Advances in Biological Regulation xxx (2012) 1–6

Contents lists available at SciVerse ScienceDirect

Advances in Biological Regulation

journal homepage: www.elsevier.com/locate/jbior

Nuclear phospholipase C β1 signaling, epigenetics and treatments in MDS

Matilde Y. Follo [a,*,1], Sandra Marmiroli [b,1], Irene Faenza [a], Roberta Fiume [a], Giulia Ramazzotti [a], Alberto M. Martelli [a], Pietro Gobbi [c], James A. McCubrey [d], Carlo Finelli [e], Francesco A. Manzoli [a], Lucio Cocco [a,*]

[a] Cellular Signalling Laboratory, Department of Human Anatomical Sciences, University of Bologna, Bologna, Italy
[b] Department of Anatomy and Histology, University of Modena and Reggio Emilia, Modena, Italy
[c] Department of Scienze della Terra, della Vita e dell'Ambiente, University "Carlo Bo", Urbino, Italy
[d] Department of Microbiology and Immunology, Brody School of Medicine, East Carolina University, Greenville, NC, USA
[e] Department of Hematology and Medical Oncology "L. e A. Seràgnoli", University of Bologna, Bologna, Italy

A B S T R A C T

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-PLCβ1 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-PLCβ1 promoter and the changes in PI-PLCβ1 expression in MDS patients treated for anemia.

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,

* Corresponding authors. Cellular Signalling Laboratory, Department of Human Anatomical Sciences, University of Bologna, via Irnerio 48, 40126, Bologna, Italy.
E-mail addresses: matilde.follo@unibo.it (M.Y. Follo), lucio.cocco@unibo.it (L. Cocco).
[1] These authors equally contributed to this work.

2212-4926/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jbior.2012.09.009

Marmiroli et al., 1994, Martelli et al., 1992). Among the enzymes of the nuclear PI cycle, phosphoinositide-specific phospholipase C (PI-PLC) β1 plays an essential role in cell cycle, as a checkpoint 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-PLCβ1 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-PLCβ1 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-PLCβ1 (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-PLCβ1 promoter hyper-methylation at diagnosis, and a decrease in PI-PLCβ1 methylation during the therapy. More interestingly, mRNA levels follow and anticipate the clinical outcome, so that the variations in PI-PLCβ1 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-PLCβ1 promoter and an increased reactivation of both PI-PLCβ1 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

Please cite this article in press as: Follo MY, et al., Nuclear phospholipase C β1 signaling, epigenetics and treatments in MDS, Advances in Biological Regulation (2012), http://dx.doi.org/10.1016/j.jbior.2012.09.009

innovative molecular mechanisms underlying the effect of epigenetic therapy have to be investigated. Recently, it has been shown that PI-PLCβ1 is affected by epigenetic therapy also in low-risk MDS (Follo et al., 2012b), where also a molecular mechanism involving PI-PLCβ1 has been analyzed. In that study, the correlation between the demethylating effect of azacitidine and the degree of recruitment to PI-PLCβ1 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-PLCβ1 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-PLCβ1 in azacitidine-induced myeloid differentiation (Fig. 1).

Nuclear PI-PLCβ1 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, Marzo 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 (Kerbauy 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-PLCγ1 (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-PLCβ1 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-PLCβ1 down-regulation (Follo et al., 2012a).

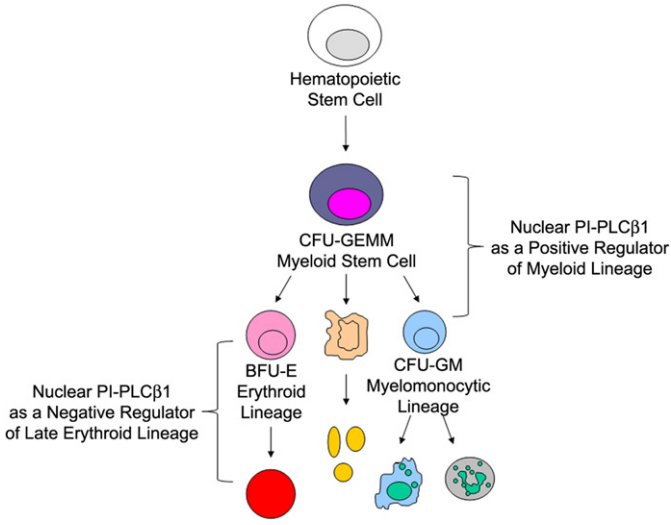

Fig. 1. Role of nuclear PI-PLCβ1 in MDS hematopoietic differentiation. PI-PLCβ1 promoter hypomethylation is associated with myeloid differentiation, whereas PI-PLCβ1 is a negative regulator of erythroid differentiation, therefore hinting at a role for PI-PLCβ1 as a modifier in MDS hematopoiesis.

