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Biochimica et Biophysica Acta 1773 (2007) 1062 – 1072
Biochimica et Biophysica Acta BBA
www.elsevier.com/locate/bbamcr

The small GTPases Rab5 and RalA regulate intracellular traffic of P-glycoprotein

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Received 13 December 2006; received in revised form 28 February 2007; accepted 27 March 2007
Available online 19 April 2007

Abstract

P-glycoprotein (P-gp) is a plasma membrane glycoprotein that can cause multidrug resistance (MDR) of cancer cells by acting as an ATP-dependent drug efflux pump. The regulatory effects of the small GTPases Rab5 and RalA on the intracellular trafficking of P-gp were investigated in HeLa cells. As expected, overexpressed enhanced green fluorescent protein (EGFP)-tagged P-gp (P-gp-EGFP) is mainly localised to the plasma membrane. However, upon cotransfection of either dominant negative Rab5 (Rab5-S34N) or constitutively active RalA (RalA-G23V) the intracellular P-gp-EGFP levels increased approximately 9 and 13 fold, respectively, compared to control P-gp-EGFP cells. These results suggest that Rab5 and RalA regulate P-gp trafficking between the plasma membrane and an intracellular compartment. In contrast, coexpression of constitutively active Rab5 (Rab5-Q79L) or dominant negative RalA (RalA-S28N) had no effect on the localisation of P-gp-EGFP. Furthermore, the intracellular accumulation of daunorubicin, a substrate for P-gp, increased significantly with an increased intracellular localisation of P-gp-EGFP. These results imply that it may be possible to overcome MDR by controlling the plasma membrane localisation of P-gp.

Keywords: P-glycoprotein; endocytosis; exocytosis; Rab5; RalA; multidrug resistance in cancer

1. Introduction

Multidrug resistance (MDR) has been a major obstacle in cancer chemotherapy. One of the major causes of MDR is over-expression of P-glycoprotein (P-gp), a membrane protein encoded by the human *mdr1* gene, whereby tumour cells become drug resistant [1,2]. Overexpression of the *mdr1* gene is found in many different cancers, including tumours not previously exposed to chemotherapy [3]. P-gp acts as a drug efflux pump to transport a wide range of chemotherapeutic drugs, differing both structurally and in their mechanism of action, out of drug resistant cancer cells. Consequently, cancer cells continue to proliferate. In order to inhibit or interrupt the transport activity of P-gp, chemosensitizers or P-gp modulators have been used but these methods give unwanted side effects. Since P-gp needs to be present on the plasma membrane to act as a drug efflux pump [4], an alternative way to regulate P-gp activity could be to interfere with its plasma membrane localisation.

Membrane proteins and cargo molecules are transported from one membrane compartment to another by mobilisation of membrane structures and vesicular transport [5,6]. Petriz and co-workers demonstrated that EGFP-tagged P-gp (P-gp-EGFP) was mainly localised at the plasma membrane and the Golgi apparatus and there was an inverse relationship between P-gp-EGFP expression and nuclear doxorubicin accumulation [7]. Our previous study showed that P-gp-EGFP is transported to the plasma membrane via the endoplasmic reticulum (ER) and the Golgi apparatus [4]. Upon reaching the plasma membrane, P-gp continuously cycles between the plasma membrane and endosomal compartments [8,9]. When the transport of newly synthesized P-gp-EGFP to the plasma membrane was blocked by brefeldin A (BFA) or monensin, we found an increased

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doi:10.1016/j.bbamcr.2007.03.023

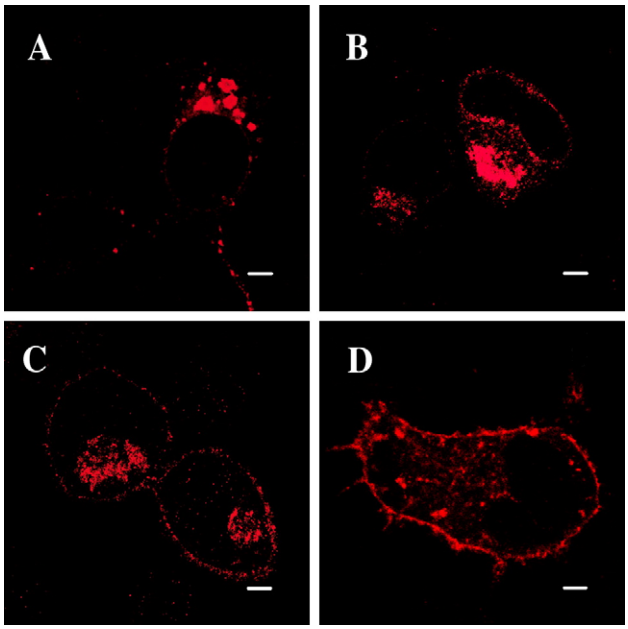

Fig. 1. Localisation of Rab5 and RalA mutants in HeLa cells. HeLa cells were transfected with HA-Rab5-Q79L (A), HA-Rab5-S34N (B), HA-RalA-G23V (C) and HA-RalA-S28N (D). Cells were fixed and immunolabelled for Rab5 (A, B) or RalA (C, D). Bar, 5 μm.

intracellular accumulation of daunorubicin, a substrate of P-gp [4]. Likewise, interfering with endocytosis of P-gp should alter the localisation, and therefore the activity of P-gp [9].

