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Research Article

High levels of the type III inorganic phosphate transporter PiT1 (*SLC20A1*) can confer faster cell adhesion

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ABSTRACT

The inorganic phosphate transporter PiT1 (SLC20A1) is ubiquitously expressed in mammalian cells. We recently showed that overexpression of human PiT1 was sufficient to increase proliferation of two strict density-inhibited cell lines, murine fibroblastic NIH3T3 and pre-osteoblastic MC3T3-E1 cells, and allowed the cultures to grow to higher cell densities. In addition, upon transformation NIH3T3 cells showed increased ability to form colonies in soft agar. The cellular regulation of PiT1 expression supports the cells that utilize the PiT1 levels to control proliferation, with non-proliferating cells showing the lowest PiT1 mRNA levels. The mechanism behind the role of PiT1 in increased cell proliferation is not known. We, however, found that compared to control cells, cultures of NIH3T3 cells overexpressing PiT1 upon seeding showed increased cell number after 24 h and had shifted more cells from G0/G1 to S+G2/M within 12 h, suggesting that an early event may play a role. We here show that expression of human PiT1 in NIH3T3 cells led to faster cell adhesion; this effect was not cell type specific in that it was also observed when expressing human PiT1 in MC3T3-E1 cells. We also show for NIH3T3 that PiT1 overexpression led to faster cell spreading. The final total numbers of attached cells did, however, not differ between cultures of PiT1 overexpressing cells and control cells of neither cell type. We suggest that the PiT1-mediated fast adhesion potentials allow the cells to go faster out of G0/G1 and thereby contribute to their proliferative advantage within the first 24 h after seeding.

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Introduction

The mammalian type III sodium-dependent inorganic phosphate (P_i[2]) symporters PiT1 (*SLC20A1*) and PiT2 (*SLC20A2*) are part of the P_i transport (PiT) family (TC#2.A.20) [1,2], which is represented in all kingdoms of life [3]. Originally, human PiT1 (formerly GLVR1) and human and rat PiT2 (formerly GLVR2 and Ram-1, respectively) were discovered as receptors for different gammaretroviruses [4–6] and were later found to transport P_i into cells [7–13]. The two PiT paralogs are highly related, they show about 60% amino acid identity and chimeras between PiT1 and PiT2 can change their retroviral specificities [14–18]. They have similar P_i transport functions [11,19,20], and are broadly expressed in mammalian tissues [21]. Moreover, knockdown of PiT1 expression can lead to upregulation of the PiT2 mRNA level [22–24]. These observations suggest that PiT1 and PiT2 possess overlapping functions in maintaining cellular phosphate homeostasis.

PiT1 and PiT2, however, also have non-overlapping functions [24–29], and P_i-transport-independent functions of PiT1 have been discovered [27,29,30]. Knockout of PiT1 in mice retards growth of embryos, slows proliferation of liver cells, and impairs erythroid and early B-cell development [24–27]. The effects of knockout of PiT1 in mice on erythroid and early B-cell development have been associated with defects in cell cycle progression [26,27]. Knockdown of PiT1 impairs proliferation of the transformed cell lines HeLa and HepG2 and tumorigenesis of HeLa cells in nude mice [29], and proliferation of the murine density-inhibited cell line MC3T3-E1 [23]. Thus, a certain level of PiT1 is important for cell proliferation. Moreover, overexpression of PiT1 is sufficient to increase proliferation of the murine density-inhibited cell lines NIH3T3 and MC3T3-E1 [23]. In agreement with that PiT1 overexpression leads to a general proliferative advantage, cultures of these cells grew to higher cell densities, but they remained density-inhibited [23]. However, when transformed, NIH3T3 cells overexpressing PiT1 formed more colonies in soft agar than control cells [23]. The cellular regulation of the endogenous PiT1 expression in NIH3T3 and MC3T3-E1 cells supports that cells utilize their PiT1 levels to control proliferation, with non-proliferating cells showing the lowest PiT1 mRNA levels [23].

