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Nuclear Inositide Signaling in Myelodysplastic Syndromes

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ABSTRACT

Myelodysplastic syndromes (MDS) are defined as clonal hematopoietic stem-cell disorders characterized by ineffective hematopoiesis in one or more of the lineages of the bone marrow. Although distinct morphologic subgroups exist, the natural history of MDS is progression to acute myeloid leukemia (AML). However, the molecular mechanisms the underlying MDS evolution to AML are not completely understood. Inositides are key cellular second messengers with well-established roles in signal transduction pathways, and nuclear metabolism elicited by phosphoinositide-specific phospholipase C (PI-PLC) β1 and Akt plays an important role in the control of the balance between cell cycle progression and apoptosis in both normal and pathologic conditions. Recent findings evidenced the role played by nuclear lipid signaling pathways, which could become promising therapeutic targets in MDS. This review will provide a concise and updated revision of the state of art on this topic. J. Cell. Biochem. 109: 1065–1071, 2010.

KEY WORDS: SIGNAL TRANSDUCTION; PI-PLCBETA1; MYELODYSPLASTIC SYNDROMES; Akt; NUCLEUS

Lipid signaling in disease is an emerging field of investigation. Inositides are key cellular second messengers with well established roles in signal transduction pathways. The identification of a distinct nuclear inositide signaling metabolism has defined a new role for these molecules [Martelli et al., 1992; Faenza et al., 2008]. Nuclear inositides are now considered essential co-factors for several nuclear processes, including DNA repair, transcription regulation, and RNA dynamics. For that reason, imbalances of the major lipid signaling pathways may contribute to disease progression in several disorders, such as chronic inflammation, cancer, metabolic, and degenerative syndromes. Lipid signaling cascades are therefore essential components of the extremely complicated, multistep process that allows one extracellular signal to be transduced inside the cell, to the nucleus. Moreover, these pathways are complex and redundant, since many of the signaling molecules, their modifying enzymes and downstream targets are common to multiple pathways, resulting in the formation of highly interconnected networks. As a consequence, many signaling pathways can be deregulated in several disease conditions, as well as in cancer. That is why signaling lipid-generating enzymes have been and are still being targeted pharmacologically, alone or in combination, to alleviate the symptoms, or even progression of the different diseases [Wymann and Schneiter, 2008].

MYELODYSPLASTIC SYNDROMES (MDS)

The myelodysplastic syndromes (MDS) are a heterogeneous group of bone marrow disorders characterized by an ineffective differentiation of the hematopoietic stem cell that causes anemia, neutropenia, bleeding problems, and infections [Tefferi and Vardiman, 2009]. The disease can result in a slow decrease in blood cell counts, but it may also have a more aggressive evolution, that is a worsening severe cytopenia or, in about 30% of all the patients, transformation into acute myeloid leukemia (AML). The phenotype of some MDS has often been associated with some AML features. However, a unique

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aspect of MDS is the notion that both bone marrow failure and the propensity towards progression into AML coexist. As both properties contribute to peripheral blood cytopenia and the natural history of the disease, it is thought that proliferative advantages lend themselves more to leukemic transformation, while initially accelerated apoptosis is responsible for the cytopenia [Corey et al., 2007]. In fact, upon evolution of MDS into AML, the progressing clonal cells present an excessive survival and decreased apoptosis.

MDS patients are usually diagnosed according to both morphologic evaluations, following either the French–American–British (FAB) [Bennett et al., 1982] or World Health Organization (WHO) [Vardiman et al., 2009] classification, and two more complex systems, based on the percentage of marrow blasts, number of cytopenias, and bone marrow cytogenetic findings, useful for the assessment of the risk of evolution into AML, that is, the International Prognostic Scoring System (IPSS) [Greenberg et al., 1997] and/or the WHO classification-based Scoring System (WPSS) [Malcovati et al., 2007]. The subdivision of MDS patients in different risk subgroups is essential for the choice of the most appropriate therapy, as for high-risk patients the main goal of treatment is the increase of survival and delay of AML evolution [Morgan and Reuter, 2006], while for low-risk patients the improvement of both peripheral cytopenia and quality of life [Jabbour et al., 2008] seem to be the key points.

In the last few years there has been an advancement in supportive care and several agents have been tested for the treatment of MDS (Table I): 5-azacytidine, decitabine, lenalidomide, farnesyltransferase inhibitors, among the others [Kaminskas et al., 2005; Fenaux et al., 2007, 2009; Faderl et al., 2008; Griffiths and Gore, 2008; Park et al., 2008; Sekeres et al., 2008; Perl et al., 2009]. With the exception of lenalidomide, which produces remarkable responses in patients with single 5q chromosomal deletions [Sekeres et al., 2008], these therapies benefit a minority of patients and the overall outcomes are still unsatisfactory [Srinivasan and Schiffer, 2008]. Moreover, being IMMUNOSUPPRESSIVE therapy helpful in a fraction of patients, allogeneic stem cell transplant remains the only curative option, even though the majority of MDS patients are not eligible because of older age and other medical problems.

