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2-Deoxy-D-glucose cooperates with arsenic trioxide to induce apoptosis in leukemia cells: Involvement of IGF-1R-regulated Akt/mTOR, MEK/ERK and LKB-1/AMPK signaling pathways

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ABSTRACT

While the anti-tumor efficacy of 2-deoxy-D-glucose (2-DG) is normally low in monotherapy, it may represent a valuable radio- and chemo-sensitizing agent. We here demonstrate that 2–10 mM 2-DG cooperates with arsenic trioxide (ATO) and other antitumor drugs to induce apoptosis in human myeloid leukemia cell lines. Using ATO and HL60 as drug and cell models, respectively, we observed that 2-DG/ATO combination activates the mitochondrial apoptotic pathway, as indicated by Bid-, and Bax-regulated cytochrome *c* and Omi/HtrA2 release, XIAP down-regulation, and caspase-9/-3 pathway activation. 2-DG neither causes oxidative stress nor increases ATO uptake, but causes inner mitochondria membrane permeabilization as well as moderate ATP depletion, which nevertheless do not satisfactorily explain the pro-apoptotic response. Surprisingly 2-DG causes cell line-specific decrease in LKB-1/AMPK phosphorylation/activation, and also causes Akt/mTOR/p70S6K and MEK/ERK activation, which is prevented by co-treatment with ATO. The use of kinase-specific pharmacologic inhibitors and/or siRNAs reveals that apoptosis is facilitated by AMPK inactivation and restrained by Akt and ERK activation, and that Akt and ERK activation mediates AMPK inhibition. Finally, 2-DG stimulates IGF-1R phosphorylation/activation, and co-treatment with IGF-1R inhibitor prevents 2-DG effects on Akt, ERK and AMPK, and facilitates 2-DG-provoked apoptosis. In summary 2-DG elicits IGF-1R-mediated AMPK inactivation and Akt and ERK activation, which facilitates or restrain apoptosis, respectively. 2-DG-provoked AMPK inactivation increases the apoptotic efficacy of ATO, while in turn ATO-provoked Akt and ERK inactivation may increase the efficacy of 2-DG as anti-tumor drug.

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Abbreviations: AMPK, AMP-activated kinase; Akt, protein kinase B; Akt_iV, Akt inhibitor V, triciribine; AMPK, AMP-activated kinase; AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; ATO, arsenic trioxide; CC, AMPK inhibitor, Compound C; DAPI, 4,6-diamino-2-phenylindole; 2-DG, 2-deoxy-D-glucose; DHE, dihydroethidium; ERK, extracellular signal-regulated kinase; GSH, reduced glutathione; H_2DCFDA, dichlorodihydrofluorescein diacetate; IGF-1, insulin-like growth factor-1; IGF-1R, insulin-like growth factor-1 receptor; LKB-1, liver kinase B1; LY294002, 2-(4-Morpholinyl)-8-phenyl-4H-1-benzopyran-4-one; mAb, monoclonal antibody; mTOR, mammalian target of rapamycin; MAPK, mitogen-activated protein kinase; MEK, mitogen-induced extracellular kinase/extracellular signal-regulated kinase; mIMP, inner mitochondrial membrane permeabilization; mOMP, outer mitochondrial membrane permeabilization; mPTP, mitochondrial transition pore; MTT, 3(4,5-dimethyl-2-thiazolyl)-2,5diphenyl-2H-tetrazolium bromide; PBLs, peripheral blood lymphocytes; PBS, phosphate buffered saline; PI3K, phosphatidylinositol 3-kinase; PI, propidium iodide; R123, rhodamine 123; PQ401, IGF-1R inhibitor II, N-(2-Methoxy-5-chlorophenyl)-*N'*-(2methylquinilin-4-yl)-urea; TNF-α, tumor necrosis factor-α; U0126, 1,4-Diamino-2,3-dicyano-1,4-*bis*(2-aminophenylthio)butadiene; z-VAD-fmk, Z-Val-Ala-Asp(OMe)-CH_2F.

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1. Introduction

Tumors are often characterized by the increased utilization of glucose as carbon source for anabolic reactions, and the preferential use of glycolysis instead of oxidative phosphorylation as source of energy. This altered metabolism confers multiple advantages for tumor growth [1], and hence provides important targets for anticancer treatments. In particular, the assumption that cancer cells are inherently glycolytic – i.e., that mainly rely on glycolysis even under high oxygen tension conditions (''aerobic glycolysis'') – led to the development of putative anti-glycolytic drugs, the best known of which is the glucose analogue 2-deoxyglucose (2-DG). 2-DG is transported through the plasma membrane of cancer cells with higher efficacy than in normal healthy cells, and phosphorylated by mitochondria-bound hexokinase II (HKII) to give 2-DG-6-P. In contrast to G-6-P, 2-DG-6-P is relatively stable and accumulates inside the cells inhibiting hexokinases and blocking the glycolytic pathway [2]. Nevertheless, this archetypal panorama requires two considerations. (i) On the one hand, aerobic glycolysis is not a universal characteristic of tumor cells, many of which mainly rely on oxidative phosphorylation as energy source, at least under normal (aerobic) culture conditions [3]. (ii) In addition, 2-DG may produce other effects which affect cell viability. This includes the following: generation of oxidative stress [4,5]; inhibition of protein glycosylation and subsequent generation of endoplasmic reticulum (ER) stress [6–8]; solubilization of mitochondria-bound HKs [9], which affects the integrity of the outer mitochondrial membrane and allows the release of apoptogenic factors [10]; and activation of growth factor receptors and/or protein kinases critical for cell survival [11].

