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Nuclear phospholipase C β 1 signaling, epigenetics and treatments in MDS

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ABSTRACT

Myelodysplastic syndromes (MDS), clonal hematopoietic stem-cell disorders mainly affecting older adult patients, show ineffective hematopoiesis in one or more of the lineages of the bone marrow. Most MDS are characterized by anemia, and a number of cases progresses to acute myeloid leukemia (AML). Indeed, the molecular mechanisms underlying the MDS evolution to AML are still unclear, even though the nuclear signaling elicited by PI-PLC β 1 has been demonstrated to play an important role in the control of the balance between cell cycle progression and apoptosis in MDS cells. Here we review both the role of epigenetic therapy on PI-PLC β 1 promoter and the changes in PI-PLC β 1 expression in MDS patients treated for anemia.

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Introduction

Phosphoinositides (PIs) regulate several important cellular processes at the plasma membrane, but also at the nuclear level, within the nuclear speckles. Indeed, nuclear inositides are essential cofactors for DNA repair, transcription regulation, and RNA dynamics (Cocco et al., 2011, Follo et al., 2011a,

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Marmioli et al., 1994, Martelli et al., 1992). Among the enzymes of the nuclear PI cycle, phosphoinositide-specific phospholipase C (PI-PLC) $\beta 1$ plays an essential role in cell cycle, as a check-point in the G1 phase (Faenza et al., 2000, Faenza et al., 2007) and in the G2/M transition (Fiume et al., 2009). The signaling pathway elicited by PI-PLC $\beta 1$ and its downstream target Cyclin D3 has been implicated in the hematopoietic system, since it can regulate the hematopoietic stem cell proliferation (Faenza et al., 2002, Suh et al., 2008) and, more importantly, the early stages of the hematopoietic differentiation (Cooper et al., 2006, Furukawa, 2002).

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematopoietic disorders that are characterized by ineffective hematopoiesis, progressive bone marrow failure, peripheral blood cytopenias, and a propensity for leukemic transformation (Lindsley and Ebert, 2012). The management of MDS has improved in recent years, with the availability of several active treatments that can alter the natural history of the disease and improve quality of life (Lyons, 2012). However, given the limited number of approved therapies for MDS, effective management of each treatment option is critical to provide each patient the best opportunity for successful treatment (Kurtin et al., 2012), above all because the identification of the MDS risk may change the therapeutic approach. In case of symptomatic anemia, especially in low-risk MDS cases, the therapy aims at the improvement of both peripheral cytopenia and quality of life (Jabbour et al., 2008), that is why these cases are mainly treated with Erythropoietin (EPO). On the other hand, high-risk MDS patients need to increase survival and delay the AML evolution, and are therefore usually administered demethylating therapies (Morgan and Reuter, 2006).

Nuclear PI-PLC $\beta 1$ and MDS: demethylating therapy

Epigenetic mechanisms contribute to regulate gene expression and assure the correct inheritance of DNA information. Among epigenetic processes, promoter DNA hypermethylation is a common hallmark of cancer that can be reversed by the epigenetic therapy with demethylating agents. In the last few years, two demethylating agents (azacitidine, decitabine), alone or in combination with histone deacetylase inhibitors (valproic acid and vorinostat) have been successfully tested in MDS therapy (Fenaux et al., 2009, Fenaux et al., 2007, Griffiths and Gore, 2008, Kaminskas et al., 2005, Park et al., 2008, Perl et al., 2009, Sekeres et al., 2008).

Azacitidine is a DNA methyltransferase inhibitor currently approved for the treatment of high-risk MDS (Kaminskas et al., 2005, Silverman and Mufti, 2005) and under experimental evaluation for low-risk MDS (Musto et al., 2010) as well as of other hematologic malignancies (Quintas-Cardama et al., 2008). Indeed, azacitidine has been reported to have a significant impact on the overall survival and delay the progression toward AML (Fenaux et al., 2009). At a molecular level, azacitidine specifically induces DNA hypomethylation, in order to resume cellular differentiation of cancer cells (Silverman, 2001). In fact, azacitidine induces the hypomethylation of several silenced genes, mostly implicated in cell cycle, such as p15/INK4B, p21WAF/Cip1 and p73 (Daskalakis et al., 2002, Raj et al., 2007). Nevertheless, these are not yet reliable markers of responsiveness, and therefore many investigators are now applying novel methods aiming at the identification of new therapeutic targets in hematological malignancies (Maraldi et al., 2011), and are studying new molecular processes affecting MDS. This is the case for PI-PLC $\beta 1$ (Follo et al., 2009), which can be considered as a specific target of azacitidine. In fact, high-risk MDS treated with this drug and showing a favorable clinical outcome frequently display a PI-PLC $\beta 1$ promoter hyper-methylation at diagnosis, and a decrease in PI-PLC $\beta 1$ methylation during the therapy. More interestingly, mRNA levels follow and anticipate the clinical outcome, so that the variations in PI-PLC $\beta 1$ expression, increase or decrease, can be detectable prior to the clinical improvement or worsening, respectively. This is particularly appealing, since some cycles of azacitidine are usually needed in order to assess the clinical response.

