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Imatinib-mesylate enhances the maintenance of chronic myeloid leukemia stem cell potential in the absence of glucose

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ABSTRACT

The introduction of BCR/Abl tyrosine kinase inhibitors (TKI), such as imatinib-mesylate (IM), has revolutioned the treatment of chronic myeloid leukemia (CML). However, although extremely effective in inducing CML remission, IM is unable to eliminate leukemia stem cells (LSC). This is largely due to the suppression of BCR/Abl protein, driven by the reduction of energy supply due to oxygen or glucose shortage, in stem cell niches of bone marrow. Here, we investigated whether, in K562 and KCL22 CML cell cultures, glucose shortage induces refractoriness of stem cell potential to IM. In the absence of glucose, IM, while maintaining its detrimental effect on CML cell bulk, actually enhanced colony formation ability and stem cell potential. This was paralleled by an increased expression of the Nanog and Sox-2 stem cell markers. These evidences stress further the importance of developing strategies alternative to TKI capable to target LSC of CML.

1. Introduction

Chronic myeloid leukemia (CML) is a hematopoietic disease driven by the t9;22 translocation, which results in the expression of the "fusion" protein BCR/Abl, a constitutively active tyrosine kinase (Holyoake and Vetrie, 2017; Gambacorti-Passerini et al., 2016). Imatinib-mesylate (IM), the prototype of tyrosine kinase inhibitors (TKI) capable to target the enzymatic activity of BCR/Abl oncoprotein, is extremely efficient in ensuring deep molecular response and prolonged survival in chronic-phase CML patients (Druker et al., 2001). However, a critical aspect of the outcome of TKI treatment is that, in most of even well-responding patients, disease relapses when TKI treatment is interrupted (Mahon et al., 2010). This is most likely due to the fact that leukemia stem cells (LSC), the CML cell subset believed to sustain minimal residual disease (MRD) and the consequent risk of recurrence (Ghiaur et al., 2012), are insensitive to TKI (Graham et al., 2002; Corbin et al., 2011).

We previously found that incubation of CML cells in atmosphere at very low oxygen tension time-dependently determines BCR/Abl protein suppression, so that LSC that are maintained under these conditions are refractory to TKI due to the lack of the TKI molecular target (Giuntoli et al., 2006; Cheloni et al., 2017a; Cheloni et al., 2017b). The point is relevant in view of the fact that normal hematopoietic stem potential is preserved in stem cell niches placed in bone marrow (BM) areas where oxygen tension is physiologically very low (Parmar et al., 2007). We envisioned on these bases a model in which MRD of CML persists due the capacity of LSC to survive under serious energy constraints independently of BCR/Abl signaling (Rovida et al., 2014). These LSC, however, remain leukemic at the BCR/abl transcript level, so that they are capable, when transferred to conditions where BCR/Abl protein can be expressed, to undergo BCR/Abl-dependent clonal expansion and thereby determine relapse of disease (Giuntoli et al., 2006; Giuntoli et al., 2011).

The shortage of glucose, like that of oxygen, represents a crucial path to energy restriction, due to the importance of glucose oxidation in cell metabolism. We recently showed that glucose shortage also leads to BCR/Abl protein suppression (Bono et al., 2016). On this basis, we decided to determine whether glucose shortage induces refractoriness of CML stem cell potential to IM. We found that, in K562 and KCL22 CML cell cultures established in the absence of glucose, IM maintains its activity and consequently reduces the total number of viable cells. However, in IM-treated cultures, the maintenance of stem cell potential was markedly enhanced. Accordingly, the expression of the Nanog

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and Sox-2 stem cell markers was increased. The possible unfavorable consequences of these facts are discussed.

2. Materials and methods

2.1. Cells and culture conditions

K562 blast-crisis CML cells (Lozzio and Lozzio, 1975) were purchased from the German Collection of Cell Cultures (Braunschweig, Germany) and routinely cultured in RPMI 1640 medium supplemented with 2 g/L D-glucose, 10% heat-inactivated foetal bovine serum, 2 mM L-glutamine, 50 units/mL penicillin and 50 μg/mL streptomycin (all from EuroClone, Paington, UK). Most experiments were carried out using RPMI 1640 medium without D-glucose (Gibco by ThermoFisher Scientific, Waltham, MA, USA) supplemented as above. IM was dissolved in PBS (EuroClone) and IM-untreated cultures were also PBS-supplemented. Experiments were performed with cells harvested from exponentially-growing routine cultures and subcultured (5×10^5/mL) in fresh medium 24 h before plating (3×10^5/mL). Incubation was carried out at 37 °C in water-saturated atmosphere containing 5% CO_2 and 21% O_2. Viable cells were counted in a hemocytometer by trypan blue exclusion.