METHYLATION IN MDS: AZACITIDINE

Epigenetic mechanisms affecting chromatin structure contribute to regulate gene expression and assure the inheritance of information, which are essential for the expression of key regulatory genes in healthy cells, tissues, and organs. In the medical field, increasing relevance has the study of altered gene expression or de-regulated gene function, leading to disease or cancer progression. Aberrant DNA methylation patterns, changes in chromatin structure and in gene expression are common in all cancer cells. However, studies on hematologic malignancies have provided examples for the functional implications of the epigenetic alterations in cancer development and progression, as well as their relevance for therapeutical targeting [Boultwood and Wainscoat, 2007].

Azacitidine is a DNA methyltransferase (DNMT) inhibitor currently approved for the treatment of MDS [Kaminskas et al., 2005; Silverman and Mufti, 2005] and under experimental evaluation for other hematologic malignancies [Quintas-Cardama et al., 2008]. Besides showing response rates of 50–80% in high-risk MDS, azacitidine has been reported to have a significant impact on the overall survival and progression towards AML [Fenaux et al., 2009]. Nevertheless, the molecular mechanisms underlying this drug are not completely understood, even though it is clear how the DNMT inhibitors can induce the re-expression of methylated silenced gene products [Griffiths and Gore, 2008]: after incorporation of demethylating agents into DNA, the methyltransferases are inhibited, but complete demethylation occurs only after several cycles of replication, thus accounting for time to response to these drugs [Stresemann and Lyko, 2008]. Low-dose regimens with azacitidine have been assumed to act by reversing the epigenetic silencing of target genes involved in the control of cell growth and differentiation. For instance, demethylation of a hyper-methylated p15/INK4B gene, as well as of other genes, such as p21WAF/Cip1 and p73, has been demonstrated in MDS patients treated with demethylating therapy [Daskalakis et al., 2002; Raj et al., 2007]. However, almost all cancer-related signaling pathways may be affected by hyper-methylation, and a growing number of silenced methylated genes involved in each major type of cancer is coming to light.

ROLE OF NUCLEAR INOSITIDE SIGNALING IN HEMATOPOIETIC MALIGNANCIES

The mechanisms regulating the growth and survival of MDS and AML cells are largely unknown. The conversion of a normal stem cell into a pre-leukemic and ultimately leukemic state is a multistep process requiring the accumulation of a number of genetic lesions. Early MDS are associated with an initial excessive apoptosis which decreases during the progression to AML [Kerbauy and Deeg, 2007]. It is still unclear which is the exact pathogenesis of the MDS

TABLE I. Current Pharmacologic Agents in MDS/AML Clinical Trials

Pharmaceutical class	Therapeutic agents in evaluation	References
Nucleoside analogs	Fludarabine, clofarabine	Faderl et al. [2008]
Demethylating agents	Azacitidine, decitabine	Fenaux et al. [2009], Kaminskas et al. [2005]
Histone deacetylase inhibitors	Vorinostat, valproic acid	Griffiths and Gore [2008]
mTOR inhibitors	Sirolimus, temsirolimus, everolimus, and PI-103	Perl et al. [2009], Park et al. [2008]
Antiangiogenic and immunomodulating agents	Lenalidomide	Sekeres et al. [2008]
Farnesyltransferase inhibitors	Tipifarnib	Fenaux et al. [2007]

progression to AML, although some recent studies indicate that the signaling pathways could be involved. In fact, over the past years, major signal transduction molecules have been identified and their genetic alterations have been extensively analyzed in both MDS and AML. These include receptors for growth factors, RAS signaling molecules, cell cycle regulators and transcription factors [Hirai, 2003]. The identification of the aberrant signaling pathways responsible for an increased survival of MDS cells is therefore of high importance, as they might represent promising targets for novel forms of therapy aimed at preventing the MDS evolution into AML.

INOSITIDE SIGNALING IN MDS/AML

Over the last years, it has been established that phosphoinositides (Pls), which are involved in the regulation of a great variety of cellular processes, both in the cytoplasm and in the plasma membrane, are present also in the inner part of the cell nucleus and that their metabolism changes during cell growth and differentiation [Martelli et al., 1992; Faenza et al., 2008]. Remarkably, it is clear that the nuclear inositide metabolism is regulated independently from the plasma membrane counterpart, suggesting that the nucleus constitutes a functionally distinct compartment of inositol lipids metabolism [Martelli et al., 2005].

Among the enzymes of the nuclear PI cycle, phosphoinositide-specific phospholipase C (PI-PLC) β1, whose hydrolysis generates diacylglycerol and inositol 1,4,5-trisphosphate as second messengers, appears to play a fundamental role as a checkpoint in the G1 phase of the cell cycle [Faenza et al., 2000], mainly targeting cyclin D3 [Faenza et al., 2007], as well as in the G2/M transition [Fiume et al., 2009].

In addition, the nucleus contains 3-phosphorylated inositol lipids and the enzymes which synthesize them, that is, phosphoinositide 3-kinase (PI3K). The PI3K/Akt signaling network is crucial to widely divergent physiologic processes that include cell cycle progression, differentiation, transcription, translation, and apoptosis [Ye, 2005]. Moreover, it is targeted by genomic aberrations including amplifications, mutations, and rearrangements more frequently than any other pathways in human cancer, with the possible exception of the p53 and retinoblastoma pathways.

