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Antiferroptotic activity of non-oxidative dopamine

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ABSTRACT

Dopamine is a neurotransmitter that has many functions in the nervous and immune systems. Ferroptosis is a non-apoptotic form of regulated cell death that is involved in cancer and neurodegenerative diseases. However, the role of dopamine in ferroptosis remains unidentified. Here, we show that the non-oxidative form of dopamine is a strong inhibitor of ferroptotic cell death. Dopamine dose-dependently blocked ferroptosis in cancer (PANC1 and HEY) and non-cancer (MEF and HEK293) cells following treatment with erastin, a small molecule ferroptosis inducer. Notably, dopamine reduced erastin-induced ferrous iron accumulation, glutathione depletion, and malondialdehyde production. Mechanically, dopamine increased the protein stability of glutathione peroxidase 4, a phospholipid hydroperoxidase that protects cells against membrane lipid peroxidation. Moreover, dopamine suppressed dopamine receptor D4 protein degradation and promoted dopamine receptor D5 gene expression. Thus, our findings uncover a novel function of dopamine in cell death and provide new insight into the regulation of iron metabolism and lipid peroxidation by neurotransmitters.

1. Introduction

Dopamine is an organic chemical of the catecholamine family that mainly acts as a neurotransmitter in the nervous system. Inside the brain, dopamine plays critical roles controlling the brain's reward and pleasure centers and movement and emotional responses [1]. Depletion of dopamine-producing cells or dopamine deficiency can cause Parkinson's disease, a neurodegenerative brain disorder that progresses slowly with ageing [2]. In addition to the central nervous system, dopamine can be produced by either additional dopaminergic neurons (such as those in the adrenal medulla and mesentery, existing in the peripheral nervous system) or immune cells [3]. Circulating dopamine is an immune regulator of the inflammatory response in multiple diseases [4]. Targeting dopaminergic signaling pathways is an emerging approach in sterile inflammation and infection [5,6].

Once released, dopamine can bind to G protein-coupled membrane receptors, namely dopamine receptors (DRDs), to activate kinases and trigger multiple signal transduction possesses. Five types of DRDs are identified and divided into two subgroups: D1-like (DRD1 and DRD5) and D2-like (DRD2, DRD3, and DRD4) [7]. DRDs are not only selectively expressed in normal cells from the nervous or immune system, but also exist in several tumor cells [8,9]. Dysfunction of dopamine metabolism is implicated in both cell survival and cell death pathways depending on its redox status, DRD type, as well as downstream effectors [10,11]. Understanding the context-dependent functions of dopamine in different types of cells is important for the development of therapeutic strategies to prevent or treat Parkinson's disease, cancer, and possibly other aging-associated diseases.

In this study, we provide the first evidence that dopamine is a potent inhibitor of ferroptosis [12], a recently recognized form of regulated cell death involved in neurodegeneration [13] and cancer cell death [14,15]. Notably, we demonstrate that dopamine limits ferroptosis in both normal and malignant cells through modulation of iron accumulation and lipid peroxidation. Moreover, we

Abbreviations: CCK-8, Cell Counting Kit-8; DRD, dopamine receptor; GPX4, glutathione peroxidase 4; GSH, glutathione; HMGB1, high mobility group box 1; LDH, lactate dehydrogenase; MDA, malondialdehyde; NRF2, nuclear factor erythroid 2-related factor; PINK1, phosphatase and tensin homolog induced putative kinase 1; PARK2, parkin RBR E3 ubiquitin protein ligase; Q-PCR, quantitative reverse transcription-polymerase chain reaction; ROS, reactive oxygen species.

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observed that dopamine inhibits DRD4 protein degradation and increases DRD5 gene expression in ferroptosis. Collectively, these findings uncover a novel function of dopamine in cell death and provide new insight into the regulation of iron metabolism and lipid peroxidation by neurotransmitters.

2. Methods

2.1. Regents

The antibody to GPX4 was obtained from Abcam (Cambridge, MA, USA). The antibodies to DRD1, DRD2, DRD3, DRD4, and DRD5 were obtained from Santa Cruz (Hercules, CA, USA). The antibody to GAPDH was obtained from Sigma Aldrich (Milwaukee, WI, USA). Erastin and ferrostatin-1 were purchased from Selleck Chemicals (Houston, TX, USA). Dopamine was purchased from Sigma Aldrich (Milwaukee, WI, USA).

2.2. Cell cultures

The human pancreatic cancer cell line PANC1, human ovarian cancer cell line HEY, mouse embryonic fibroblast cell line (MEFs) and human embryonic kidney cell line (HEK293 cells) were obtained from American Type Culture Collection. These cells were grown in Dulbecco's Modified Eagle's Medium with 10% fetal bovine serum, 2 mM L-glutamine, and 100 U/ml of penicillin and streptomycin.