2.2. Colony Formation Ability (CFA) assay

Cells rescued from cultures established as described above in the absence of glucose and treated or not with IM (Liquid Culture 1 - LC1) were washed free of drug and replated (3×10^3/35-mm dish) at different times of incubation into IM-free, methylcellulose-containing secondary semisolid cultures (#04432, Stem Cell Technology, Vancouver, BC, Canada) supplemented with standard glucose concentration. Colonies were counted after 10 days.

2.3. Culture Repopulation Ability (CRA) assay

The CRA assay is an *in vitro* substitute for the marrow repopulation ability assay *in vivo* where the estimate of stem cell potential is obtained, rather than transplanting cells into animals, via cell transfer to liquid cultures (LC2) (Cheloni and Tanturli, 2016; Cipolleschi et al., 2000, 2013; Giuntoli et al., 2006, 2011; Ivanovic et al., 2000; Ivanovic et al., 2002).

Cells from LC1 established as described above were washed free of drug and replated (3×10^4 cells/mL), at different times of incubation in LC1, into IM-free secondary LC2 containing standard glucose concentration. Culture medium was never changed during LC1 or LC2. The kinetics of viable cell number in LC2 provides an estimate of the CRA of LC1 cells.

2.4. Cell protein content analysis

Cells were washed once with ice-cold phosphate buffered saline (PBS) containing 100 μM Na_3VO_4. Total cell lysates were obtained in Laemmli buffer (62.5 mM Tris/HCl, pH 6.8, 10% glycerol, 0.005% bromophenol blue, and 2% SDS), while hypotonic buffer (10 mM HEPES, 10 mM NaCl, 5 mM $NaHCO_3$, 1 mM $CaCl_2$-$2H_2O$, 0.5 mM $MgCl_2$-$6H_2O$, 5 mM Na_2EDTA, 20 mM $Na_4P_2O_7$, 10 mM NaF, supplemented with protease inhibitors: 100 μM Na_3VO_4, 1 mM PMSF, 0.1 U/mL Aprotinin, 4 μg/mL Pepstatin, 10 μg/mL TPCK, all from Sigma-Aldrich, St. Louis, MO, USA) was used for cytosol/nucleus separation. Protein concentration was determined by the BCA method (#23225, Pierce™ BCA Protein Assay Kit by Thermo Fisher Scientific) and 50–80 μg protein/sample were subjected to SDS-PAGE. Separated proteins were transferred onto nitrocellulose membranes (#10600002, Amersham™ Protran™ 0.45 μm NC by GE Healthcare Life Sciences, Little Chalfont, Buckinghamshire, UK) by electroblotting. Membranes were blocked in a 1:1 dilution of Odyssey (LI-COR® Biosciences, Lincoln, NE, USA) blocking buffer (OBB) with PBS for 1 h at room temperature (RT) and then incubated (overnight, 4 °C) with primary antibody in a 1:1 dilution of OBB with PBS-0,1% Tween (T-PBS). Primary antibodies used were: anti-c-Abl (K-12), rabbit polyclonal (#sc-131), anti-Sox-2 (E-4), mouse monoclonal (#sc-365823), anti-Fibrillarin (D-14), goat polyclonal (#sc-11336) (all from Santa Cruz Biotechnology, Santa Cruz, CA, USA); anti-Nanog (D73G4) XP, rabbit monoclonal (#4903, Cell Signaling Technology, Danvers, MA, USA), anti-Vinculin (clone hVIN-1), mouse monoclonal (#V9131, Sigma-Aldrich). Washed membranes were incubated for 1 h at RT in 1:1 OBB/T-PBS containing an IRDye®800CW- or IRDye®680-conjugated secondary antibody (Lulli et al., 2012). Antibody-coated protein bands were visualized and quantified by the Odyssey Infrared Imaging System (LI-COR® Biosciences), as previous reported (Witort et al., 2013).

2.5. Statistical analysis

Data are presented as the mean ± SD (unless indicated otherwise) of independent experiments and were compared by using a Student's *t*-test. P values (p) of <0.05 were considered statistically significant.

3. Results

3.1. IM affects K562 and KCL22 cell survival and growth under glucose shortage

The effects of IM on K562 cell growth under our standard incubation conditions are shown in Fig. 1A. Viable cell number underwent a 5-fold increase, peaking on day 10, to decline thereafter as an effect of culture exhaustion. IM addition on day 3 determined a minor, although significant, effect until day 5, to induce thereafter a marked decrease of cell number, as expected. The effects of IM addition on day 2, 4 or 7 were then evaluated under glucose shortage (Fig. 1B), a condition where CML cell growth is reduced (Bono et al., 2016). IM treatment reduced cell number, the effect being the more marked, the earlier the IM administration. Such an outcome is in keeping with the progressive suppression, under our experimental conditions, of BCR/Abl protein, the molecular target of IM. Indeed, we previously demonstrated in K562 cells a reduction of about 25, 70 or 100% of BCR/Abl protein expression after 2, 4 or 7 days, respectively, of glucose shortage (Bono et al., 2016). Thus, IM activity was maintained under glucose shortage, although the effect of the drug was progressively quenched during culture decline, so that IM administered on day 7 of incubation was ineffective at least until day 14. A similar result was obtained in KCL22 cells, the growth of which under glucose shortage was markedly reduced by IM addition on day 2 (Bono et al., 2018).

