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Research Report

Role of protein histidine phosphatase for viability of neuronal cells

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ABSTRACT

We recently found protein histidine phosphatase (PHP) in eukaryotes and identified ATP-citrate lyase (ACL) and the β-subunit of G-proteins as its substrates. The aim of the present study was to get information on the significance of PHP for cellular function and viability. PHP was overexpressed by a viral vector in SH-SY5Y cells, a human neuroblastoma cell line, and in primary cultures of cortical neurons from embryonic (E19) rats. Furthermore, PHP was downregulated by siRNA in SH-SY5Y cells. We could demonstrate that overexpression of PHP decreased the phosphorylation state of ACL. Accordingly, the activity of ACL seemed to be reduced and subsequently, the viability of the cells was diminished. On the other hand, downregulation of PHP did not clearly influence phosphorylation and activity of ACL as well as viability of the cells. The results suggest that an increased activity of PHP impairs cellular function whereas downregulation of PHP does not.

1. Introduction

The importance of reversible protein phosphorylation was first described by Fischer et al. (1959). Nowadays, it is well established that kinases and phosphatases play a crucial role in cellular signaling (Cohen, 2001). The amino acids involved in reversible protein phosphorylation are serine, threonine and tyrosine on the one hand and histidine, lysine and aspartate on the other (Klumpp and Krieglstein, 2005). A lot of evidence has been accumulated on the phosphorylation of serine, threonine and tyrosine residues. N-bound phosphates of proteins escaped detection under the acidic conditions which were normally applied. Therefore, in eukaryotes much less is known about N-phosphates, protein histidine kinases and histidine phosphatases. For some time, it was assumed that O-phosphorylation exclusively takes place in eukaryotes and N-phosphorylation occurs in prokaryotes only (Kennelly and Potts, 1996).

Meanwhile, two laboratories discovered independently from each other PHP in mammals (Hermesmeier and Klumpp, 1999; Klumpp et al., 2002; Ek et al., 2002). It is a monomeric 14 kDa protein which is not present in bacteria and fungi but is ubiquitously expressed in a variety of mammalian tissues. Looking for substrates of PHP, we found a 440 kDa protein which was identified as ACL (Klumpp et al., 2003) with an impact on fatty acid and energy metabolism as well as on cholesterol and acetylcholine synthesis. Overexpression of PHP might inhibit cellular metabolism and viability whereas its downregulation might improve cellular viability by a reduced dephosphorylation of ACL (Krieglstein et al., 2008).

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Thus, in the present paper, we made an attempt to study viability of cultured neurons changed by up- and down-regulation of PHP.

2. Results

2.1. *Knockdown of PHP*

Investigating the physiological importance of PHP, we first addressed the question whether the viability of SH-SY5Y cells could be affected by reducing the expression of PHP.

Transient downregulation of PHP was successfully achieved by siRNA. Several siRNA constructs were tested for their potency to prevent translation of PHP. The sequence encoding the nucleotides 142–164 corresponding to the amino acid sequence KWAEYHAD (aa 48–55) yielded best results. Reduction of the amount of PHP was observed on the mRNA level. Two days after transfection with siRNA, the amount of PHP mRNA was drastically reduced (Fig. 1A). In line with these data, Western blot analysis using a polyclonal antibody against PHP showed a significant reduction of PHP in SH-SY5Y cells 48 h after transfection (Fig. 1B). Unspecific down-regulation of PHP was excluded using siRNA containing 4 substituted nucleotides. Transfection of SH-SY5Y cells with this scrambled siRNA by means of electroporation did not affect the amount of PHP protein (Fig. 1B). Cell viability determined by staining with Hoechst 33258 was not affected by PHP knockdown (data not shown).

ACL with an impact on fat and energy metabolism is a substrate of PHP (Klumpp et al., 2003). Therefore, we examined whether downregulation of PHP has an influence on ACL activity due to reduced dephosphorylation of the enzyme. To demonstrate a putative higher phosphorylation state of ACL in the cells with a reduced amount of PHP we incubated the cell homogenates with [γ-^{32}P]ATP in the presence of 5 mM EDTA (Noiman and Shaul, 1995) for 1 h at 37 °C. ACL in the homogenate of SH-SY5Y cells transfected with siRNA against PHP was phosphorylated to the same extent as the cells transfected with scrambled siRNA. Upon electroporation, phosphorylation of ACL was reduced compared to control (Fig. 2A). The specific activity of ACL and the viability of cells also were not affected by downregulation of PHP in SH-SY5Y cells (Fig. 2B,C).

