Downregulation of PPP2R5E is a common event in acute myeloid leukemia that affects the oncogenic potential of leukemic cells

Genetic aberrations affecting kinases have been widely studied in acute myeloid leukemia (AML). However, the role of phosphatases remains underexplored. Protein phosphatase 2A (PP2A) is a major serine/threonine phosphatase that participates in many signaling pathways.[1] Transformed cells present a wide variety of mechanisms to inactivate PP2A.[2,3] Our group reported that PP2A inhibition is a common event in AML, and that restoration of PP2A activity induces cell growth arrest and caspase-dependent apoptosis. This suggests that PP2A inactivation plays a crucial role in AML[4] and confirms the potential of PP2A as a therapeutic target in hematologic malignancies.[5] Moreover, we found deregulated expression of PP2A endogenous inhibitors and/or PP2A subunits, including the regulatory subunits PPP2R5B and PPP2R5C, as mechanisms that could contribute to PP2A inhibition in AML.[4] However, we did not observe any aberration in several AML cases with inhibited PP2A, suggesting that other unidentified mechanisms might be involved. The regulatory subunit PPP2R5E/B56ε is involved in multiple signaling pathways and plays a critical role during early development.[6-8] Moreover, PPP2R5E is an essential regulator of apoptosis,[9] and acts as a negative regulator of MAP4K3, mediating its ability to signal to mTORC1.[10] Thus, we hypothesized that downregulation of PPP2R5E could contribute to PP2A inhibition in AML.

To know the prevalence of PPP2R5E downregulation in AML, we first analyzed 12 cell lines and 41 AML patient samples. PPP2R5E was down-regulated at mRNA and protein level in 4 cell lines (4 of 12, 33%) (*Online Supplementary Figure S1*). We found PPP2R5E mRNA downregulation in 25 cases (25 of 41, 61%), and decreased levels of the protein in 10 cases (10 of 16, 63%), all with either hyperphosphorylation or reduced expression of the PP2A catalytic subunit (Figure 1A and *Online Supplementary Table S1*). These results strongly suggest that PPP2R5E downregulation is a common event in AML, and that PP2A activity is affected in these cases. The high prevalence of PPP2R5E downregulation in our AML cases led us to investigate its biological relevance. PP2A is a heterotrimeric complex including catalytic, scaffold, and regulatory subunits. Regulatory subunits determine the substrate specificity and the intracellular location of the PP2A complex.[1] Four families of PP2A regulatory subunits have been described, therefore different sets of genomic aberrations in tumor formation require the loss of different PP2A complexes during tumor progression. The actual challenge is not only to explore the potential therapeutic value of PP2A activators,[4,5,11] but to identify the particular PP2A complexes affected in each disease in order to develop more efficient therapeutic strategies.[12] MTS assays in HEL cells showed decreased proliferation in cells transfected with PPP2R5E (Figure 1B); these results were confirmed in HL-60 and KG-1 (*Online Supplementary Figure S2*). Moreover, PPP2R5E-expressing cells formed a significantly lower number of colonies than controls (*Online Supplementary Figure S3A*). Furthermore, consistent with its ability to suppress cell proliferation, PPP2R5E overexpression had a significant caspase-dependent proapoptotic effect (*Online Supplementary Figure S3B*). The role of PPP2R5E in apoptosis seems to be through p53 regulation.[9] Therefore, we analyzed the p53 status in our series. We observed a good correlation between PPP2R5E downregulation and p53 levels, suggesting that the molecular effects of PPP2R5E in AML could occur, at least in part, via p53 (*Online Supplementary Figure S4*).

PP2A regulatory subunits PPP2R5B and PPP2R5C dephosphorylate AKT.[13] Therefore, we analyzed the effects of PPP2R5E ectopic expression in HEL cells, observing decreased phosphorylation of the PP2A target AKT, without affecting its expression levels (Figure 1C). These results were confirmed in HL-60 and KG-1 (*Online Supplementary Figure S2*). B56 regulatory subunits are involved in the PI3K/Akt, Wnt/beta-catenin, and Hedgehog pathways.[6,7] Interestingly, a recent study shows that low expression of another regulatory subunit,