The Rab family of small GTPases are known to regulate most vesicular transport events, principally by regulating vesicle docking and fusion [10–14]. Rab proteins form the largest branch of small GTPases, and more than 60 Rab proteins have been discovered in mammalian cells [15]. All Rab proteins cycle between an active GTP-bound and an inactive GDP-bound conformation, but distinct Rab proteins are believed to be associated with regulation of vesicle traffic from a specific organelle or along a specific pathway [16]. Rab4, Rab5 and Rab11 have been shown to be involved in endocytosis and recycling to the plasma membrane [17].

Rab5 is the key regulator of membrane transport along the endocytic pathway. Rab5 interacts with its effector early endosome antigen 1 (EEA1), to regulate early endosome fusion [18]. In addition, Rab5 has been shown to play a role in endocytosis, as overexpression of dominant-negative Rab5 mutants inhibit endocytosis of several receptors, such as the neurokinin 1 receptor, epidermal growth factor (EGF) receptor, protease-activated receptor 2, β_2-adrenergic receptor and M4 muscarinic acetylcholine receptor [19–23]. Conversely, overexpression of wild-type Rab5 or constitutively active Rab5 has been shown to accelerate endocytic transport [19–23], including endocytosis of P-gp [8]. Another small GTPase, Ral, has been shown to regulate endocytosis. There are two Ral genes, RalA and RalB, and their encoded proteins have the same structural organisation and share ~85% protein sequence identity [24,25] . Ral plays a role in endocytosis of a variety of receptors such as EGF, transferrin (Tf), insulin, activin type II, and metabotropic glutamate receptors [26–30], but the nature of this role is poorly understood. Conflicting reports of overexpression of Ral mutants on endocytosis of some of these receptors have created uncertainty [31].

In order to alter the plasma membrane localisation of P-gp, we examined the effects of Rab5 and RalA on the intracellular trafficking of P-gp. We found that overexpression of dominant negative Rab5 increased the intracellular localisation of P-gp. Furthermore, overexpression of a constitutively active RalA also caused accumulation of P-gp in large intracellular structures. A concomitant increase in intracellular levels of daunorubicin, a substrate of P-gp, was found upon an increased intracellular localisation of P-gp. These results suggest altering the intracellular trafficking of P-gp by modulation of its small GTPase regulators can be a potential strategy to overcome MDR.

2. Materials and methods

2.1. Materials

HeLa cells and MCF-7 cells were purchased from the American Type Culture Collection (ATCC, Rockville, MD). Multidrug resistance MCF-7/Adr cells which have overexpressed wild-type P-gp were obtained from Dr. Mary Bebawy (Faculty of Pharmacy, University of Sydney). Dulbecco's Modified Eagle Medium (DMEM) and foetal calf serum were obtained from Invitrogen (Carlsbad, CA). Paraformaldehyde was from ProSciTech (Thuringowa Central, Australia). Antibody against Rab5A was from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-RalA and anti-EEA1 were from BD Biosciences Pharmingen (California, USA). Anti-TfR (H68.4) was from Zymed and P-gp antibody was from Sigma. Texas Red conjugated dextran (dextran-TxR) and Texas Red conjugated EGF (EGF-TxR) were from Molecular Probes (Eugene, OR). CY3 or Texas Red conjugated secondary antibodies were obtained from Jackson ImmunoResearch (West Grove, PA). FuGENE 6 was from Roche and Lipofectin was from Invitrogen (Carlsbad, CA). All other materials were obtained from Sigma (St. Louis, MO).

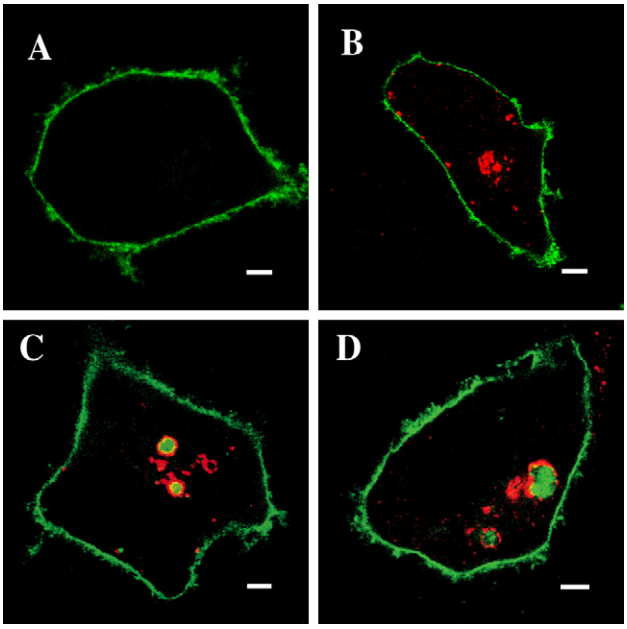

Fig. 2. Rab5-Q79L does not affect the localisation of P-gp-EGFP. HeLa cells were transfected with P-gp-EGFP alone (A) or P-gp-EGFP and HA-Rab5-Q79L (B–D). Cells were fixed and immunostained for Rab5 (B–C) or EEA1 (D) (red). Merged images are shown. Bar, 5 μm.