Activation of the PI3K/Akt signaling results in altering the control of cell proliferation and apoptosis, ensuing in competitive growth advantage for tumor cells. Furthermore, it is now clear that the up-regulation of the PI3K/Akt axis may be one of the major factors undermining successful anti-neoplastic treatments, thus portending a poor prognosis in many cancer types. Therefore, the PI3K/Akt pathway is an attractive target for the development of novel therapeutic strategies in patients with various tumor types.

ACTIVATION of PI3K/Akt/mTOR PATHWAY IN HIGH-RISK MDS

The PI3K/Akt signaling pathway is involved in many different cellular processes, such as proliferation, differentiation, and apoptosis [Engelman et al., 2006]. An impaired regulation of the PI3K/Akt axis has been strongly implicated in carcinogenesis. In particular, the activation of the PI3K/Akt survival pathway is often associated with hematologic malignancies, including acute and chronic human leukemias. In particular, recent reports indicate that the Ras/Raf/MEK/ERK pathway is usually associated with proliferation and drug resistance of hematopoietic cells, and that the up-regulation of the PI3K/PTEN/Akt/mTOR axis is observed frequently in AML samples and associated with a poorer prognosis than patients lacking these changes [Scholl et al., 2008]. That is why several clinical trials are now targeting either PI3K or Akt, in order to prevent their activation in AML cases.

As for MDS, recent reports demonstrated a constitutive activation of the Akt pathway. First of all, it was demonstrated that the activated (phosphorylated) form of Akt was highly expressed in bone marrow and peripheral blood mononuclear cells from high-risk MDS patients, while it was almost absent in low-risk MDS as well as in healthy donors [Nyakern et al., 2006].

Other studies reported that not only Akt but also some of its molecular targets are implicated in the leukemic progression. One of the downstream targets of Akt is represented by the mammalian Target of Rapamycin (mTOR), a highly conserved Ser/Thr protein kinase that is essential for the regulation of cell growth and proliferation, by controlling these processes at the translational level and by acting on the cell cycle progression [Dunlop and Tee, 2009]. Indeed, mTOR is capable of regulating the synthesis of key proteins such as retinoblastoma protein, p27Kip1, cyclin D1, c-myc, or STAT-3 [Yazbeck et al., 2008]. Furthermore, some studies have demonstrated that mTOR is also involved in cell death, so that a deregulation of this kinase could lead to the activation of anti-apoptotic mechanisms [Wangpaichitr et al., 2008].

A recent investigation reported that in high-risk MDS patients the Akt/mTOR pathway is over-activated, thus leading to an imbalance in the apoptotic processes [Follo et al., 2007]. Therefore, this survival network is likely to play an important role in the MDS pathogenesis and contribute to the malignant growth of MDS cells. Furthermore, there is a specific up-regulation of the mTOR pathway in the hematopoietic myeloid progenitors of high-risk MDS patients, since rapamycin, targeting mTOR, influenced the survival of $CD33^{+}$ cells, and the clonogenic ability of $CD34^{+}$ MDS cells.

Taken together, the above-mentioned findings may provide the rationale for using pharmacologic inhibitors of the PI3K/Akt/mTOR network, not only for the treatment of AML, but also for high-risk MDS. In fact, at present, leukemic patients can be treated with drugs targeting PI3K and/or mTOR, such as PI-103 [Park et al., 2008] and rapamycin [Perl et al., 2009] (sirolimus and its derivatives temsirolimus and everolimus), and current studies are focusing on Akt inhibitors, for example, perifosine, whose role in hematologic malignancies has not been completely understood yet [Chiarini et al., 2008; Papa et al., 2008]. That is why all of the aforementioned studies can be complementary to the current clinical studies and strengthen the concept that the inositide signaling pathways could become in the future an important target for the development of innovative strategies even for the treatment of high-risk MDS.

PHOSPHOINOSITIDE-PHOSPHOLIPASE C BETA1 (PI-PLCβ1)

PI-PLCβ1 is a key enzyme for the nuclear signaling pathways, since it is implicated in many cellular processes, such as proliferation and differentiation [Suh et al., 2008]. Moreover, it has been demonstrated that PI-PLCβ1 is involved in the hematopoietic differentiation, suggesting that this enzyme could affect the generation of MDS blasts [Cocco et al., 2009]. As a consequence, several studies have been performed to demonstrate the involvement of PI-PLC signaling pathways in MDS [Cocco et al., 2010], as it is shown in Figure 1.

