ΘΕΑΓΕΝΕΙΟ ΑΝΤΙΚΑΡΚΙΝΙΚΟ ΝΟΣΟΚΟΜΕΙΟ ΘΕΣΣΑΛΟΝΙΚΗΣ

ΑΡΙΣΤΟΤΕΛΕΙΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΘΕΣΣΑΛΟΝΙΚΗΣ

Expression and localization of EGFP-tagged DJ-1 in various cancer cell lines

Areti Kantzioura[1], Anastasia Koukiali[2], Theodora Choli-Papadopoulou[2] and Eleni Nikolakaki[2]

[1] Theagenio Cancer Hospital Thessaloniki Greece, Hellenic Society of Medical Oncology

[2] Laboratory of Biochemistry, Department of Chemistry, Aristotle University,Thessaloniki 54124, Greece

ABSTRACT

DJ-1 is overexpressed in most known human cancers, which is consistent with its role as an oncogene. The cloning of the DJ-1 cDNA in the plasmid vector pEGFP-N1, in order to study the expression of the GFP-tagged protein in HeLa, U87 and MCF-7 cancer cell lines showed different location and also partial extracellular secretion that depends on the cell type. These data suggest that DJ-1 overexpression and secretion are a frequent event in cancer cells and emphasize its potential prognostic value as a survival marker in patients with different tumor types. Thus, DJ-1 might be a promising serum marker for cancer diagnosis, monitoring, and prognosis.

INTRODUCTION

DJ-1 is involved in various cellular processes, including transcriptional regulation, oxidative stress response, fertilization, mitochondrial regulation, and cellular metabolism. An increasing number of studies have found that the amount of DJ-1 is overexpressed in most known human cancers, which is consistent with its role as an oncogene. Interestingly, DJ-1 could be secreted by cancer cells and thus could serve as a biomarker for cancer monitoring. In this particular experiment, we tried to investigate the mechanism of DJ-1 secretion in cancer cells and its invasive potential to different tumor cell lines.

METHODS AND MATERIALS

To isolate DJ-1 cDNA, RT-PCR was performed on RNA isolated from K562 cells that express high levels of DJ-1. Following cDNA synthesis, DJ-1 cDNA was amplified by PCR. DJ-1 was cloned into pEGFP-N1 for fusing Enhanced Green Fluorescent Protein (EGFP) to the C-terminus of DJ-1 protein. An immunofluorescent approach was then employed to detect DJ-1. To this end HeLa, MCF-7 and U87 cells cultured in DMEM medium supplemented with 10% (v/v) fetal bovine serum (FBS) and antibiotics, were transfected with the chimeric cDNA using the Xfect™ transfection kit of Clontech, according to the manufacturer's instructions. Cells were grown on glass coverslips for 24-72 h. After the incubation period the cell coverslips were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 20 min at room temperature, followed by washes with PBS and QUENS (100 mM Tris-HCl pH 7.5 in PBS) to remove excess formaldehyde and stop the fixing. DNA was stained with propidium iodide (PI) following incubation with RNAse to remove residual RNA. After 3x washing with PBS, the coverslips were mounted in 90% glycerol and visualized in a Nikon confocal microscope using the EZ-C1 3.20 software.

RESULTS

DJ-1-EGFP was found to localize both intracellularly and extracellularly in the three cell lines tested (Figures 1, 2 and 3). The extracellular localization was more prominent in HeLa cells (Figure 1). As shown in Figures 1, 2, 3 the DJ-1-GFP protein localized mainly at the periphery of cells. A small percentage was found in the cytoplasm, while the largest fraction was found in the extracellular space between the adjacent cells.