Please cite this article in press as: Follo MY, et al., Nuclear phospholipase C β1 signaling, epigenetics and treatments in MDS, Advances in Biological Regulation (2012), http://dx.doi.org/10.1016/j.jbior.2012.09.009

In that study, EPO responder patients showed an activation of Akt, as expected, whereas the same cases displayed a PI-PLCβ1 decrease.

Interestingly, the decrease of PI-PLCβ1 was statistically significant after 4–6 months of therapy, which is consistent with previous findings showing that PI-PLCβ1, 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-PLCβ1 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-PLCβ1 and Akt, but also hint at a role for PI-PLCβ1 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-PLCβ1 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-PLCβ1 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-PLCβ1 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-PLCβ1 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.

Acknowledgments

This work was supported by Italian MIUR-FIRB (Human Proteome Net and Accordi di Programma 2010), Italian MIUR PRIN and Celgene Corp.

References

Bejar R, Levine R, Ebert BL. Unraveling the molecular pathophysiology of myelodysplastic syndromes. J Clin Oncol 2011;29:504–15.
Cocco L, Follo MY, Faenza I, Fiume R, Ramazzotti G, Weber G, et al. Physiology and pathology of nuclear phospholipase C beta1. Adv Enzyme Regul 2011;51:2–12.
Cooper AB, Sawai CM, Sicinska E, Powers SE, Sicinski P, Clark MR, et al. A unique function for cyclin D3 in early B cell development. Nat Immunol 2006;7:489–97.
Cortelezzi A, Colombo G, Pellegrini C, Silvestris I, Moronetti Mazzeo L, Bosari S, et al. Bone marrow glycophorin-positive erythroid cells of myelodysplastic patients responding to high-dose rHuEPO therapy have a different gene expression pattern from those of nonresponders. Am J Hematol 2008;83:531–9.
Daskalakis M, Nguyen TT, Nguyen C, Guldberg P, Kohler G, Wijermans P, et al. Demethylation of a hypermethylated P15/INK4B gene in patients with myelodysplastic syndrome by 5-Aza-2′-deoxycytidine (decitabine) treatment. Blood 2002;100:2957–64.
di Giacomo V, Matteucci A, Stellacci E, Battistini A, Di Baldassarre A, Capitani S, et al. Expression of signal transduction proteins during the differentiation of primary human erythroblasts. J Cell Physiol 2005;202:831–8.
Elliott S. Erythropoiesis-stimulating agents. Cancer Treat Res 2011;157:55–74.
Faenza I, Matteucci A, Bavelloni A, Marmiroli S, Martelli AM, Gilmour RS, et al. Nuclear PLCbeta(1) acts as a negative regulator of p45/NF-E2 expression levels in Friend erythroleukemia cells. Biochim Biophys Acta 2002;1589:305–10.
Faenza I, Matteucci A, Manzoli L, Billi AM, Aluigi M, Peruzzi D, et al. A role for nuclear phospholipase Cbeta 1 in cell cycle control. J Biol Chem 2000;275:30520–4.
Faenza I, Ramazzotti G, Bavelloni A, Fiume R, Gaboardi GC, Follo MY, et al. Inositide-dependent phospholipase C signaling mimics insulin in skeletal muscle differentiation by affecting specific regions of the cyclin D3 promoter. Endocrinology 2007;148:1108–17.

Please cite this article in press as: Follo MY, et al., Nuclear phospholipase C β1 signaling, epigenetics and treatments in MDS, Advances in Biological Regulation (2012), http://dx.doi.org/10.1016/j.jbior.2012.09.009