By using fluorescence in situ hybridization (FISH) analysis, the PI-PLCβ1 gene has been mapped on chromosome 20p12 [Peruzzi et al., 2000]. Using the same probe, a large number of MDS patients (80 cases belonging to all of the IPSS risk groups) has been tested for the detection of the PI-PLCβ1 allelic status: FISH analyses disclosed the presence of a mono-allelic deletion of the PI-PLCβ1 gene in about 30% of all of the MDS cases analyzed [Follo et al., 2009a]. On the contrary, PI-PLCβ4, another gene coding for a signaling molecule and located on 20p12.3 at a distance as far as less than 1Mb from PI-PLCβ1, was unaffected, even in MDS patients with the deletion of PI-PLCβ1 gene, hinting at a specific and interstitial deletion of the PI-PLCβ1 gene. Interestingly, MDS patients bearing the mono-allelic deletion, both at high and low risk of evolution into AML, rapidly evolved to AML, suggesting not only that PI-PLCβ1 mono-allelic deletion is associated with the MDS progression towards AML, but also that it could have a prognostic role, through the identification of a sub-group of patients at a poorer prognosis also among low-risk cases, usually considered as patients with a better outcome.

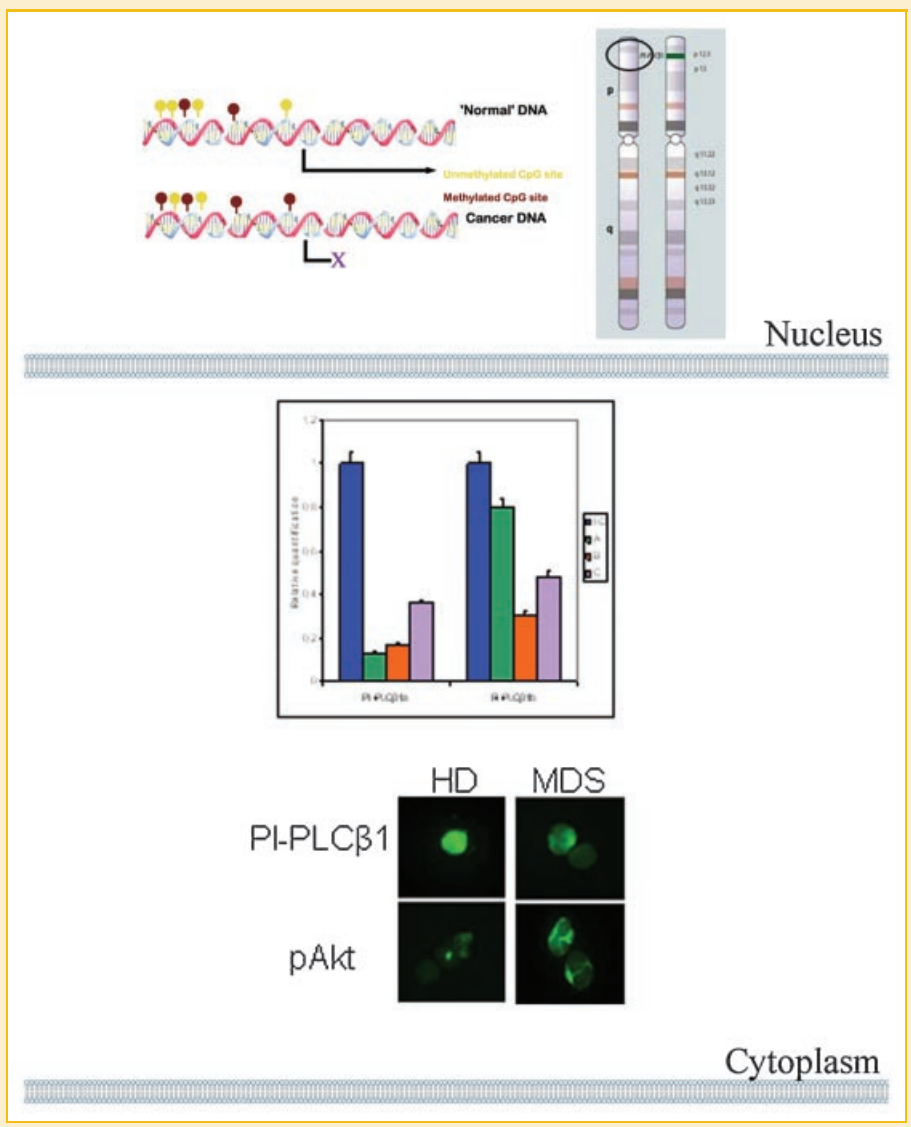

Fig. 1. Role of inositide signaling in MDS progression to AML. At a nuclear level, the presence of the PI-PLCβ1 promoter hyper-methylation and the PI-PLCβ1 mono-allelic deletion in MDS patients can be linked to PI-PLCβ1gene silencing and the identification of a sub-group of patients at higher risk of evolution into AML. At a cytoplasmatic level, the deregulation of PI-PLCβ1 gene and protein expression, as well as the activation of Akt, could play an important role in the MDS progression to AML. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

In another study, it has been shown that also the expression profile of both PI-PLCβ1a and PI-PLCβ1b mRNAs, the two alternative splicing subtypes of PI-PLCβ1, is altered in high-risk MDS, as compared to healthy donors [Follo et al., 2006]. In particular, it was suggested that a reduced expression of PI-PLCβ1 mRNAs is frequent in patients affected by high-risk MDS, since all of the cases analyzed showed a strong decrease in the amount of PI-PLCβ1a, whilst most of the patients demonstrated low levels of PI-PLCβ1b. Interestingly, MDS cells always expressed higher levels of PI-PLCβ1b mRNA, as if the splicing isoform 1b, which is completely localized within the nucleus, was somehow partially preserved, hinting at a possible imbalance of the nuclear versus the cytoplasmatic PI-PLCβ1 signaling which, in turn, could affect the cell cycle progression mechanisms of MDS cells.

