 and Cdc42 induces stress fibers, lamellipodia and filopodia, respectively (Nobes and Hall, 1999). Other cellular processes are observed during cell migration, such as ruffles, pseudopodia or blebs and invadopodia in the case of cell invasion (Ridley, 2011). All these protrusions induce dynamic interactions between the plasma membrane and the extracellular matrix and regulate cell migration. Two different modes of individual cell movement have been characterized for adherent cells: a mesenchymal mode and an amoeboid mode. Cells migrating in mesenchymal mode are elongated and require extracellular proteolysis. Cells migrating in amoeboid fashion are rounded, undergo considerable shape changes when moving through a three-dimensional extracellular matrix-network and exhibit reduced affinity for the matrix. This type of motility is virtually independent on proteases and relies on high Rho-kinase signaling to drive elevated levels of actomyosin contractility (Friedl, 2004). In another situation, invasive feet made of cell membrane protrusions extending from the ventral side of the plasma membrane into the extracellular matrix have been characterized in some highly motile cells or invasive and metastatic cancer cells (Buccione et al., 2004). These specialized integrin-rich F-actin microdomains collectively termed invadosomes (podosomes for normal cells and invadopodia for tumor cells), support local membrane attachment to permit initiation of invasion and intravasation through the action of metalloproteases. Formation of invadopodia is correlated with invasiveness of cancer cells (Seals et al., 2005). Cdc42 appears to play a major role in the formation of these structures (Nakahara et al., 2003).

The molecular mechanisms associated with the high-speed migration of hematopoietic cells are less well documented. Using a model of murine pro-B cell line Ba/F3 cells, we previously showed that $p210^{bcr\text{-}abl}$ (Ba/F3p210 cells) drives an amoeboid type motility while $p190^{bcr\text{-}abl}$ (Ba/F3p190 cells) induces a rolling type motility (Daubon et al., 2008). This latter motility type has not been previously reported except at the onset of the multistep process of leukocyte extravasation (Nourshargh et al., 2010). Ba/F3p190 cells use pseudopodia to roll into the 3D Matrigel. At the GTPase level, $p210^{bcr\text{-}abl}$ activates Rac1 and RhoA, whereas $p190^{bcr\text{-}abl}$ regulates Rac1 only. In both cases, Rac1 is activated through Vav1 phosphorylation. The only structural difference between $p190^{bcr\text{-}abl}$ and $p210^{bcr\text{-}abl}$ resides in the unique presence of a DH/PH domain, which displays a GEF activity for RhoA (Harnois et al., 2003). The activation status of Cdc42 has never been assessed.

In order to better understand the high invasive potential of $p190^{bcr\text{-}abl}$-expressing lymphoblasts in ALL, we designed experiments to address the role of GEF activities linked to $p190^{bcr\text{-}abl}$ or $p210^{bcr\text{-}abl}$ in determining the actin processes associated with their migration pattern. Rac1 activation, mediated through Bcr-Abl induced Vav1 phosphorylation, triggers leukemic cell motility in both $p190^{bcr\text{-}abl}$ or $p210^{bcr\text{-}abl}$ cells. Here we described that $p190^{bcr\text{-}abl}$ expressing cells exhibit F-actin microdomains at the plasma membrane, which are absent from $p210^{bcr\text{-}abl}$ cells. These structures display the structural and functional characteristics of invadopodia, which by providing local degradation assist Bcr-Abl transformed leukemic cells invasion. Specific activation of RhoA carried by $p210^{bcr\text{-}abl}$ DH/PH domain is associated with decreased Cdc42 activity levels, which seem insufficient for invadopodia formation in cells expressing this chimera. These alterations coincide with a switch from a rolling motility type mode toward an amoeboid one. Thus, RhoA plays a pivotal role in the adaptive switch in migration/invasion pattern and mechanism, defining and distinguishing tumor cell invasion strategies.