3.2. IM enhances Colony Formation Ability of K562 cells under glucose shortage

IM treatment was also tested by CFA assay (Fig. 2). K562 cells rescued from glucose-free liquid cultures treated or not with IM from different times of incubation were transferred on day 10 or day 14 to non-selective (standard glucose concentration, no IM) secondary semisolid cultures. In either case, IM enhanced CFA, the effect being the more marked, the earlier the IM treatment. Like in the experiments of Fig. 1B, the effect of IM was zeroed when treatment was applied after cell number in culture started to decline (day 7). The yield of cells transferred to semisolid cultures on day 14 (Fig. 2B) was lower than that of cells transferred on day 10 (Fig. 2A), most likely as result of the progressive disappearance of clonogenic progenitors in liquid cultures.

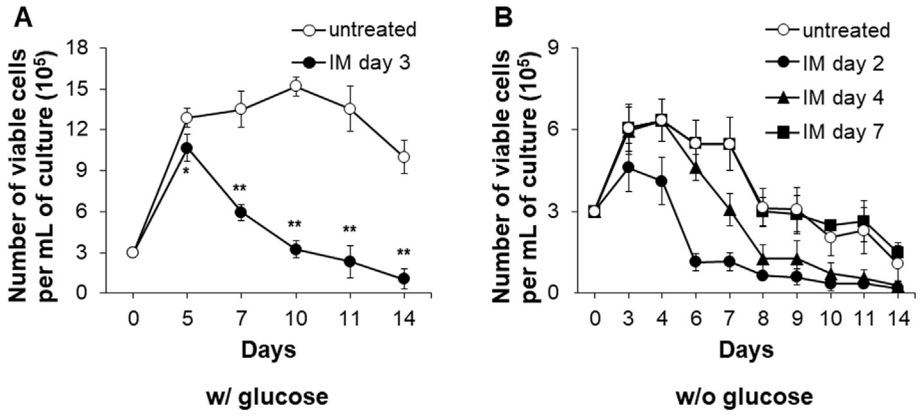

Fig. 1. Effects of IM on K562 cell survival and growth in the presence or the absence of glucose. K562 cells were plated at 3×10^5 cells/mL and incubated under standard conditions in the presence (A) or the absence (B) of glucose: (o) untreated control; (●) IM administered on day 3 (A) or day 2 (B); (▲) IM on day 4; (■) IM on day 7. Viable cells were counted by trypan blue exclusion at the indicated times. Data represent the mean ± SD of 10 independent experiments. A: **p < 0.01 (●) vs (o) from day 7, *p < 0.05 (●) vs (o) day 5. B: **p < 0.01 (●) vs (o) from day 6, *p < 0.05 (●) vs (o) day 4; **p < 0.01 (▲) vs (o) from day 7 to day 9, *p < 0.05 (▲) vs (o) from day 10 (two-tailed Student's *t*-test).

3.3. IM enhances Culture Repopulation Ability of K562 and KCL22 cells under glucose shortage

The effects of glucose shortage and IM treatment on the maintenance of CML stem cell potential in culture were then tested by CRA assay (Fig. 3 and Bono et al., 2018). K562 cells from cultures established in the absence of glucose and treated with IM on different times of incubation (LC1) like in Fig. 1B, were transferred on day 10 (Fig. 3A) or 14 (Fig. 3B) to non-selective (standard glucose concentration, no IM) secondary LC2. With IM-treated LC1 cells, the increase of viable cell number in LC2 was the more rapid and marked, the earlier the IM treatment of LC1. Here again, the effect of IM was zeroed when treatment was applied during the decline phase of LC1. This effect was magnified in the experiments of Fig. 3B, where day-14 LC1 cells were transferred to LC2. In IM-untreated cells rescued from day-14 LC1, unlike in those from day-10, stem cell potential was undetectable. A similar result was obtained with KCL22 cells, where IM treatment on day 2 of LC1 determined a marked increase of viable cell number in LC2 compared to IM-untreated cells (Bono et al., 2018). Therefore, IM treatment during the florid phase of LC1 (day 2 or 4 for K562 cells, day 2 for KCL22 cells) determined a massive LC2 repopulation, indicating that IM protected stem cell potential from its suppression in glucose-free cultures.