Finally, our latest studies demonstrated not only that demethylating agents specifically target nuclear PI-PLCβ1 signaling pathways, but also that there is an inverse correlation between PI-PLCβ1 and activated Akt levels [Follo et al., 2008]. In fact, we firstly showed that PI-PLCβ1 expression could predict the degree of azacitidine effectiveness, in that we observed a responsive MDS patient who displayed higher PI-PLCβ1 levels whenever the clinical status was improving. Interestingly, along with an increase in PI-PLCβ1 levels, the patient demonstrated a reduction in activated Akt levels, thus indicating that PI-PLCβ1 and Akt could play opposite roles. These observations were subsequently confirmed by a larger investigation about azacitidine effect on high-risk MDS [Follo et al., 2009b]. In that study, the PI-PLCβ1 gene expression profile of 18 high-risk MDS patients was systematically analyzed during azacitidine administration and compared to some patients treated with only best supportive care as well as healthy subjects. The results obtained indicate not only that it would be possible to monitor the effect of azacitidine in MDS patients under demethylating therapy, but also that the molecular response anticipates the clinical one, since the increase or decrease in PI-PLCβ1 gene expression would be detectable about two months before the clinical improvement or worsening.

These molecular findings, together with the clinical data of the patients, are therefore consistent with the hypothesis that in high-risk MDS cases PI-PLCβ1 gene silencing could play an essential role in MDS progression towards AML, as well as the activation of Akt, whose pathways are correlated to the imbalance of the apoptotic processes in MDS cells. Moreover, it is reasonable to hypothesize

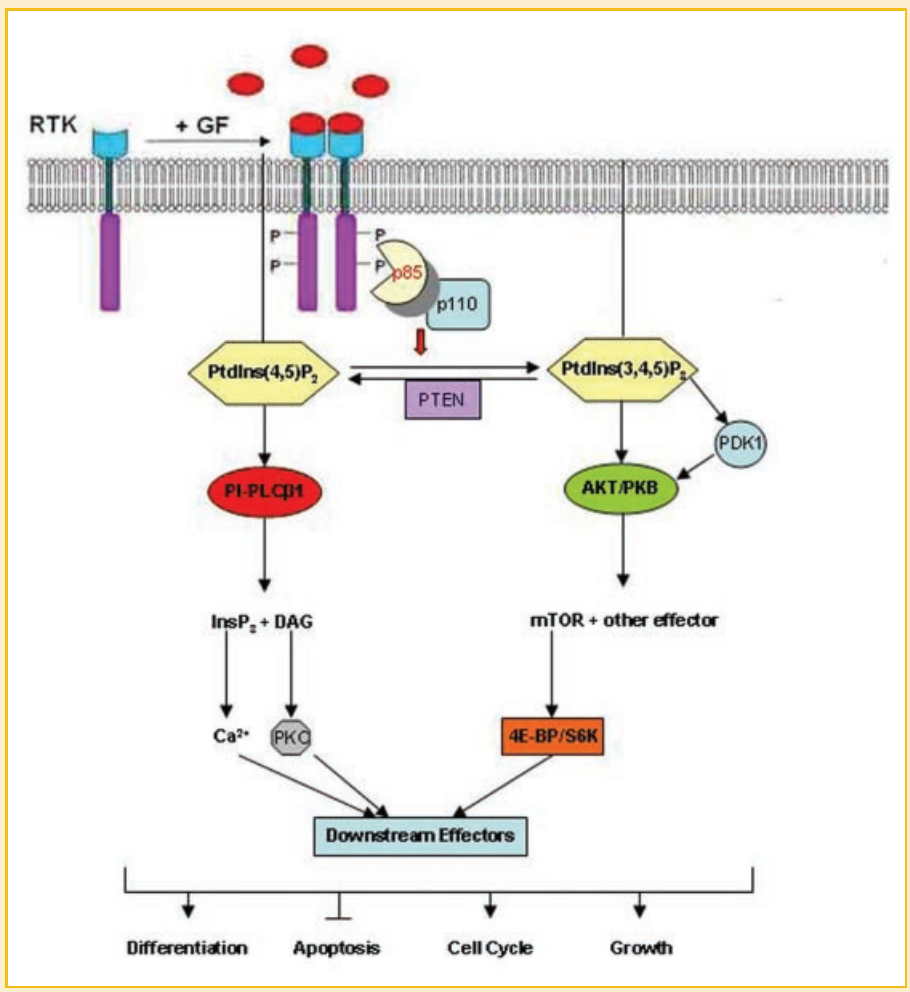

Fig. 2. Inverse correlation between PI-PLCβ1 and Akt signaling in MDS. PI-PLCβ1 and Akt signaling pathways can be inter-connected and inversely related in the activation of cell cycle progression, differentiation, and apoptotic processes. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

that the balance between these two molecules could be directly related to PIP_2 levels, which is both substrate for PI-PLCβ1 and key player in the activation of the PI3K/Akt axis.