Results

Ba/F3p190 but not Ba/F3p210 cells form F-actin structures in addition to cellular protrusions

Since $p190^{bcr\text{-}abl}$ and $p210^{bcr\text{-}abl}$ differ in their pattern of RhoGTPase activation (Daubon et al., 2008), we undertook to compare the impact of each of these two chimera on cytoskeleton organization in the model of murine pro-B cell line Ba/F3 cells. We first set out to examine the cytoskeletal structures and associated behavior of Ba/F3 $p190^{bcr\text{-}abl}$ as compared to Ba/F3 $p210^{bcr\text{-}abl}$ cells when plated onto a fibronectin matrix, a substrate which was previously reported to promote firm cell adhesion (Salgia et al., 1997). Under brightfield microscopy, $p210^{bcr\text{-}abl}$ cells exhibited a stretched body with heterogeneous shapes (Fig. 1A). In contrast, Ba/F3 $p190^{bcr\text{-}abl}$ cells appeared roughly spherical but most cells also showed one (45% of the cells) or even two (10% of the cells) protrusions (1–8 μm in length) (Fig. 1A). Strikingly, in the fluorescence microscopy mode, a single dense F-actin dot was detected in most Ba/F3p190 cells ($80 \pm 8\%$, $n = 6$ experiments) but not in Ba/F3p210 cells. Intriguingly, this structure did not coincide with the cellular protrusions seen in the brightfield mode (Fig. 1A). When cells were embedded in Matrigel, both cellular protrusions and F-actin dots could be observed in 3D in the Ba/F3p190 and not in the Ba/F3p210 cells (Fig. 1B). The immunofluorescence approach detected the hematopoietic (lineage cell-specific protein1) homolog of cortactin, HS1 at the F-actin structure but not at cellular protrusions (Fig. 1C). Merging brightfield and fluorescence images revealed that the prominent F-actin structures and the cellular protrusions, both observable in either 2D or 3D environments, represent independent entities. Under brightfield microscopy, Ba/F3 $p190^{bcr\text{-}abl}$ presented a fast, rolling motility mode in 3D Matrigel (Daubon et al., 2008). In living Ba/F3 $p190^{bcr\text{-}abl}$ cells, actin was visualized after transfection of a construct encoding Actin-cherry. Cell speed measured for these cells was not found significantly different from that of control untransfected cells or cells expressing GFP (data not shown). In most cases, the dense F-actin dots were seen in cells presenting reduced speed in the 2D (Fig. 1D and F and Movie 1) as well as in the 3D (Fig. 1E and F and Movie 2) situations. The cellular protrusions could still be seen, dynamically expanding in any direction whilst the F-actin dot appeared more static (Movies 1 and 2 and Fig. 1F). We speculate that in 2D, these F-actin dots could be used as attachment points to stabilize the cells on the substrate, perform matrix degradation and thereby assist the invasion process through tissues in a 3D environment.

Characterization of F-actin-based structures formed in Ba/F3p190 cells

The formation of invadopodia has been linked to tumor cell invasion across tissue boundaries and metastasis. We therefore examined whether the F-actin based structures found in Ba/F3p190 cells presented the typical features of invadopodia. The structures detected on fibronectin were also seen, although to a lesser extent, on collagen I, collagen IV, laminin or gelatin coated coverslips but not on glass to which cells did not adhere (Fig. 2A). In addition to HS1, immunostaining of Ba/F3 $p190^{bcr\text{-}abl}$ cells revealed the presence of other typical invadopodial markers such as WASp, the Src substrate and adaptor protein Tks5, fascin, the transmembrane metalloprotease MT1-MMP, the active (autophosphorylated form) of Focal adhesion kinase (P-FAK-Y397) (Fig. 2B). Remarkably, no specific accumulation of vinculin was detected at the structures. This finding is consistent with the fact that vinculin is not usually present at invadopodia (Artym et al., 2006). In GFP-$p190^{bcr\text{-}abl}$-Ba/F3 transfected cells, the tagged oncoprotein was also found localized at these F-actin enriched structures (Fig. 2B).