Editorial: **Pro-matrix metalloproteinase-9 in tumor B lymphocytes: balancing migration and homing**

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RECEIVED MARCH 10, 2014; REVISED MARCH 27, 2014; ACCEPTED MARCH 28, 2014. DOI: 10.1189/jlb.3CE0314-143R

SEE CORRESPONDING ARTICLE ON PAGE 185

Since the discovery of human MMP-9 (also known as gelatinase B) in polymorphonuclear leukocytes and monocytes in 1979 [1], researchers have focused their attention on the abnormal expression of this secreted protease in inflammation and cancer. MMP-9, expressed by various cell types, has emerged as a biomarker in many hematological and nonhematological malignancies [2, 3]. Retrospective clinical studies and experiments on in vitro and in vivo models have shown that overexpression of MMP-9 is often correlated with a malignant cancer phenotype (associated with tumor cell growth, survival, migration, and invasion) [2, 3]. In most cancers, MMP-9 is secreted as 92 kDa-inactive zymogen (pro-MMP-9) [2, 3]. Much effort has gone into understanding how pro-MMP-9 contributes to tumor progression. An elegant, pivotal study by Dufour and colleagues [4] demonstrated that the proteolytic activity of MMP-9 is not required for MMP-mediated migration. Several research groups have shown that pro-MMP-9 binds to various integral membrane proteins (such as integrins and CD44) at the surface of leukocytes and epithelial and endothelial cells [5] and thus, directly triggers intracellular signaling pathways controlling tumor cell growth, survival, migration, and invasion [6] (**Fig. 1A**).

Over the past decade, Dr. Ángeles García-Pardo's group at the Centro de Investigaciones Biológicas (Madrid, Spain) has made major contributions to our understanding of the function of MMP-9 in CLL. This disease is characterized by clonal expansion of $CD5^+$/$CD23^+$ B lymphocytes, which can be detected in the blood, bone marrow, and secondary lymphoid tissues [7]. The accumulation of (mostly quiescent) leukemic cells results from: (1) the latter's inability to develop an apoptotic program and (2) survival signals delivered by the tumor microenvironment [8]. There is a growing body of evidence to suggest that cell trafficking has a critical role in the physiopathology of CLL [7]. In contrast to normal, resting B cells, tumor B lymphocytes synthesize and secrete pro-MMP-9 [6]. García-Pardo's group [8] has demonstrated that endogenous pro-MMP-9 is involved in CLL blood–cell invasion (through Matrigel) and transendothelial migration (through HUVECs) in a PI3K/AKT- and ERK1/2-dependent manner. García-Pardo and colleagues [9] also found that the hemopexin C-terminal domain of pro-MMP-9 binds to the $\alpha4\beta1$ integrin (CD49d/CD29) and the high molecular weight CD44 isoform CD44v. Given that cells isolated from the lymph nodes or bone marrow of CLL patients express more surface-bound pro-MMP-9 than their peripheral blood counterparts [10], García-Pardo and colleagues have further explored the mechanisms of CLL cell migration and invasion when surface-bound pro-MMP-9 is present at high levels. In their latest study (published in this issue of the *Journal of Leukocyte Biology* [11]), Bailón and colleagues highlight a new role for pro-MMP-9 in CLL cell motility and (probably) retention in lymphoid tissues. The researchers showed that elevated levels of pro-MMP-9 significantly inhibit CLL cell migration and invasion in vitro and in vivo using xenograft models in NOD/SCID mice [11]. Moreover, their data indicate that pro-MMP-9 (likely as a complex with $\alpha4\beta1$ integrin) reduces the activation of RhoA GTPase, the downstream kinase effectors ERK and AKT, and focal adhesion kinase—all signaling molecules reportedly involved in the modulation of cell adhesion and motility [11]. Importantly, the observations of Bailón and colleagues imply that the function of pro-MMP-9 in cell migration and invasion in CLL is more complicated than currently envisaged, with both stimulatory and inhibitory actions on cell motility (Fig. 1B). These differences may depend on the level of pro-MMP-9 bound to malignant B cells (Fig. 1B). The source of surface-bound pro-MMP-9 may be endogenous and/or exogenous, as stromal cells in lymphoid tissues also produce pro-MMP-9 [10]. García-Pardo's group [8, 12] had shown previously that the chemokines CXCL12 and CCL21 significantly increase pro-MMP-9 production by CLL cells. In turn, pro-MMP-9 is involved in CCL21-driven CLL cell migration [12]. CXCL12 is produced by stromal cells in the lymph nodes and bone marrow, whereas CCL21 is expressed in high endothelial venules—a major route for lymphocyte homing to lymphoid tissues. Like pro-MMP-9, chemokines, such as CCL21 and CXCL12, have a decisive role in CLL cell migration and contribute to the latter's survival through various antiapoptotic and/or