3.4. Nanog and Sox-2 protein expression is increased by IM in K562 cells under to glucose shortage

The impact of IM on the maintenance of stem cell phenotype of K562 cells under glucose shortage was evaluated not only at the functional, but also at the molecular level. Fig. 4A (upper blot) shows, first of all, that BCR/Abl protein is suppressed in the absence of glucose, an event we extensively described and characterized (Bono et al., 2016). In the same experiments, the effects of IM on the expression of stem cell markers were also addressed. IM enhanced Nanog expression (Fig. 4A), the effect being visible when treatment was applied on day 2 or day 4, but not day 7, with either day-10 or day-14 cells. Sox-2 expression was evaluated following cytosol/nucleus separation (Fig. 4B). IM markedly enhanced nuclear Sox-2 expression, again with similar outcome for day-10 (left) and day-14 cells (right). These data well explain the effects of IM on stem cell potential shown in Fig. 3.

4. Discussion

We had previously demonstrated that, under energy restriction, BCR/Abl protein is time-dependently suppressed (Bono et al., 2016), while a CML cell subset endowed with progenitor/stem cell properties is maintained (Giuntoli et al., 2006, 2011). The study reported here showed that a similar cell subset was maintained when energy restriction was driven via glucose shortage, and addressed the effects of IM applied when (days 2 or 4 for K562, day 2 for KCL22) cells still express

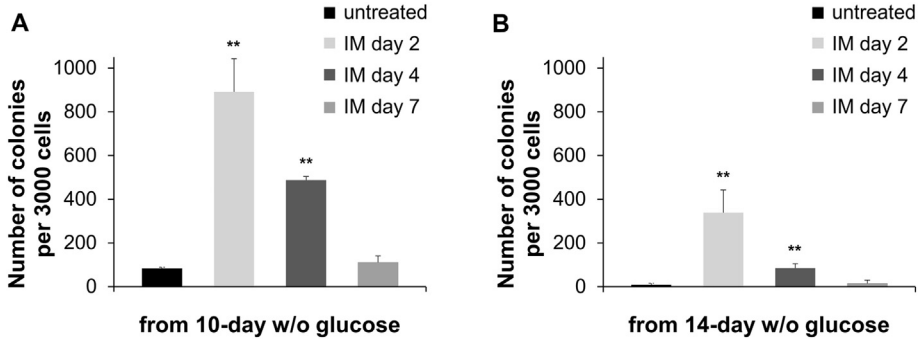

Fig. 2. Effects of IM on CFA assay of glucose-free K562 cell cultures. K562 cells were cultured (3×10^5/mL) in glucose-free liquid medium and treated or not with IM from the indicated times to day 10 (A) or 14 (B) of incubation. Cells were then washed free of drug and replated (3×10^3/35-mm dish) into IM-free, methylcellulose-containing semisolid medium supplemented with standard glucose concentration. Colonies were counted after 10 days. Data represent the mean + SD of 4 independent experiments. **p < 0.01 vs untreated control (two-tailed Student's *t*-test).

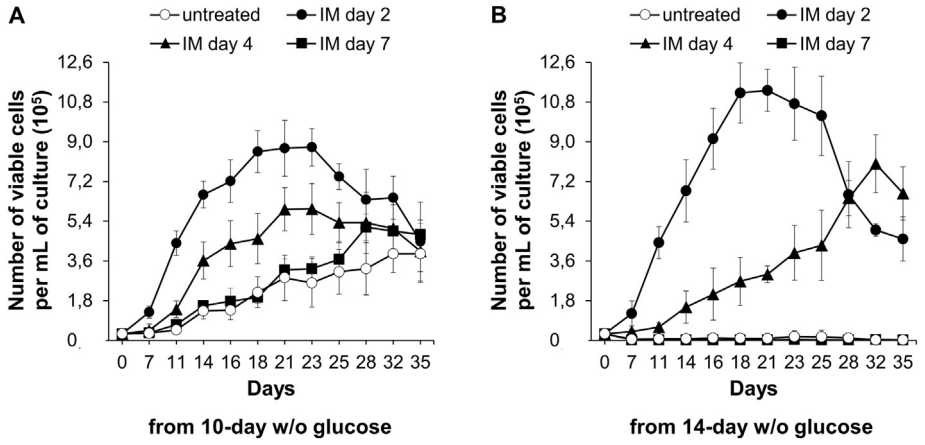

Fig. 3. Effects of IM on stem cell potential (CRA assay) of glucose-free K562 cell cultures. K562 cells were cultured (3×10^5/mL) in glucose-free liquid cultures (LC1) and treated or not (o) with IM from day 2 (●), day 4 (▲) or day 7 (■). On day 10 (A) or 14 (B) of incubation in LC1, cells were washed free of drug and replated (3×10^4/mL) into IM-free LC2 supplemented with standard glucose concentration. The maintenance of stem cell potential at the end of LC1 was determined by counting viable cells (trypan blue exclusion) at the indicated times of incubation in LC2. Data represent the mean ± SD of 7 independent experiments. A: $^{**}p < 0.01$ (●) vs (o) from day 11 to day 23, $^{*}p < 0.05$ (●) vs (o) from day 25 to day 32; $^{**}p < 0.01$ (▲) vs (o) from day 14 to day 21, $^{*}p < 0.05$ (▲) vs (o) from day 23 to day 28. B: $^{**}p < 0.01$ (●) vs (o) from day 11; $^{**}p < 0.01$ (▲) vs (o) from day 18 (two-tailed Student's *t*-test).