Further investigations are in any case needed to fully understand the molecular mechanisms underlying the pathogenesis of the disease and the role of azacitidine on the lipid signaling pathways. Nevertheless, these findings have great significance for high-risk MDS patients, since the altered expression of nuclear PI-PLCβ1 and the amount of activated Akt could be involved in a deregulation of the cell cycle and negatively influence the apoptotic processes, thereby affecting the survival of primary MDS cells. Moreover, the interconnections between PI3K/Akt/mTOR and PI-PLCβ1 signaling (Fig. 2), could lead to combinatorial approaches aiming at inhibiting PI3K/Akt/mTOR activation as well as increasing PI-PLCβ1 expression.

CONCLUSIONS

Nuclear metabolism elicited by PI-PLCβ1 and Akt plays an important role in the control of the balance between cell cycle progression and apoptosis. Recent findings indicate that the lipid signaling pathways can become therapeutic targets in MDS. In fact, the recent discovery of a possible involvement of PI-PLCβ1 in the progression of high-risk MDS into AML [Follo et al., 2008, 2009a,b], as well as an over-activation of the Akt/mTOR axis [Follo et al., 2007], strengthens the contention that the nuclear lipid signaling is essential for physiologic processes such as cell growth and differentiation in MDS.

Moreover, these data might contribute to the further clarification of the therapeutic activity of some drugs currently used in high-risk MDS, such as azacitidine, and pave the way for new therapeutic approaches in these patients, as the quantification of the expression of PI-PLCβ1 and levels of activated Akt could represent an attractive new predictive factors for the responsiveness to demethylating agents. Further investigations are needed to fully understand the molecular mechanisms underlying the MDS progression into AML, but it is now clear that signal transduction pathways can be considered as innovative therapeutic targets in MDS treatments.

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REFERENCES

Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, Sultan C. 1982. Proposals for the classification of the myelodysplastic syndromes. Br J Haematol 51:189–199.

Boultwood J, Wainscoat JS. 2007. Gene silencing by DNA methylation in haematological malignancies. Br J Haematol 138:3–11.

Chiarini F, Del Sole M, Mongiorgi S, Gaboardi GC, Cappellini A, Mantovani I, Follo MY, McCubrey JA, Martelli AM. 2008. The novel Akt inhibitor, perifosine, induces caspase-dependent apoptosis and downregulates P-glycoprotein expression in multidrug-resistant human T-acute leukemia cells by a JNK-dependent mechanism. Leukemia 22:1106–1116.

Cocco L, Faenza I, Follo MY, Billi AM, Ramazzotti G, Papa V, Martelli AM, Manzoli L. 2009. Nuclear inositides: PI-PLC signaling in cell growth, differentiation and pathology. Adv Enzyme Regul 49:2–10.

Cocco L, Follo MY, Faenza I, Billi AM, Ramazzotti G, Martelli AM, Manzoli L, Weber G. 2010. Inositide signaling in the nucleus: From physiology to pathology. Adv Enzyme Regul 50:1–10.

Corey SJ, Minden MD, Barber DL, Kantarjian H, Wang JC, Schimmer AD. 2007. Myelodysplastic syndromes: the complexity of stem-cell diseases. Nat Rev Cancer 7:118–129.

Daskalakis M, Nguyen TT, Nguyen C, Guldberg P, Kohler G, Wijermans P, Jones PA, Lubbert M. 2002. Demethylation of a hypermethylated P15/INK4B gene in patients with myelodysplastic syndrome by 5-Aza-2′-deoxycytidine (decitabine) treatment. Blood 100:2957–2964.

Dunlop EA, Tee AR. 2009. Mammalian target of rapamycin complex 1: signalling inputs, substrates and feedback mechanisms. Cell Signal 21:827–835.

Engelman JA, Luo J, Cantley LC. 2006. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nat Rev Genet 7:606–619.

Faderl S, Ravandi F, Huang X, Garcia-Manero G, Ferrajoli A, Estrov Z, Borthakur G, Verstovsek S, Thomas DA, Kwari M, Kantarjian HM. 2008. A randomized study of clofarabine versus clofarabine plus low-dose cytarabine as front-line therapy for patients aged 60 years and older with acute myeloid leukemia and high-risk myelodysplastic syndrome. Blood 112: 1638–1645.

Faenza I, Matteucci A, Manzoli L, Billi AM, Aluigi M, Peruzzi D, Vitale M, Castorina S, Suh PG, Cocco L. 2000. A role for nuclear phospholipase Cbeta 1 in cell cycle control. J Biol Chem 275:30520–30524.

Faenza I, Ramazzotti G, Bavelloni A, Fiume R, Gaboardi GC, Follo MY, Gilmour RS, Martelli AM, Ravid K, Cocco L. 2007. Inositide-dependent phospholipase C signaling mimics insulin in skeletal muscle differentiation by affecting specific regions of the cyclin D3 promoter. Endocrinology 148:1108–1117.