Abbreviations: CLL=chronic lymphocytic leukemia, MMP-9=matrix metalloproteinase-9, pro-MMP-9=pro-matrix metalloproteinase-9

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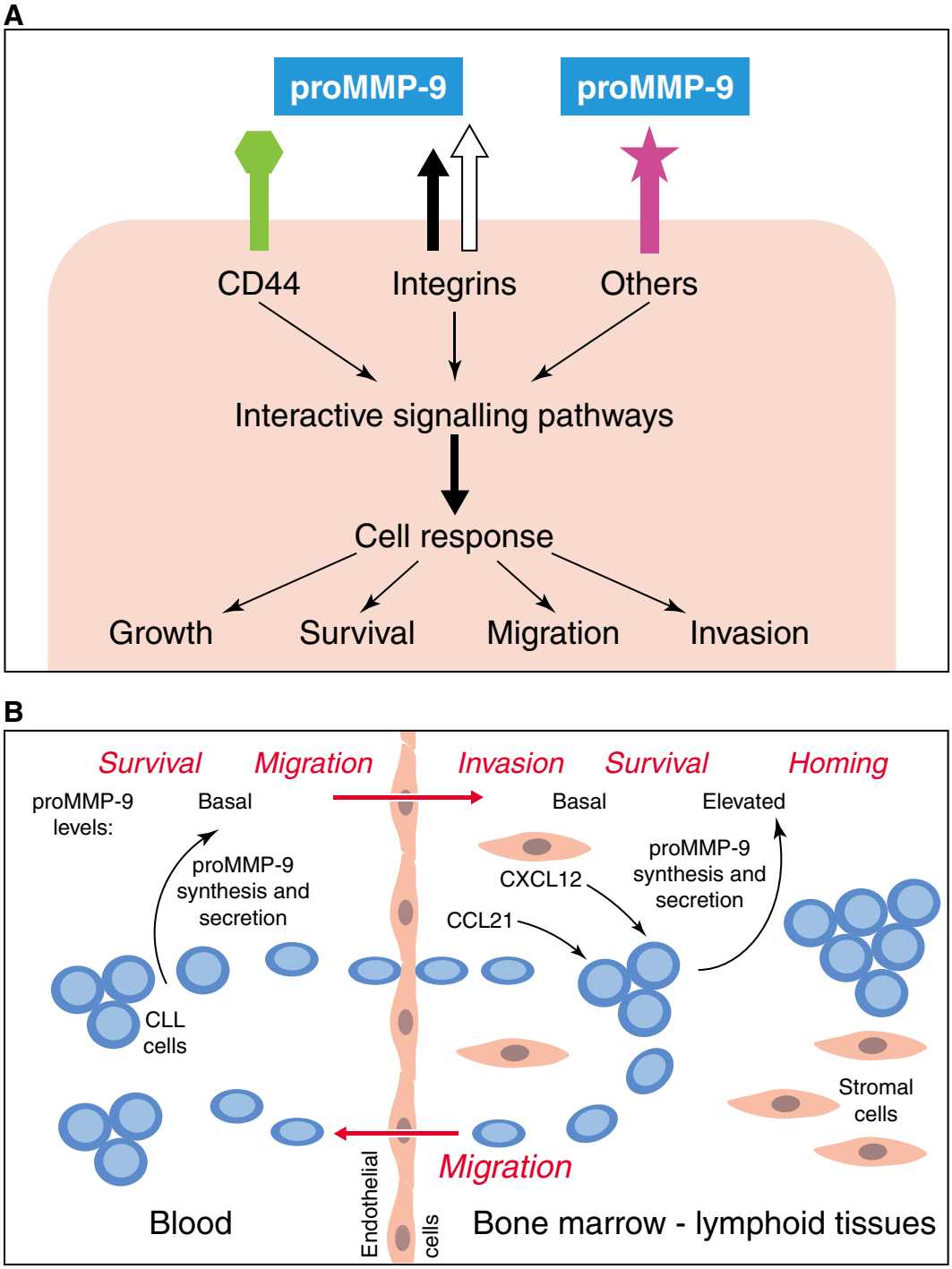