BCR/Abl protein and are thereby sensitive to the treatment. That CML cell bulk reacts differently from progenitor/stem cells to energy restriction and that glucose shortage is adequate to highlight their different response to IM is witnessed by the opposite effects determined by day-2 or day-4 IM treatment in Fig. 1B when compared to Figs. 2 and 3. While IM decreased cell bulk, as expected, it increased the yield of progenitor/stem cell assays. This is a remarkable finding, as in a previous study where energy restriction was driven via incubation of K562 cells in low-oxygen atmosphere (Giuntoli et al., 2011), stem cell potential was proved to be refractory to IM, but by no means enhanced by IM like in the experiments of Fig. 3. This indicates that, while stem cell potential maintained under energy restriction is generally insensitive to IM, different types of metabolic pressure impact differently the response to IM.

CFA was also markedly enhanced in the presence of IM (Fig. 2), a finding that matches closely that obtained for stem cell potential as determined by CRA assay (Fig. 3 and Bono et al., 2018). These results do not contradict the notion that CML non-stem progenitors capable to form colony in semisolid medium are sensitive to IM (Belle et al., 2012). In our experiments, indeed, CFA assays were not established with cells directly recovered from routine cultures, but following a 10 or 14 day-long selective incubation in liquid medium under glucose shortage. This implies that clonal expansion in semisolid medium was measured after a 20- to 24-day total transit time from the beginning of IM treatment. This time is closer to that of CRA assay (30 to 34 days to peak of LC2), which provides an estimate of stem cell potential, than to that of standard CFA assay (colonies scored on day 10). Thus, in our study, CFA assays reflect clonogenic progenitors of significantly higher hierarchical level than that of standard *in vitro* colony-forming cells. We believe that this conclusion is appropriate to explain the similar outcome of the experiments of Figs. 2 and 3.

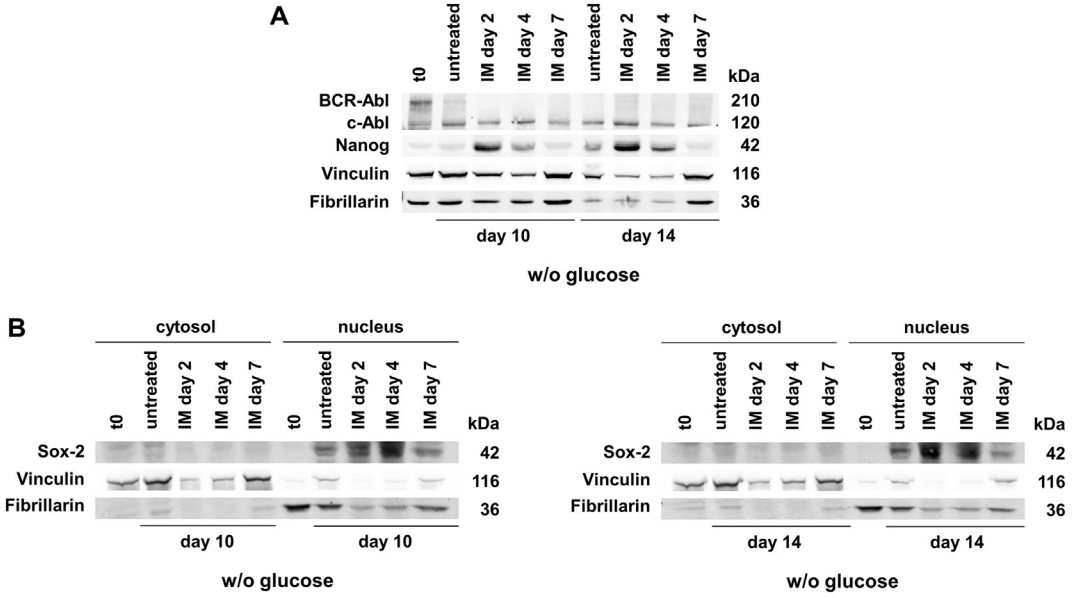

Fig. 4. Effects of IM on the expression of BCR/Abl and stem cell markers in glucose-free K562 cell cultures. K562 cells were cultured (3×10^5/mL) in glucose-free liquid cultures treated or not with IM from the indicated times and lysed on day 10 or 14 of incubation. Total cell lysates (A) or lysates subjected to cytosol/nucleus separation (B) were processed for immunoblotting with the indicated antibodies. Vinculin (cytosolic) and fibrillarin (nuclear) were used as loading control.