At a clinical level, also the combination of azacitidine and valproic acid has been tested, because it might offer a better efficacy by modulating the methylation and acetylation states of silenced genes (Fenaux et al., 2009). At a molecular level, this combination therapy has been shown to induce a major demethylation of PI-PLC $\beta 1$ promoter and an increased reactivation of both PI-PLC $\beta 1$ gene and protein expression in responder patients, as compared with azacitidine alone (Follo et al., 2011b).

As mentioned above, azacitidine can now be administered to all subsets of MDS, even though there is very little data in the use of this drug in lower risk MDS (Garcia-Manero, 2011). That is why

innovative molecular mechanisms underlying the effect of epigenetic therapy have to be investigated. Recently, it has been shown that PI-PLC β 1 is affected by epigenetic therapy also in low-risk MDS (Follo et al., 2012b), where also a molecular mechanism involving PI-PLC β 1 has been analyzed. In that study, the correlation between the demethylating effect of azacitidine and the degree of recruitment to PI-PLC β 1 promoter of some transcription factors implicated in hematopoietic stem cell proliferation and differentiation was investigated, by applying a chromatin immunoprecipitation method. In particular, MDS patients responding to azacitidine therapy were reported to show a specific recruitment to PI-PLC β 1 promoter of myeloid zinc finger (MZF)-1, but not c-myb. This is particularly appealing, since MZF-1 plays a role in myeloid differentiation (Morris et al., 1995), whereas c-myb is specifically associated with hematopoietic stem cell proliferation (Lidonnici et al., 2008), therefore confirming the involvement of PI-PLC β 1 in azacitidine-induced myeloid differentiation (Fig. 1).

Nuclear PI-PLC β 1 and MDS: EPO therapy

EPO is currently used in the treatment of low-risk MDS patients, mainly with the aim of correcting anemia (Elliott, 2011), since it regulates cell metabolism by balancing cell cycle activation and apoptosis (Bejar et al., 2011, Marzò et al., 2008). Indeed, this is particularly important for low-risk MDS patients, who usually show an increased apoptosis and a low proliferation rate, which may be reversed in case of leukemic evolution (Kerbaui and Deeg, 2007).

Little is known about the exact molecular mechanisms underlying the effect of EPO in low-risk MDS cells and the reasons why some patients do not respond to this treatment, even though some studies recently investigated whether EPO responder and non responder patients have different gene expression profiles (Cortelezzi et al., 2008). At a molecular level, EPO activates the EPO receptor, which is in turn linked to the activation of both Akt and PI-PLC γ 1 (Marshall et al., 2000, Wang et al., 2006), whose signaling pathways are associated with proliferation and leukemogenesis (Martelli et al., 2011). In high-risk MDS patients, our group demonstrated the specific activation of Akt, mTOR, and its downstream targets (Follo et al., 2007, Nyakern et al., 2006). Moreover, by analyzing the same case series, an inverse correlation between Akt and PI-PLC β 1 was also postulated (Follo et al., 2008). This hypothesis was confirmed by recent investigations, performed on low-risk MDS under treatment with EPO and demonstrating that Akt activation is linked to PI-PLC β 1 down-regulation (Follo et al., 2012a).

Fig. 1. Role of nuclear PI-PLC β 1 in MDS hematopoietic differentiation. PI-PLC β 1 promoter hypomethylation is associated with myeloid differentiation, whereas PI-PLC β 1 is a negative regulator of erythroid differentiation, therefore hinting at a role for PI-PLC β 1 as a modifier in MDS hematopoiesis.

In that study, EPO responder patients showed an activation of Akt, as expected, whereas the same cases displayed a PI-PLC β 1 decrease.

Interestingly, the decrease of PI-PLC β 1 was statistically significant after 4–6 months of therapy, which is consistent with previous findings showing that PI-PLC β 1, after an early transient increase, is down-regulated in primary human erythroblasts treated with EPO for up to 96 hours (di Giacomo et al., 2005), therefore suggesting that PI-PLC β 1 could be required at the beginning of erythroid differentiation but is dispensable, if not inhibitory, at later stages (Fig. 1). At the same time, also the Akt phosphorylation which we detected in EPO responder cases is in agreement with other previous in vitro studies showing that EPO can induce a nuclear translocation of active Akt, which is required for erythroid differentiation (Missiroli et al., 2009). Taken together, these results not only confirm the inverse correlation between PI-PLC β 1 and Akt, but also hint at a role for PI-PLC β 1 as a negative regulator of erythroid differentiation, as also previously hypothesized by in vitro studies in erythroleukemia cells (Faenza et al., 2002).

Conclusions

Nuclear PI-PLC β 1 plays an important role in cell proliferation and differentiation, in normal and pathological conditions. Indeed, recent findings indicate that the nuclear inositide signaling pathways might contribute to the further clarification of the therapeutic activity of some drugs currently used in MDS, such as azacitidine or EPO. In fact, not only PI-PLC β 1 promoter hypermethylation has been associated with the progression of high-risk MDS into AML, but also the effect of EPO treatment on Akt activation and PI-PLC β 1 expression strengthens the contention that a correct nuclear lipid signaling is essential for physiological processes such as cell growth and differentiation in MDS. Further investigations are needed to fully understand the molecular mechanisms underlying the MDS progression into AML, but it is now clear that PI-PLC β 1 is a modifier in MDS pathogenesis, since it is a positive regulator of myeloid differentiation and a negative regulator of erythroid differentiation.

Disclosure of conflicts of interest

All the authors declare no conflict of interest.

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