Fenaux P, Mufti GJ, Hellstrom-Lindberg E, Santini V, Finelli C, Giagounidis A, et al. Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. The lancet oncology 2009;10:223–32.
Fenaux P, Raza A, Mufti GJ, Aul C, Germing U, Kantarjian H, et al. A multicenter phase 2 study of the farnesyltransferase inhibitor tipifarnib in intermediate- to high-risk myelodysplastic syndrome. Blood 2007;109:4158–63.
Fiume R, Ramazzotti G, Teti G, Chiarini F, Faenza I, Mazzotti G, et al. Involvement of nuclear PLCbeta1 in lamin B1 phosphorylation and G2/M cell cycle progression. FASEB J 2009;23:957–66.
Follo MY, Faenza I, Fiume R, Ramazzotti G, McCubrey JA, Martelli AM, et al. Revisiting nuclear phospholipase C signalling in MDS. Adv Enzyme Regul 2011a.
Follo MY, Finelli C, Bosi C, Martinelli G, Mongiorgi S, Baccarani M, et al. PI-PLCbeta-1 and activated Akt levels are linked to azacitidine responsiveness in high-risk myelodysplastic syndromes. Leukemia 2008;22:198–200.
Follo MY, Finelli C, Mongiorgi S, Clissa C, Bosi C, Testoni N, et al. Reduction of phosphoinositide-phospholipase C beta1 methylation predicts the responsiveness to azacitidine in high-risk MDS. Proc Natl Acad Sci U S A 2009;106:16811–6.
Follo MY, Finelli C, Mongiorgi S, Clissa C, Chiarini F, Ramazzotti G, et al. Synergistic induction of PI-PLCbeta1 signaling by azacitidine and valproic acid in high-risk myelodysplastic syndromes. Leukemia 2011b;25:271–80.
Follo MY, Mongiorgi S, Bosi C, Cappellini A, Finelli C, Chiarini F, et al. The Akt/mammalian target of rapamycin signal transduction pathway is activated in high-risk myelodysplastic syndromes and influences cell survival and proliferation. Cancer Res 2007;67:4287–94.
Follo MY, Mongiorgi S, Clissa C, Paolini S, Martinelli G, Martelli AM, et al. Activation of nuclear inositide signalling pathways during erythropoietin therapy in low-risk MDS patients. Leukemia 2012a.
Follo MY, Russo D, Finelli C, Mongiorgi S, Clissa C, Fili C, et al. Epigenetic regulation of nuclear PI-PLCbeta1 signaling pathway in low-risk MDS patients during azacitidine treatment. Leukemia 2012b;26:943–50.
Furukawa Y. Cell cycle control genes and hematopoietic cell differentiation. Leuk Lymphoma 2002;43:225–31.
Garcia-Manero G. Myelodysplastic syndromes: 2011 update on diagnosis, risk-stratification, and management. Am J Hematol 2011;86:490–8.
Griffiths EA, Gore SD. DNA methyltransferase and histone deacetylase inhibitors in the treatment of myelodysplastic syndromes. Seminars in hematology 2008;45:23–30.
Jabbour E, Kantarjian HM, Koller C, Taher A. Red blood cell transfusions and iron overload in the treatment of patients with myelodysplastic syndromes. Cancer 2008;112:1089–95.
Kaminskas E, Farrell A, Abraham S, Baird A, Hsieh LS, Lee SL, et al. Approval summary: azacitidine for treatment of myelodysplastic syndrome subtypes. Clin Cancer Res 2005;11:3604–8.
Kerbauy DB, Deeg HJ. Apoptosis and antiapoptotic mechanisms in the progression of myelodysplastic syndrome. Exp Hematol 2007;35:1739–46.
Kurtin SE, Demakos EP, Hayden J, Boglione C. Treatment of myelodysplastic syndromes. Clin J Oncol Nurs 2012;16:23–35.
Lidonnici MR, Corradini F, Waldron T, Bender TP, Calabretta B. Requirement of c-Myb for p210(BCR/ABL)-dependent transformation of hematopoietic progenitors and leukemogenesis. Blood 2008;111:4771–9.
Lindsley RC, Ebert BL. Molecular pathophysiology of myelodysplastic syndromes. Annu Rev Pathol 2012.
Lyons RM. Myelodysplastic syndromes: therapy and outlook. Am J Med 2012;125:S18–23.
Maraldi T, Bertacchini J, Benincasa M, Guida M, De Pol A, Liotta LA, et al. Reverse-phase protein microarrays (RPPA) as a diagnostic and therapeutic guide in multidrug resistant leukemia. Int J Oncol 2011;38:427–35.
Marmiroli S, Ognibene A, Bavelloni A, Cinti C, Cocco L, Maraldi NM. Interleukin 1 alpha stimulates nuclear phospholipase C in human osteosarcoma SaOS-2 cells. J Biol Chem 1994;269:13–6.
Marshall AJ, Niiro H, Yun TJ, Clark EA. Regulation of B-cell activation and differentiation by the phosphatidylinositol 3-kinase and phospholipase Cgamma pathway. Immunol Rev 2000;176:30–46.
Martelli AM, Evangelisti C, Chappell W, Abrams SL, Basecke J, Stivala F, et al. Targeting the translational apparatus to improve leukemia therapy: roles of the PI3K/PTEN/Akt/mTOR pathway. Leukemia 2011;25:1064–79.
Martelli AM, Gilmour RS, Bertagnolo V, Neri LM, Manzoli L, Cocco L. Nuclear localization and signalling activity of phosphoinositidase C beta in Swiss 3T3 cells. Nature 1992;358:242–5.
Marzo F, Lavorgna A, Coluzzi G, Santucci E, Tarantino F, Rio T, et al. Erythropoietin in heart and vessels: focus on transcription and signalling pathways. J Thromb Thrombolysis 2008;26:183–7.
Missiroli S, Etro D, Buontempo F, Ye K, Capitani S, Neri LM. Nuclear translocation of active AKT is required for erythroid differentiation in erythropoietin treated K562 erythroleukemia cells. Int J Biochem Cell Biol 2009;41:570–7.
Morgan MA, Reuter CW. Molecularly targeted therapies in myelodysplastic syndromes and acute myeloid leukemias. Ann Hematol 2006;85:139–63.
Morris JF, Rauscher 3rd FJ, Davis B, Klemsz M, Xu D, Tenen D, et al. The myeloid zinc finger gene, MZF-1, regulates the CD34 promoter in vitro. Blood 1995;86:3640–7.
Musto P, Maurillo L, Spagnoli A, Gozzini A, Rivellini F, Lunghi M, et al. Azacitidine for the treatment of lower risk myelodysplastic syndromes: a retrospective study of 74 patients enrolled in an Italian named patient program. Cancer 2010;116:1485–94.
Nyakern M, Tazzari PL, Finelli C, Bosi C, Follo MY, Grafone T, et al. Frequent elevation of Akt kinase phosphorylation in blood marrow and peripheral blood mononuclear cells from high-risk myelodysplastic syndrome patients. Leukemia 2006;20:230–8.
Park S, Chapuis N, Bardet V, Tamburini J, Gallay N, Willems L, et al. PI-103, a dual inhibitor of Class IA phosphatidylinositide 3-kinase and mTOR, has antileukemic activity in AML. Leukemia 2008;22:1698–706.
Perl AE, Kasner MT, Tsai DE, Vogl DT, Loren AW, Schuster SJ, et al. A phase I study of the mammalian target of rapamycin inhibitor sirolimus and MEC chemotherapy in relapsed and refractory acute myelogenous leukemia. Clin Cancer Res 2009;15:6732–9.
Quintas-Cardama A, Tong W, Kantarjian H, Thomas D, Ravandi F, Kornblau S, et al. A phase II study of 5-azacitidine for patients with primary and post-essential thrombocythemia/polycythemia vera myelofibrosis. Leukemia 2008;22:965–70.
Raj K, John A, Ho A, Chronis C, Khan S, Samuel J, et al. CDKN2B methylation status and isolated chromosome 7 abnormalities predict responses to treatment with 5-azacytidine. Leukemia 2007;21:1937–44.