Immunofluorescence: HeLa

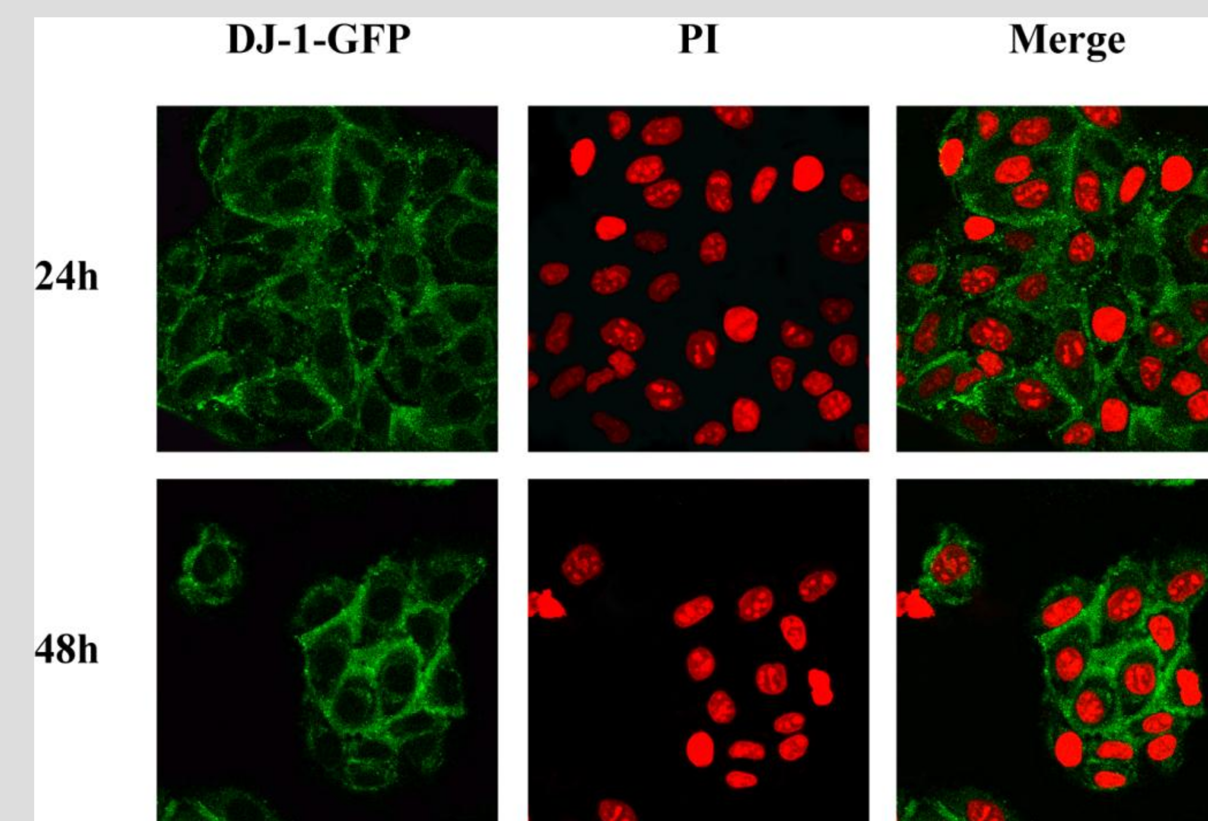

Figure 1. Localization of transfected DJ-1-GFP in HeLa cells. Nuclei were stained with PI.