Figure 1. Schematic representation of the newly discovered signaling properties of pro-MMP-9 and its involvement in CLL trafficking. (A) Pro-MMP-9 binds to integrins ($\alpha\beta1$, $\alpha\beta2$, $\alpha v\beta3$, etc.), CD44, and other proteins (such as DNA repair protein Ku, the low-density lipoprotein receptor-related proteins, and reversion-inducing, cysteine-rich protein with Kazal motifs) on human leukocytes and epithelial and endothelial cells [5]. Specific pro-MMP-9–cell interactions trigger intracellular signaling pathways that favor cell growth, survival, migration, and/or invasion [6]. (B) Peripheral blood CLL cells release basal levels of pro-MMP-9, and the interaction of pro-MMP-9 with $\alpha4\beta1$ and CD44 on tumor B cells induces intracellular survival signals (A) [10]. The CLL cells are attracted to bone marrow and secondary lymphoid tissues by pro-MMP-9 and chemokines (such as CCL21 and CXCL12, produced by endothelial cells and stromal cells, respectively) [7]. Chemokines and pro-MMP-9 bind to chemokine receptors and $\alpha4\beta1$ on CLL cells, respectively. This leads to CLL cell transmigration across the endothelium into lymphoid tissues, where the tumor cells are protected by prosurvival factors, including pro-MMP-9 and chemokines [7, 10]. The chemokines CCL21 and CXCL12 stimulate pro-MMP-9 production by CLL cells [8, 12]. Elevated levels of pro-MMP-9, bound to CLL cells, then activate an intracellular pathway that prevents migration and thus, ultimately contributes to CLL homing [11]. This model highlights a new role for pro-MMP-9 in balancing CLL cell migration and homing.

prosurvival mechanisms [7] (Fig. 1B). Taken as a whole, these data suggest that pro-MMP-9 has a lot in common with chemokines; one can speculate that both pro-MMP-9 and chemokines contribute to the extravasation, survival, and homing of neoplastic B cells in vivo (Fig. 1B).

Although conventional chemotherapy is often effective at killing CLL cells in the peripheral blood, residual leukemia stem cells remain in the bone marrow and lymphoid tissues [7, 13]. Recent evidence indicates that the chemokines produced by bone marrow stromal cells concentrate within microenvironmental "niches", which favor the growth of CLL stem cells and contribute to the induction of drug resistance [7, 13]. The findings of Bailón and colleagues [11] suggest that elevated levels of pro-MMP-9 in these niches can also contribute to CLL cell engraftment and thus, facilitate the cells' survival and acquisition of drug resistance. Integrins and chemokines are already considered to be targets for improving treatment efficacy in CLL [8, 13]. In view of the above-mentioned roles of pro-MMP-9 in CLL [8–11], can this protein become a drug target? Initial trials of antiprotease drugs targeting the catalytic site of MMPs in cancer patients have failed [6]. The enzyme-inhibitor approach may be no longer sufficient, as it does not address the interaction of pro-MMP-9 with its "receptors" and the subsequent cell signaling. Hence, novel therapeutic strategies already involve newly designed inhibitors, such as peptides that block pro-MMP-9 cell-surface interactions, and function-blocking anti-MMP-9 antibodies [6]. In principle, these novel treatment options for CLL could be combined with conventional therapies.

The work of Bailón et al. [11] raises two exciting questions with respect to pro-MMP-9 as a novel, "bidirectional" regulator of cell motility. Firstly, is this novel concept relevant in other disease states characterized by pro-MMP-9 overexpression (such as other leukemias, solid tumors, inflammatory disease, and cardiovascular disease)? Secondly, does the dual ability of pro-MMP-9 to stimulate or inhibit cell migration extend to other secreted proteases that are, like pro-MMP-9, overexpressed in cancer and involved in cell invasion, cell-surface binding, and the modulation of signaling pathways? For example, cathepsin G, serine elastase, pro-MMP-1, pro-MMP-2, and MMP-19 all interact with cell-surface receptors [6, 14]. Pro-MMP-2 binds to integrins and may be just as critical as pro-MMP-9 for activating signaling cascades related to cell survival and transmigration [6]. It remains to be determined whether this novel concept is relevant for these other proteases.

In conclusion, the study by Bailón et al. [11], described in the current issue, constitutes a major step forward in our understanding of the function of pro-MMP-9 and emphasizes the need for further investigation of this protein's physiological and pathological roles in cell motility.

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KEY WORDS:
leukemia · function · gelatinase · cell-surface binding · signaling