The comparison of results of Figs. 2 and 3 obtained with K562 cells rescued from day-10 (A) or day-14 (B) LC1 provides additional information. The two different incubation times led to different combinations of stem/progenitor cells, as indicated by the fact that, from day 10 to day 14 of incubation under glucose shortage, CFA declined (Fig. 2), while CRA increased (Fig. 3). This is most probably the result of the progressive concentration in culture of stem/progenitor cells of higher hierarchical level. Irrespective of those differences, IM enhanced the yield of either assay, provided it was administered before BCR/Abl suppression (day 2 or 4). Fig. 3 requires a further comment. The differences between experimental variants were magnified in B, where, with respect to A, the curves of LC2 repopulation were much more scattered and, more importantly, the stem cell potential in the absence of IM (or with IM addition on day 7) was completely suppressed instead of reduced. Thus, the longer stem cell selection in culture, the higher the detrimental effects of glucose shortage (control cultures) as well as the stimulatory effects of IM.

LC2 repopulation reflects the growth-promoting effect of BCR/Abl re-expression following the transfer of LC1 cells into the growth-permissive LC2 environment (Giuntoli et al., 2006, 2011). When conditions suppressing BCR/Abl signaling (for instance, IM treatment or oxygen and/or glucose shortage) are established, CML cells capable to survive in its absence are primed to rescue BCR/Abl protein expression, once conditions permissive for clonal expansion are restored, more rapidly than at the steady-state turnover (Giuntoli et al., 2006; Weisberg and Griffin, 2000). However, the differences between the results shown in A and B of Fig. 3 seem related, rather than to a question of enhanced BCR/Abl protein re-expression, to a better maintenance of stem cell potential. A strong indication in this direction is provided by the data relative to the expression of Nanog and Sox-2 proteins, stem cell markers in different hematological malignancies (Gelebart et al., 2012; Kakiuchi et al., 2017; Xin et al., 2013), which was enhanced in the presence of IM, again only when the IM target BCR/Abl was expressed (Fig. 4).

That the block of BCR/Abl signaling by IM enhanced the maintenance of stem cell potential under glucose shortage is the main result of our study. This is especially evident in Fig. 3B, where stem cell potential was completely suppressed under control conditions. Our hypothesis to explain the finding that IM protected stem cell compartment under energy restrictions is as follows. We previously demonstrated that sustained IL3-induced proliferation of normal BMC steers the cycling of metabolically-selected stem cells to boost clonal expansion, while constraints on those stimuli privilege self-renewal (Ivanovic et al., 2002). In CML, the maintenance of the IL3-mimetic BCR/Abl signaling (Jiang et al., 2002) is apparently detrimental to stem cell adaptation to energy restriction (Rovida et al., 2014), so that stem cell potential is better capable to stand glucose shortage when excessive BCR/Abl-dependent growth stimulation is quenched by IM. It is to underscore again here that this can be obtained only if the CML stem cell subset involved in the phenomenon (I) expresses the molecular target of IM (day 2 or 4 of incubation) and (II) is capable to survive when BCR/Abl signaling is suppressed or strongly reduced (day 7 and beyond). CML cell bulk, on the contrary, decreases under IM treatment because addicted to full BCR/Abl-dependent growth stimulation (Cheloni et al., 2017a). The relationship between hematopoietic growth factors, glucose supply and cell survival is long-known (Dexter et al., 1984; Whetton et al., 1984).

The physiological occurrence of low-glucose areas in hematopoietic tissue *in vivo* is under question. Areas where glucose gets close to exhaustion have been shown in solid tumors (Tannock, 1968) and are very likely to characterize the hyperplastic BM of CML patients. Glucose shortage also appears compatible with normal BM, where areas at very low blood perfusion host the most primitive HSC (Winkler et al., 2010). In any case, we consider the findings reported here as a proof of concept that the treatment with IM or other TKI, while remarkably effective in CML debulking and induction of remission, may be detrimental to a favorable long-term outcome of disease, not because just inactive on the LSC compartment (Giuntoli et al., 2006, 2011; Cheloni et al., 2017a), but because actually capable to protect its maintenance.

Finally, it is necessary to comment here on the fact that permanent suppression of MRD following TKI treatment seems to be achieved in a small fraction of patients. Our hypothesis to explain such a favorable outcome lies in the dual CML stem cell model we proposed, where a BCR/Abl-independent LSC subset resistant to energy restriction and dedicated to MRD maintenance is dynamically related to another, downstream BCR/Abl-dependent LSC subset committed to clonal expansion (Rovida et al., 2014). In some patients, TKI-induced stress on the latter subset might be temporarily compensated by the former, until LSC capable to sustain MRD themselves are exhausted.

5. Conclusions

We show here that under glucose shortage IM treatment results in an enhancement of CML stem cell potential. This represents a proof of concept that the treatment with IM or other TKI may be detrimental to a favorable long-term outcome of CML due to adverse effects of therapy related to an enhanced persistence of MRD.