Immunofluorescence: U87

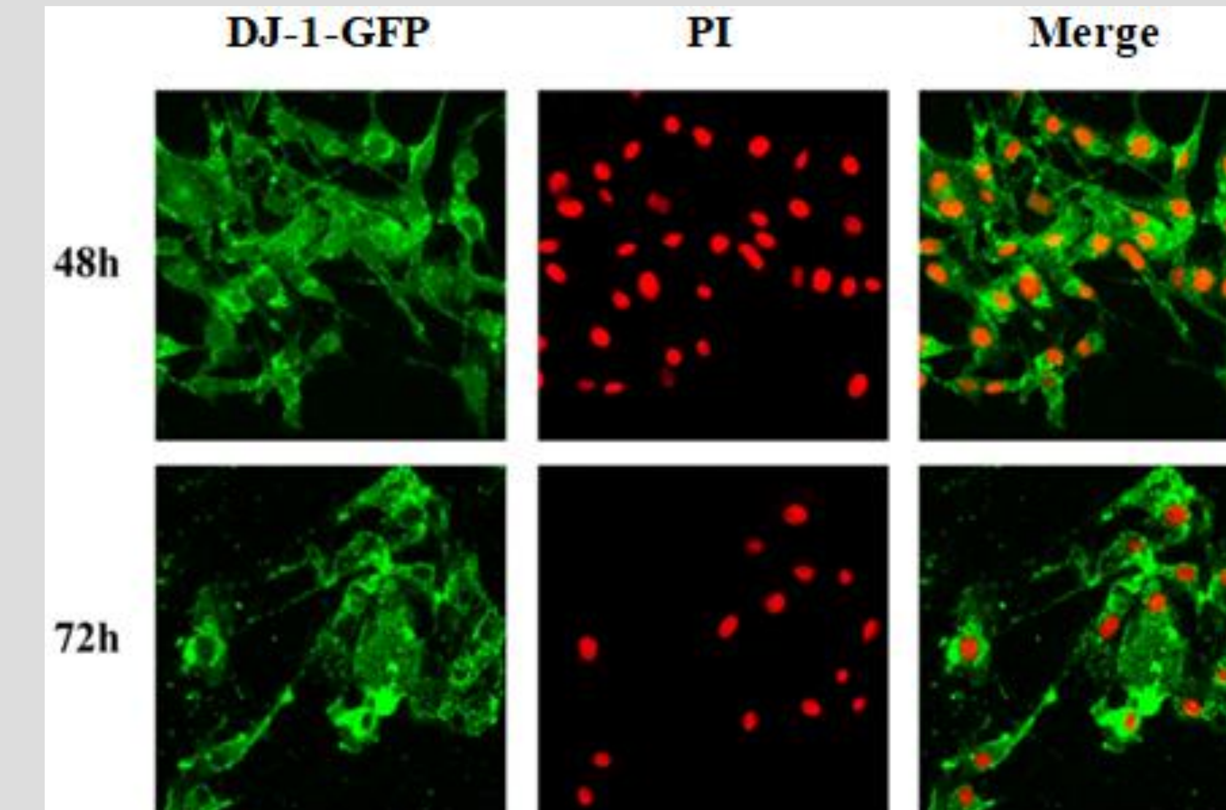

Figure 2. Localization of transfected DJ-1-GFP in U87 cells. Nuclei were stained with PI.

Immunofluorescence: MCF7

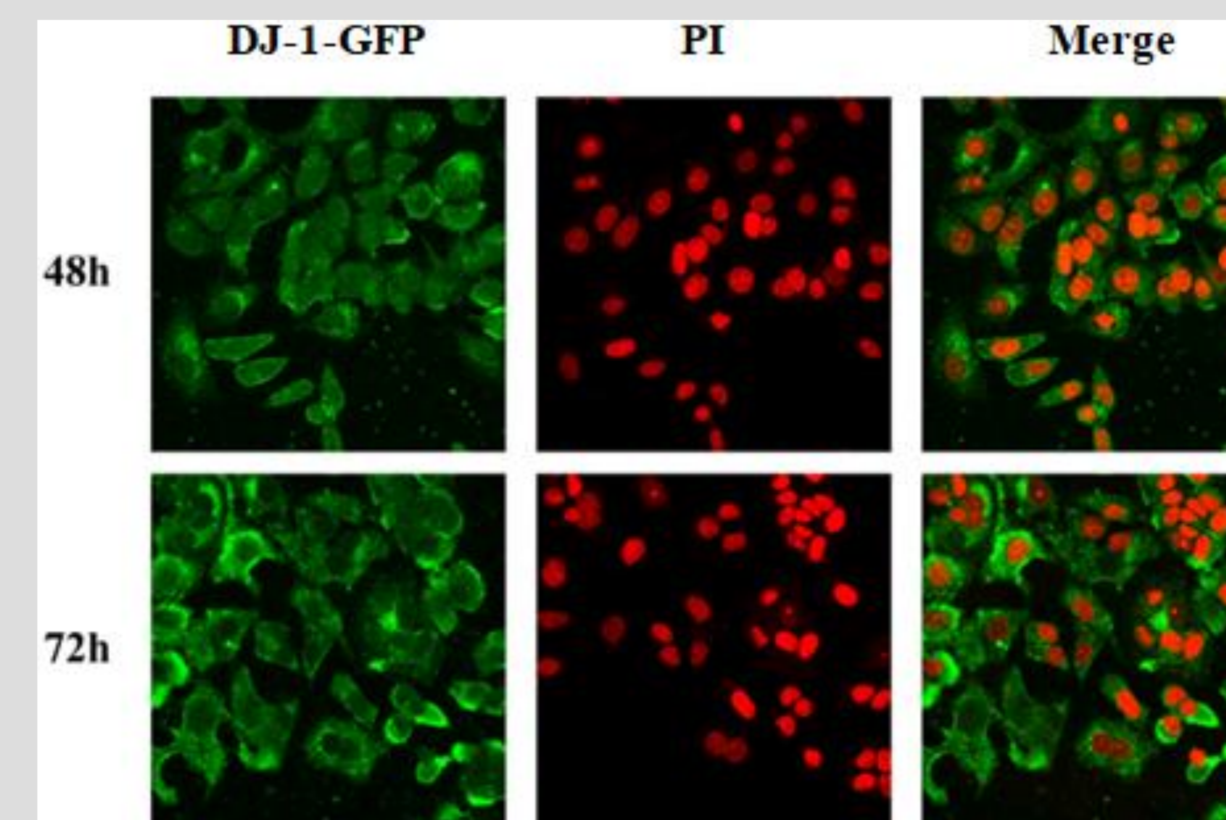

Figure 3. Localization of transfected DJ-1-GFP in MCF-7 cells. Nuclei were stained with PI.