2.3. Cell viability assay

Cell viability was evaluated using the Cell Counting Kit-8 (CCK-8) (Dojindo Laboratories, Tokyo, Japan) according to the manufacturer's instructions. CCK-8 uses WST-8 [(2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium. monosodium salt)] to evaluate cell viability.

2.4. Quantitative reverse transcription-polymerase chain reaction (Q-PCR) analysis

Total RNA was extracted and purified from cultured cells using the RNeasy Plus Mini Kit (Qiagen, Valencia, CA, USA), according to the manufacturer's instructions. The RNA was quantified by determining absorbance at 260 nm. One μg of total RNA from each sample was reverse transcribed into cDNA using the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA, USA) in a volume of 20 μl cDNA from cell samples was amplified with specific primers (DRD4:5′-TGCTGCCGCTCTTCGTCTACTC -3'and 5′- ACAGGTTGAA-GATGGAGGCGGT -3′; DRD5: 5′- CCGACGTGAATGCAGAGAACTG -3′ and 5′- TAGATGCGCGTGTAGGTCACGA -3′). Quantitative real time PCR was performed using ssoFast EvaGreen Supermix (Bio-Rad, Hercules, CA, USA) on the C1000 Touch Thermocycler CFX96 Real Time System (Bio-Rad, Hercules, CA, USA) according to the manufacturer's protocol. Analysis was performed using the Bio-Rad CFX Manager software (Bio-Rad, Hercules, CA, USA).

2.5. Western blot

Proteins in the cell lysate or supernatants were resolved on 4%–12% Criterion XT Bis-Tris gels (Bio-Rad, Hercules, CA, USA) and transferred to a nitrocellulose membrane (pore size 0.22 μM) as previously described [16–18]. After blocking with 5% milk, the membrane was incubated for two hours at 25 °C or overnight at 4 °C with various primary antibodies. After incubation with peroxidase-conjugated secondary antibodies (1:3000) for one hour at routine temperature, the signals were visualized using chemiluminescence (Pierce, Rockford, IL, USA).

2.6. Iron assay

Intracellular ferrous iron level was determined using an iron assay kit purchased from Sigma Aldrich (Milwaukee, WI, USA) according to the manufacturer's instructions.

2.7. Lipid peroxidation assay

The concentration of malondialdehyde, one of end products of lipid peroxidation, was assessed using a lipid peroxidation assay kit purchased from Sigma Aldrich (Milwaukee, WI, USA) according to the manufacturer's instructions.

2.8. Glutathione assay

The glutathione concentration in cell lysates was assessed using a glutathione assay kit purchased from Sigma Aldrich (Milwaukee, WI, USA) according to the manufacturer's instructions.

2.9. Lactate dehydrogenase and HMGB1 assay

The release of lactate dehydrogenase and HMGB1 were assessed using ELISA kits purchased from Abcam (Cambridge, MA, USA) and Shino-Test Corporation (Japan) respectively according to the manufacturer's instructions.

2.10. Statistical analysis

Data are expressed as means ± SD. Significance of differences between groups was determined using two-tailed Student's *t*-test. A p-value of <0.05 was considered significant.

3. Results

3.1. Dopamine inhibits erastin-induced ferroptosis

Erastin is a classical inducer of ferroptosis by acting on mitochondrial voltage-dependent anion channels or inhibition of cystine-glutamate exchange at the plasma membrane [12]. We first investigated the effects of dopamine on erastin-induced cell death in cancer (PANC1 and HEY) and non-cancer (MEF and HEK293) cell lines. Cell viability assay using the CCK-8 kit showed that dopamine dose-dependently inhibited erastin-induced cell death in these cell lines (Fig. 1A). Light microscopy of cell morphology assay confirmed that dopamine treatment significantly increased the number of PANC1 and HEY cells following erastin treatment (Fig. 1B). Moreover, dopamine inhibited the erastin-induced release of lactate dehydrogenase (LDH, a marker enzyme of cytosol, which is not released upon physiological condition) (Fig. 1C) and high mobility group box 1 (HMGB1, a marker of cell death) (Fig. 1D) in PANC1 and MEF cells. These findings indicate that dopamine increases cell survival and reduces cell death in both cancer and non-cancer cells following erastin treatment.

3.2. Dopamine reduces erastin-induced iron accumulation, lipid peroxidation, and GSH depletion in ferroptosis

Ferroptosis is an iron- and reactive oxygen species (ROS)-dependent form of regulated cell death [19]. In particular, ferrous iron (Fe^{2+}) contributes to ferroptosis through induction of ROS production by Fenton reaction. Finally, lipid peroxidation from ROS-mediated oxidative injury can be a central event in ferroptosis [20]. To determine the molecular mechanisms of the action of

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