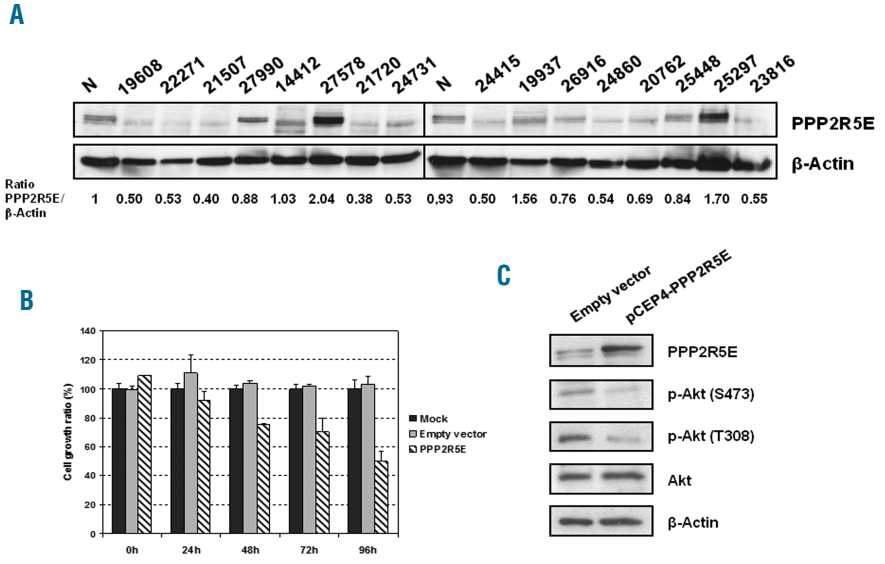

Figure 1. Functional significance of PP2R5E in AML cells. (A) Western blot and densitometric analysis of the expression levels of PPP2R5E in 16 AML patient samples. (B) MTS assay showing proliferation in HEL cells transfected with PPP2R5E compared to mock-transfected cells or cells transfected with an empty vector. (C) Western blot showing the effect of PPP2R5E overexpression on AKT activity and expression in the HEL cell line.

PPP2R2A/B55α, correlates with AKT-T308 dephosphorylation and is an adverse prognostic factor in AML.[3] Moreover, Wnt/beta-catenin is required for the development of leukemia stem cells in AML.[14] Thus, loss of PPP2R5E function could provide an additional mechanism to activate this pathway in AML cells. It has been shown that PPP2R5E knockdown does not induce a transformed phenotype in HEK-TER cells.[12] However, we observed that PPP2R5E negatively affects the clonogenic potential of AML cells (*Online Supplementary Figure S3A*), indicating that the importance of the PP2A complexes would be different depending on the cellular model. In addition, as we have indicated before, the cases analyzed present several alterations affecting PP2A (*Online Supplementary Table S1*).[4] Altogether, these results would suggest that PPP2R5E downregulation contributes to PP2A inhibition in AML, although it would need other additional alterations.

We had previously shown that downregulation of the PP2A subunits PPP2R1B and PPP2R5B/C by genomic deletions could be mechanisms contributing to PP2A inhibition in AML.[4] However, our results showed that this was probably not the mechanism of PPP2R5E downregulation in AML, since only the HEL cell line had downregulation of PPP2R5E and deletion of its locus (14q23.1). In fact, in the case of PPP2R2A, it may involve a post-transcriptional component.[3] Therefore we looked for the expression of miRNAs predicted to target PPP2R5E. We found no correlation between the expression of these miRNAs and PPP2R5E in AML cell lines (*Online Supplementary Table S2*). Recently, Mavrakis *et al.* found that the oncogenic properties of miR-19b in acute lymphoblastic leukemia were due, at least in part, to its inhibitory effect on PP2A via downregulation of PPP2R5E.[15] Thus, we analyzed the expression of miR-19b and PPP2R5E in 12 AML cell lines and in 35 AML patient samples. However, we found no correlation either in cell lines or samples (Figure 1A, *Online Supplementary Figure S1*, *Online Supplementary Tables S1 and S3*). In addition, functional studies with miR-19b in HEL, F-36P and KG-1 cells showed that miR-19b, described as a regulator of PPP2R5E in lymphocytes,[15] does not regulate PPP2R5E in AML cells (*Online Supplementary Figure S5*). Further studies are needed to clarify the alternative mechanisms that lead to PPP2R5E downregulation in AML.

In conclusion, the high frequency of PPP2R5E downregulation in AML cell lines and patient samples at both mRNA and protein level, and its functional relevance, suggest that this alteration represents a novel contributing mechanism to PP2A inhibition in this disease. PPP2R5E impairs cell proliferation, induces caspase-dependent apoptosis, affects the activation status of AKT and reduces the colony-forming ability of the leukemic cells. These results indicate that PPP2R5E downregulation has relevance in AML, and could help distinguish a subgroup of patients who might benefit from receiving future treatments with PP2A activators.

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doi:10.3324/haematol.2013.084731

Funding: Ministerio de Ciencia e Innovación (PI11/02443), Departamento Salud del Gobierno de Navarra (78/2012), ISCIII-RTICC (RD12/0036/0063) and Fundación para la Investigación Médica Aplicada y UTE (Spain).

Key words: PPP2R5E, PP2A, AML, AKT.

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

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