Please cite this article in press as: Follo MY, et al., Nuclear phospholipase C β1 signaling, epigenetics and treatments in MDS, Advances in Biological Regulation (2012), http://dx.doi.org/10.1016/j.jbior.2012.09.009

Sekeres MA, Maciejewski JP, Giagounidis AA, Wride K, Knight R, Raza A, et al. Relationship of treatment-related cytopenias and response to lenalidomide in patients with lower-risk myelodysplastic syndromes. J Clin Oncol 2008;26:5943–9.
Silverman LR. Targeting hypomethylation of DNA to achieve cellular differentiation in myelodysplastic syndromes (MDS). Oncologist 2001;6(Suppl. 5):8–14.
Silverman LR, Mufti GJ. Methylation inhibitor therapy in the treatment of myelodysplastic syndrome. Nat Clin Pract Oncol 2005; 2(Suppl. 1):S12–23.
Suh PG, Park JI, Manzoli L, Cocco L, Peak JC, Katan M, et al. Multiple roles of phosphoinositidespecific phospholipase C isozymes. BMB Rep 2008;41:415–34.
Wang Y, Wu J, Wang Z. Akt binds to and phosphorylates phospholipase C-gamma1 in response to epidermal growth factor. Mol Biol Cell 2006;17:2267–77.

Please cite this article in press as: Follo MY, et al., Nuclear phospholipase C β1 signaling, epigenetics and treatments in MDS, Advances in Biological Regulation (2012), http://dx.doi.org/10.1016/j.jbior.2012.09.009