There is direct and indirect evidence that the role of PiT1 in cell proliferation is not dependent on its P_i transport function [23,24,29]. Thus although mouse embryonic fibroblasts from PiT1 knockout mice [24] and MC3T3-E1 cells with knocked down PiT1 expression [23] exhibited increased PiT2 expression, they still showed impaired proliferation. In addition, overexpression of PiT2 leading to increased P_i uptake, did not rescue impaired proliferation of HeLa cells caused by reduced PiT1 expression, while expression of a PiT1 transport knockout mutant did [29]. Moreover, overexpression of PiT1 in NIH3T3 and MC3T3-E1 cells in general does not lead to regulation of PiT2, and while it does lead to increased proliferation of both cell types, only NIH3T3 cells show increased ability to import P_i [23]. Together these results suggest that PiT1's role in regulation of cell proliferation is independent on its P_i-uptake ability.

PiT1 overexpression was found to confer increased proliferation upon NIH3T3 and MC3T3-E1 cells under standard cultivation conditions, i.e., in standard growth media containing 10% bovine serum and tissue-culture treated plastic ware, all negatively charged (when wetted) tissue-culture treated polystyrene (TCPS[3]) [23]. When cells are seeded in serum-containing cell medium onto TCPS, the proteins of the serum will very quickly adsorb to the negative charges of the polystyrene. Depending on the quality of the TCPS (i.e., charge density and topology of the polystyrene) adsorption of more or less adhesion-promoting proteins will occur. The seeded cells adhere to the adhesion-promoting proteins, which predominantly are vitronectin (spreading factor) and plasma fibronectin, or via adhesion-promoting protein already attached to the cells. Specific contact with the adhesion-promoting proteins through integrins allows the cells to spread out [31–36] (reviewed in Elbert et al. [37] and Wilson et al. [38]). It is presently not known why cultures of cells with increased expression of PiT1 show enhanced proliferation. We have, however, previously observed that after just one day in culture, fibroblastic NIH3T3 cells overexpressing PiT1 had proliferated faster, and already 12 h after seeding, a lower percentage of the cells were in G0/G1 in the PiT1 overexpressing cultures compared to cultures of control cells [23]. We have here addressed how a high expression of PiT1 allows the cells to increase proliferation within the first day in culture. Using the same conditions as in the proliferation experiments, i.e., standard cultivation conditions, we found that overexpression of PiT1 in NIH3T3 cells leads to faster adhesion and spreading compared to control cells. The adhesion advantage of PiT1 overexpressing NIH3T3 cells compared to control cells was, moreover, found to be independent of serum concentrations during cultivation prior to seeding and during seeding, and of the quality of the TCPS, which the cells had been cultivated on prior to seeding. In addition, adhesion of MC3T3-E1 cells was also investigated under standard cultivation conditions, and overexpression of PiT1 was also found to confer faster adhesion on this pre-osteoblastic cell line.

Materials and methods

Constructs

The vector pLXSN [39] was modified. The original HindIII site in the plasmid was removed by site-directed mutagenesis and HindIII and NotI sites introduced in the multiple cloning region; the modified pLXSN vector is referred to as pLXSN-ΔHindIII+HindIII+NotI and the retroviral vector as LXSN+HindIII+NotI. The PiT1 encoding sequence was cloned as a HindIII-XhoI fragment from pOJ75 [16] into the HindIII – XhoI sites of pLXSN-ΔHindIII+HindIII+NotI resulting in the plasmid pLPiT1SN-ΔHindIII+HindIII+NotI; the retroviral vector is referred to as LPiT1SN+HindIII+NotI.

Malachite green based assay for p_i determination

To determine the contribution of P_i from 10% newborn calf serum (NCS) (Gibco BRL) and 10% fetal bovine serum (FBS) (Gibco BRL) supplemented to Dulbecco's Modified Eagles Medium (DMEM) and Minimum Essential Medium alpha (α-MEM) (Gibco BRL), respectively, the total P_i contents of P_i-free DMEM containing 10% NCS or 10% FBS were determined using a malachite green based method basically as described by Baykov et al. [40] with the exception that 50 µl samples were added to 100 µl assay solutions in a 96-well plate and the developed malachite green-phosphomolybdate complex was measured at 650 nm.

[2] Inorganic phosphate, P_i

[3] Tissue-culture treated polystyrene, TCPS.

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