Western blot: E-Cadherin

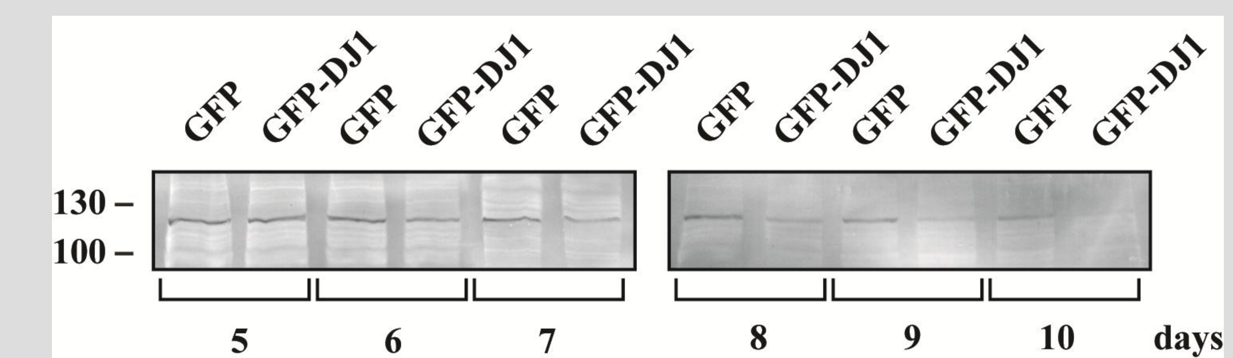

Figure 4. Western blotting analysis, using an anti-E-Cadherin monoclonal antibody, of extracts from MCF-7 cells that were incubated for the indicated time periods with the culture supernatants of GFP- or GFP-DJ1-overexpressed HeLa cells.

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Western blot: β-Catenin

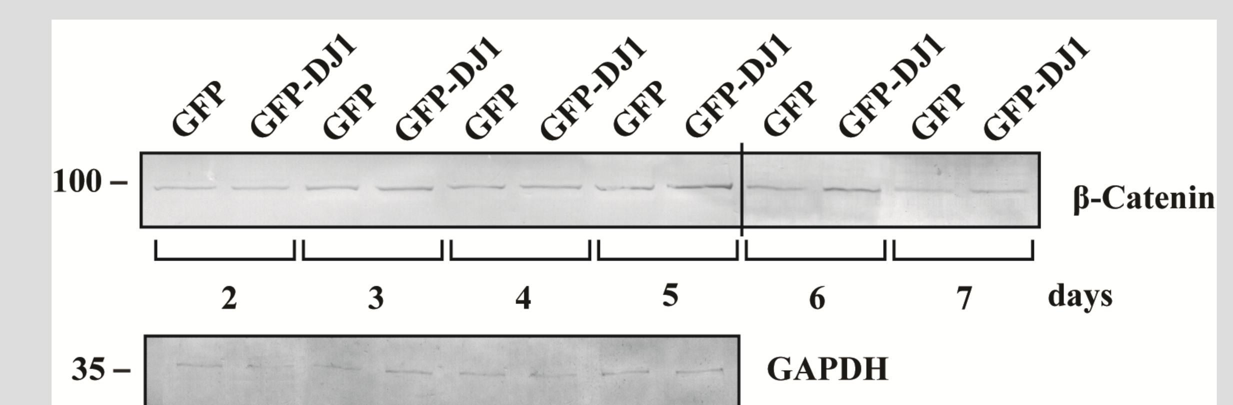

Figure 5. Western blotting analysis, using an anti-β-Catenin monoclonal antibody, of extracts from MCF-7 cells that were incubated for the indicated time periods with the culture supernatants of GFP- or GFP-DJ1-overexpressed HeLa cells.

DISCUSSION

The aim of the present study was the cloning of the DJ-1 cDNA in the plasmid vector pEGFP-N1, in order to study the expression of the GFP-tagged protein in HeLa, U87 and MCF-7 cancer cell lines. We noticed different location and also partial extracellular secretion that depends on the cell type. These data suggest that DJ-1 overexpression and secretion are a frequent event in cancer cells and emphasize its potential prognostic value as a survival marker in patients with different tumor types. Thus, DJ-1 might be-a promising serum marker for cancer diagnosis, monitoring, and prognosis.

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CONTACT

Areti Kantzioura
Email: aretikan@gmail.com