Conflicts of interest

The authors declare no conflict of interest.

Author contributions

S.B. ideated and performed the experiments, analyzed the data and wrote the manuscript. P.D.S. and M.L. conceived and supervised the study, and critically revised the manuscript. All authors read and approved the final manuscript.

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References

Belle, L., Bruck, F., Foguenne, J., Gothot, A., Beguin, Y., Baron, F., Briquet, A., 2012. Imatinib and nilotinib inhibit hematopoietic progenitor cell growth, but do not prevent adhesion, migration and engraftment of human cord blood CD34+ cells. PLoS One 7, e52564.

Bono, S., Lulli, M., D'Agostino, V.G., Di Gesualdo, F., Loffredo, R., Cipolleschi, M.G., Provenzani, A., Rovida, E., Dello Sbarba, P., 2016. Different BCR/Abl protein suppression patterns as a converging trait of chronic myeloid leukemia cell adaptation to energy restriction. Oncotarget 7, 84810–84825.

Bono, S., Dello Sbarba, P., Lulli, M., 2018. Data on the effect of Imatinib on survival/growth and stem cell potential of KCL22 cells subjected to glucose shortage. (Data in Brief submitted).

Cheloni, G., Tanturli, M., 2016. The Culture Repopulation Ability (CRA) assay and incubation in low oxygen to test antileukemic drugs on imatinib-resistant CML stem-like cells. Methods Mol. Biol. 1465, 73–85.

Cheloni, G., Poteti, M., Bono, S., Masala, E., Mazure, N.M., Rovida, E., Lulli, M., Dello Sbarba, P., 2017a. The leukemic stem cell niche: adaptation to "hypoxia" versus oncogene addiction. Stem Cells Int. 4979474.

Cheloni, G., Tanturli, M., Tusa, I., DeSouza, N.H., Shan, Y., Gozzini, A., Mazurier, F., Rovida, E., Li, S., Dello Sbarba, P., 2017b. Targeting chronic myeloid leukemia stem cells with the hypoxia-inducible factor inhibitor acriflavine. Blood 130, 655–665.

Cipolleschi, M.G., Rovida, E., Ivanovic, Z., Praloran, V., Olivotto, M., Dello Sbarba, P., 2000. The expansion of murine bone marrow cells preincubated in hypoxia as an in vitro indicator of their marrow-repopulating ability. Leukemia 14, 735–739.

Cipolleschi, M.G., Rovida, E., Dello Sbarba, P., 2013. The Culture-Repopulating Ability assays and incubation in low oxygen: a simple way to test drugs on leukaemia stem or progenitor cells. Curr. Pharm. Des. 19, 5374–5383.

Corbin, A.S., Agarwal, A., Loriaux, M., Cortes, J., Deininger, M.W., Druker, B.J., 2011. Human chronic myeloid leukemia stem cells are insensitive to imatinib despite inhibition of BCR-ABL activity. J. Clin. Invest. 121, 396–409.

Dexter, T.M., Whetton, A.D., Bazill, G.W., 1984. Haemopoietic cell growth factor and glucose transport. Its role in cell survival and the relevance of this in normal haemopoiesis and leukaemia. Differentiation 27, 163–167.

Druker, B.J., Sawyers, C.L., Kantarjian, H., Resta, D.J., Reese, S.F., Ford, J.M., Capdeville, R., Talpaz, M., 2001. Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. N. Engl. J. Med. 344, 1038–1042.

Gambacorti-Passerini, C., Aroldi, A., Cordani, N., Piazza, R., 2016. Chronic myeloid leukemia: second-line drugs of choice. Am. J. Hematol. 91, 9.

Gelebart, P., Hegazy, S.A., Wang, P., Bone, K.M., Anand, M., Sharon, D., Hitt, M., Pearson, J. D., Ingham, R.J., Ma, Y., Lai, R., 2012. Aberrant expression and biological significance of Sox2, an embryonic stem cell transcriptional factor, in ALK-positive anaplastic large cell lymphoma. Blood Cancer J. 2, e82.

Ghiaur, G., Gerber, J., Jones, R.J., 2012. Concise review: cancer stem cells and minimal residual disease. Stem Cells 30, 89–93.

Giuntoli, S., Rovida, E., Barbetti, V., Cipolleschi, M.G., Olivotto, M., Dello Sbarba, P., 2006. Hypoxia suppresses BCR/Abl and selects imatinib-insensitive progenitors within clonal CML populations. Leukemia 20, 1291–1293.

Giuntoli, S., Tanturli, M., Di Gesualdo, F., Barbetti, V., Rovida, E., Dello Sbarba, P., 2011. Lucose availability in hypoxia regulates the selection of chronic myeloid leukemia progenitor subsets with different resistance to imatinib-mesylate. Haematologica 96, 204–212.