Faenza I, Bregoli L, Ramazzotti G, Gaboardi G, Follo MY, Mongiorgi S, Billi AM, Manzoli L, Martelli AM, Cocco L. 2008. Nuclear phospholipase C beta1 and cellular differentiation. Front Biosci 13:2452–2463.

Fenaux P, Raza A, Mufti GJ, Aul C, Germing U, Kantarjian H, Cripe L, Kerstens R, De Porre P, Kurzrock R. 2007. A multicenter phase 2 study of the farnesyltransferase inhibitor tipifarnib in intermediate- to high-risk myelodysplastic syndrome. Blood 109:4158–4163.

Fenaux P, Mufti GJ, Hellstrom-Lindberg E, Santini V, Finelli C, Giagounidis A, Schoch R, Gattermann N, Sanz G, List A, Gore SD, Seymour JF, Bennett JM, Byrd J, Backstrom J, Zimmerman L, McKenzie D, Beach C, Silverman LR. 2009. 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. Lancet Oncol 10:223–232.

Fiume R, Ramazzotti G, Teti G, Chiarini F, Faenza I, Mazzotti G, Billi AM, Cocco L. 2009. Involvement of nuclear PLCbeta1 in lamin B1 phosphorylation and G2/M cell cycle progression. Faseb J 23:957–966.

Follo MY, Bosi C, Finelli C, Fiume R, Faenza I, Ramazzotti G, Gaboardi GC, Manzoli L, Cocco L. 2006. Real-time PCR as a tool for quantitative analysis of PI-PLCbeta1 gene expression in myelodysplastic syndrome. Int J Mol Med 18:267–271.

Follo MY, Mongiorgi S, Bosi C, Cappellini A, Finelli C, Chiarini F, Papa V, Libra M, Martinelli G, Cocco L, Martelli AM. 2007. The Akt/mammalian target of rapamycin signal transduction pathway is activated in high-risk myelodysplastic syndromes and influences cell survival and proliferation. Cancer Res 67:4287–4294.

Follo MY, Finelli C, Bosi C, Martinelli G, Mongiorgi S, Baccarani M, Manzoli L, Blalock WL, Martelli AM, Cocco L. 2008. PI-PLCbeta-1 and activated Akt levels are linked to azacitidine responsiveness in high-risk myelodysplastic syndromes. Leukemia 22:198–200.

Follo MY, Finelli C, Clissa C, Mongiorgi S, Bosi C, Martinelli G, Baccarani M, Manzoli L, Martelli AM, Cocco L. 2009a. Phosphoinositide-phospholipase C beta1 mono-allelic deletion is associated with myelodysplastic syndromes evolution into acute myeloid leukemia. J Clin Oncol 27:782–790.

Follo MY, Finelli C, Mongiorgi S, Clissa C, Bosi C, Testoni N, Chiarini F, Ramazzotti G, Baccarani M, Martelli AM, Manzoli L, Martinelli G, Cocco L. 2009b. Reduction of phosphoinositide-phospholipase C beta1 methylation predicts the responsiveness to azacitidine in high-risk MDS. Proc Natl Acad Sci U S A 106:16811–16816.

Greenberg P, Cox C, LeBeau MM, Fenaux P, Morel P, Sanz G, Sanz M, Vallespi T, Hamblin T, Oscier D, Ohyashiki K, Toyama K, Aul C, Mufti G, Bennett J. 1997. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood 89:2079–2088.

Griffiths EA, Gore SD. 2008. DNA methyltransferase and histone deacetylase inhibitors in the treatment of myelodysplastic syndromes. Semin Hematol 45:23–30.

Hirai H. 2003. Molecular mechanisms of myelodysplastic syndrome. Jpn J Clin Oncol 33:153–160.

Jabbour E, Kantarjian HM, Koller C, Taher A. 2008. Red blood cell transfusions and iron overload in the treatment of patients with myelodysplastic syndromes. Cancer 112:1089–1095.

Kaminskas E, Farrell A, Abraham S, Baird A, Hsieh LS, Lee SL, Leighton JK, Patel H, Rahman A, Sridhara R, Wang YC, Pazdur R. 2005. Approval summary: azacitidine for treatment of myelodysplastic syndrome subtypes. Clin Cancer Res 11:3604–3608.

Kerbauy DB, Deeg HJ. 2007. Apoptosis and antiapoptotic mechanisms in the progression of myelodysplastic syndrome. Exp Hematol 35:1739–1746.

Malcovati L, Germing U, Kuendgen A, Della Porta MG, Pascutto C, Invernizzi R, Giagounidis A, Hildebrandt B, Bernasconi P, Knipp S, Strupp C, Lazzarino M, Aul C, Cazzola M. 2007. Time-dependent prognostic scoring system for predicting survival and leukemic evolution in myelodysplastic syndromes. J Clin Oncol 25:3503–3510.

Martelli AM, Gilmour RS, Bertagnolo V, Neri LM, Manzoli L, Cocco L. 1992. Nuclear localization and signalling activity of phosphoinositidase C beta in Swiss 3T3 cells. Nature 358:242–245.

Martelli AM, Follo MY, Evangelisti C, Fala F, Fiume R, Billi AM, Cocco L. 2005. Nuclear inositol lipid metabolism: more than just second messenger generation? J Cell Biochem 96:285–292.