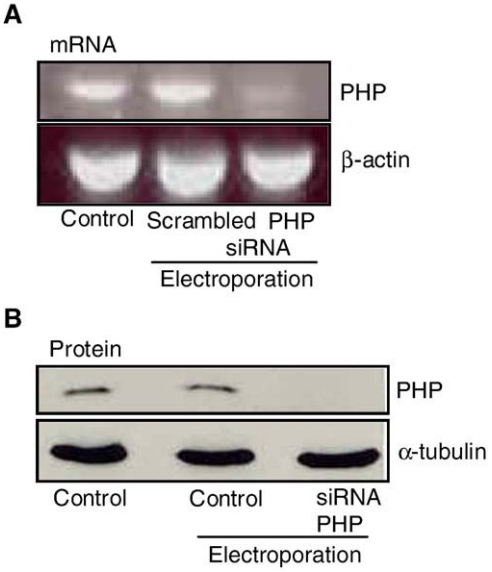

Fig. 1 – Knockdown of PHP in SH-SY5Y cells. Downregulation of PHP mRNA and protein was achieved by the RNAi technique. The levels of PHP mRNA (A) and PHP protein (B) were not influenced in the cells treated with scrambled siRNA. When siRNA against PHP was used the mRNA (A) and protein (B) of PHP were downregulated 48 h after transfection. The housekeeping proteins β-actin (A) and α-tubulin (B) remained unchanged.

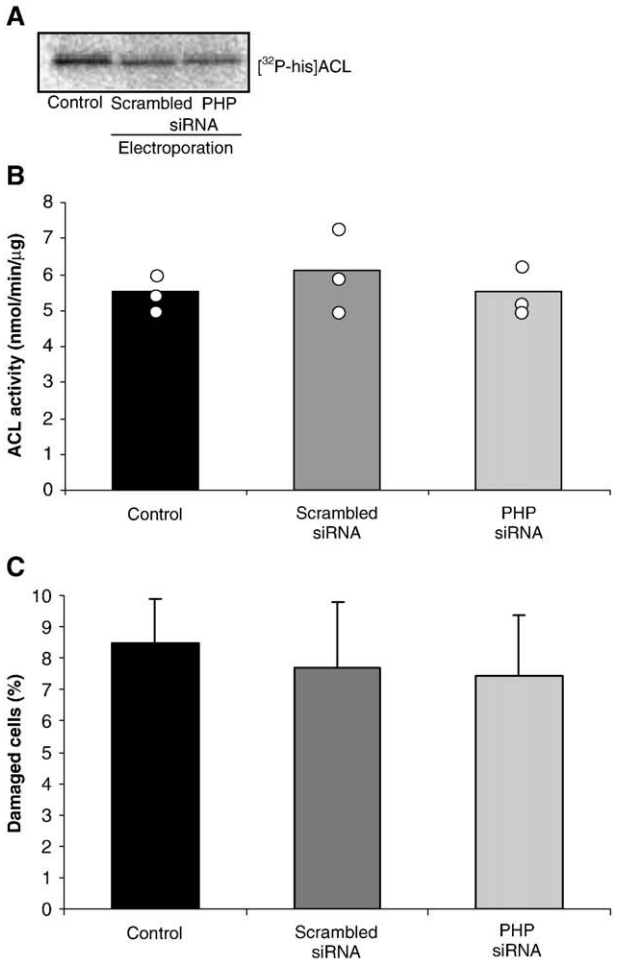

Fig. 2 – Viability of SH-SY5Y cells after knockdown of PHP. The amount of [^{32}P-his]ACL decreased after RNAi treatment by 40% whereas the difference between scrambled RNAi- and PHP RNAi-treated cells was about 15% (A). ACL activity (B) and the percentage of damaged cells (C) were not affected by PHP downregulation. ACL activity values are given as means of 3 experiments and the percentages of damaged cells are means of 5 experiments ± SD.

2.2. *Overexpression of PHP*

As described before, knockdown of PHP did not affect cell viability, ACL phosphorylation and ACL activity. As a next step we studied whether overexpression of PHP would reduce cell