Graham, S.M., Jorgensen, H.G., Allan, E., Pearson, C.M., Alcorn, J., Richmond, L., Holyoake, T. L., 2002. Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 in vitro. Blood 99, 319–325.

Holyoake, T.L., Vetrie, D., 2017. The chronic myeloid leukemia stem cell: stemming the tide of persistence. Blood 129, 12.

Ivanovic, Z., Bartolozzi, B., Bernabei, P.A., Cipolleschi, M.G., Rovida, E., Milenkovic, P., Praloran, V., Dello Sbarba, P., 2000. Incubation of murine bone marrow cells in hypoxia ensures the maintenance of marrow-repopulating ability together with the expansion of committed progenitors. Br. J. Haematol. 108, 424–429.

Ivanovic, Z., Belloc, F., Faucher, J.L., Cipolleschi, M.G., Praloran, V., Dello Sbarba, P., 2002. Hypoxia maintains and interleukin-3 reduces the pre-colony-forming cell potential of dividing CD34(+) murine bone marrow cells. Exp. Hematol. 30, 67–73.

Jiang, X., Ng, E., Yip, C., Eisterer, W., Chalandon, Y., Stuible, M., Eaves, A., Eaves, C.J., 2002. Primitive interleukin 3 null hematopoietic cells transduced with BCR-ABL show accelerated loss after culture of factor-independence in vitro and leukemogenic activity in vivo. Blood 100, 3731–3740.

Kakiuchi, S., Minami, Y., Miyata, Y., Mizutani, Y., Goto, H., Kawamoto, S., Yakushijin, K., Kurata, K., Matsuoka, H., Minami, H., 2017. NANOG expression as a responsive biomarker during treatment with hedgehog signal inhibitor in acute myeloid leukemia. Int. J. Mol. Sci. 18, 486.

Lozzio, C.B., Lozzio, B.B., 1975. Human chronic myelogenous leukemia cell-line with positive Philadelphia chromosome. Blood 45, 321–334.

Lulli, M., Witort, E., Papucci, L., Torre, E., Schiavone, N., Dal Monte, M., Capaccioli, S., 2012. Coenzyme Q10 protects retinal cells from apoptosis induced by radiation in vitro and in vivo. J. Radiat. Res. 53, 695–703.

Mahon, F.X., Rea, D., Guilhot, J., Guilhot, F., Huguet, F., Nicolini, F., Legros, L., Charbonnier, A., Guerci, A., Varet, B., Etienne, G., Reiffers, J., Rousselot, P., 2010. Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular remission for at least 2 years: the prospective, multicentre Stop Imatinib (STIM) trial. Lancet Oncol. 11, 1029–1035.

Parmar, K., Mauch, P., Vergilio, J.A., Sackstein, R., Down, J.D., 2007. Distribution of hematopoietic stem cells in the bone marrow according to regional hypoxia. Proc. Natl. Acad. Sci. U. S. A. 104 (13):5431–5436. https://doi.org/10.1073/pnas.0701152104.

Rovida, E., Peppicelli, S., Bono, S., Bianchini, F., Tusa, I., Cheloni, G., Marzi, I., Cipolleschi, M. G., Calorini, L., Dello Sbarba, P., 2014. The metabolically-modulated stem cell niche: a dynamic scenario regulating cancer cell phenotype and resistance to therapy. Cell Cycle 13, 3169–3175.

Tannock, I.F., 1968. The relation between cell proliferation and the vascular system in a transplanted mouse mammary tumour. Br. J. Cancer 22, 258–273.

Weisberg, E., Griffin, J.D., 2000. Mechanism of resistance to the ABL tyrosine kinase inhibitor STI571 in BCR/ABL-transformed hematopoietic cell lines. Blood 95, 3498–3505.

Whetton, A.D., Bazill, G.W., Dexter, T.M., 1984. Haemopoietic cell growth factor mediates cell survival via its action on glucose transport. EMBO J. 3, 409–413.

Winkler, I.G., Barbier, V., Wadley, R., Zannettino, A.C., Williams, S., Levesque, J.P., 2010. Positioning of bone marrow hematopoietic and stromal cells relative to blood flow in vivo: serially reconstituting hematopoietic stem cells reside in distinct nonperfused niches. Blood 116, 375–385.

Witort, E., Lulli, M., Carloni, V., Capaccioli, S., 2013. Anticancer activity of an antisense oligonucleotide targeting TRADD combined with proteasome inhibitors in chemoresistant hepatocellular carcinoma cells. J. Chemother. 25, 292–297.

Xin, H., Kong, Y., Jiang, X., Wang, K., Qin, X., Miao, Z.H., Zhu, Y., Tan, W., 2013. Multi-drug-resistant cells enriched from chronic myeloid leukemia cells by Doxorubicin possess tumor-initiating-cell properties. J. Pharmacol. Sci. 122, 299–304.