Morgan MA, Reuter CW. 2006. Molecularly targeted therapies in myelodysplastic syndromes and acute myeloid leukemias. Ann Hematol 85:139–163.

Nyakern M, Tazzari PL, Finelli C, Bosi C, Follo MY, Grafone T, Piccaluga PP, Martinelli G, Cocco L, Martelli AM. 2006. Frequent elevation of Akt kinase phosphorylation in blood marrow and peripheral blood mononuclear cells from high-risk myelodysplastic syndrome patients. Leukemia 20:230–238.

Papa V, Tazzari PL, Chiarini F, Cappellini A, Ricci F, Billi AM, Evangelisti C, Ottaviani E, Martinelli G, Testoni N, McCubrey JA, Martelli AM. 2008. Proapoptotic activity and chemosensitizing effect of the novel Akt inhibitor perifosine in acute myelogenous leukemia cells. Leukemia 22:147–160.

Park S, Chapuis N, Bardet V, Tamburini J, Gallay N, Willems L, Knight ZA, Shokat KM, Azar N, Viguie F, Ifrah N, Dreyfus F, Mayeux P, Lacombe C, Bouscary D. 2008. PI-103, a dual inhibitor of Class IA phosphatidylinositide 3-kinase and mTOR, has antileukemic activity in AML. Leukemia 22:1698–1706.

Perl AE, Kasner MT, Tsai DE, Vogl DT, Loren AW, Schuster SJ, Porter DL, Stadtmauer EA, Goldstein SC, Frey NV, Nasta SD, Hexner EO, Dierov JK, Swider CR, Bagg A, Gewirtz AM, Carroll M, Luger SM. 2009. 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 15:6732–6739.

Peruzzi D, Calabrese G, Faenza I, Manzoli L, Matteucci A, Gianfrancesco F, Billi AM, Stuppia L, Palka G, Cocco L. 2000. Identification and chromosomal localisation by fluorescence in situ hybridisation of human gene of phosphoinositide-specific phospholipase C beta(1). Biochim Biophys Acta 1484: 175–182.

Quintas-Cardama A, Tong W, Kantarjian H, Thomas D, Ravandi F, Kornblau S, Manshouri T, Cortes JE, Garcia-Manero G, Verstovsek S. 2008. A phase II study of 5-azacitidine for patients with primary and post-essential thrombocythemia/polycythemia vera myelofibrosis. Leukemia 22:965–970.

Raj K, John A, Ho A, Chronis C, Khan S, Samuel J, Pomplun S, Thomas NS, Mufti GJ. 2007. CDKN2B methylation status and isolated chromosome 7 abnormalities predict responses to treatment with 5-azacytidine. Leukemia 21:1937–1944.

Scholl C, Gilliland DG, Frohling S. 2008. Deregulation of signaling pathways in acute myeloid leukemia. Semin Oncol 35:336–345.

Sekeres MA, Maciejewski JP, Giagounidis AA, Wride K, Knight R, Raza A, List AF. 2008. Relationship of treatment-related cytopenias and response to lenalidomide in patients with lower-risk myelodysplastic syndromes. J Clin Oncol 26:5943–5949.

Silverman LR, Mufti GJ. 2005. Methylation inhibitor therapy in the treatment of myelodysplastic syndrome. Nat Clin Pract Oncol 2(Suppl 1):S12–23.

Srinivasan S, Schiffer CA. 2008. Current treatment options and strategies for myelodysplastic syndromes. Expert Opin Pharmacother 9:1667–1678.

Stresemann C, Lyko F. 2008. Modes of action of the DNA methyltransferase inhibitors azacytidine and decitabine. Int J Cancer 123:8–13.

Suh PG, Park JI, Manzoli L, Cocco L, Peak JC, Katan M, Fukami K, Kataoka T, Yun S, Ryu SH. 2008. Multiple roles of phosphoinositide-specific phospholipase C isozymes. BMB Rep 41:415–434.

Tefferi A, Vardiman JW. 2009. Myelodysplastic syndromes. N Engl J Med 361:1872–1885.

Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, Harris NL, Le Beau MM, Hellstrom-Lindberg E, Tefferi A, Bloomfield CD. 2009. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: Rationale and important changes. Blood 114:937–951.

Wangpaichitr M, Wu C, You M, Kuo MT, Feun L, Lampidis T, Savaraj N. 2008. Inhibition of mTOR restores cisplatin sensitivity through down-regulation of growth and anti-apoptotic proteins. Eur J Pharmacol 591:124–127.

Wymann MP, Schneiter R. 2008. Lipid signalling in disease. Nat Rev Mol Cell Biol 9:162–176.

Yazbeck VY, Buglio D, Georgakis GV, Li Y, Iwado E, Romaguera JE, Kondo S, Younes A. 2008. Temsirolimus downregulates p21 without altering cyclin D1 expression and induces autophagy and synergizes with vorinostat in mantle cell lymphoma. Exp Hematol 36:443–450.

Ye K. 2005. PIKE/nuclear PI 3-kinase signaling in preventing programmed cell death. J Cell Biochem 96:463–472.