While the anti-tumor efficacy of 2-DG is normally low when used as single agent, it may represent a useful radio- and chemo-sensitizing drug. Thus, 2-DG overcame resistance or potentiated cyto-reduction by some conventional antitumor treatments in cancer cells in culture and animal models [12–14], without damage or even with protective effect for normal healthy cells [15]. The efficacy of 2-DG as radio-sensitizing agent was also corroborated in phase I and II clinical trials [16]. However, the results may depend on the used drug, cell model and experimental conditions, and hence 2-DG was reported to potentiate, inhibit or not affect anti-tumor drug toxicities [12–14,17,18].

Arsenic trioxide (ATO, Trisenox) is a clinically established drug for the treatment of acute promylocytic leukemia (APL) [19], and also potentially useful against other hematological malignancies [20]. Nonetheless its efficacy is frequently limited by the requirement of high doses to effectively induce apoptosis, pointing to the necessity of introducing sensitizing strategies. An earlier report indicated that 2-DG did not affect ATO toxicity in several tumor cell models [12]. Nevertheless we recently showed that lonidamine, a glycolytic inhibitor [21] improved the apoptotic efficacy of ATO in leukemia cells [22]. With this precedents in mind, in the present report we examine the capacity of 2-DG to cooperate with ATO and other antitumor drugs to induce apoptosis in HL60 and other human myeloid leukemia cell lines, as well as the behavior of factors such as ATP levels, oxidative stress, mitochondrial dysfunction, and protein kinase signaling pathways, critical for apoptosis regulation and execution. The results indicate that ATO and 2-DG efficaciously cooperate to induce apoptosis by mechanisms involving attenuation by ATO of 2-DG-provoked IGF-1R, MEK/ERK and Akt/mTOR activation, as well as occasional inactivation by 2-DG of the LKB-1/AMPK pathway.

2. Materials and methods

2.1. Reagents and antibodies

All components for cell culture were obtained from Invitrogen, Inc. (Carlsbad, CA, USA). 4,6-diamino-2-phenylindole (DAPI) was obtained from Serva (Heidelberg, Germany). Dichlorodihydrofluorescein diacetate (H_2DCFDA) and monochlorobimane were obtained from Molecular Probes, Inc. (Eugene, OR, USA). Dihydroethidium (DHE, supplied as a 5 mM solution in dimethyl sulfoxide) was obtained from Invitrogen, Inc. The kinase inhibitors Compound C (AMPK inhibitor, CC), 1,4-Diamino-2,3-dicyano-1,4-*bis*(2-aminophenylthio)butadiene (U0126), 2-(4-Morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002), triciribine (Akt inhibitor V, Akt$_i$V), N-(2-Methoxy-5-chlorophenyl)-*N′*-(2methylquinilin-4-yl)-urea (IGF-1R inhibitor, PQ401), and the caspase inhibitor Z-Val-Ala-Asp(OMe)-CH_2F (z-VAD-fmk), were obtained from Calbiochem (Darmstad, Germany). Rabbit anti-human AMPKα, p44/42 MAPK, phospho-p44/p42 MAPK (Thr^{202}/Tyr^{204}), Akt, phospho-Akt (Ser^{473}), phospho-mTOR (Ser2448), phospho-S6 ribosomal protein (Ser$^{235/236}$) (rpS6), HtrA2, and caspase-3 polyclonal antibodies (pAbs), rabbit anti-human phospho-AMPKα (Thr172), phospho-LKB1 (Ser428) (C6743), and mTOR (7C10) monoclonal antibodies (mAbs), and mouse anti-human phospho-p70 S6 kinase (Thr^{389}) (1A5) (p70S6K) mAb, were obtained from Cell Signaling Technology Inc (Danvers, MA, USA). Mouse anti-pigeon cytochrome *c* mAb clone 7H8.2C12 was obtained from BD PharMingen (San Diego, CA, USA). Rabbit anti-human phospho-IGF-1R (Tyr1165/1166), Bax (N-20), and caspase-9 p35 (H-170) pAbs; and goat anti-human Bid (C-20) pAb, were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Mouse anti-XIAP (clone 2F1) mAb was obtained from MBL International Corporation (Woburn, MA, USA). Peroxidase-conjugated immunoglobulin G antibodies were obtained from DAKO Diagnostics, S.A. (Barcelona, Spain). Small interfering RNA (siRNA) against AMPK (AMPK1/2 siRNA (h)) and control scramble siRNA were obtained from Santa Cruz Biotechnology, Inc. All other non-mentioned reagents and antibodies were from Sigma (Madrid, Spain).

2.2. Cells and treatments

The human cell lines HL60 and U937 (acute myeloid leukemia, AML), NB4 (acute promyelocytic leukemia, APL), and THP-1 (promonocytic leukemia) were grown in standard RPMI 1640 medium (containing 2.05 mM L-glutamine and 11.11 mM L-glucose) supplemented with 10% (v/v) heat-inactivated calf serum, 0.2% sodium bicarbonate and antibiotics in a humidified 5% CO_2 atmosphere at 37 °C. Cells were routinely maintained under logarithmic growth by passing them every 2–3 days. Under these conditions, HL60, U937, and NB4 cells exhibited an approximate doubling time of 18 h, and THP-1 of 24–36 h. Except when necessary, to avoid manipulations which could per se affect basal kinase activation, 24 h before treatments the cells were adjusted at 10^5 (for 16–24 h treatments) or 2×10^5 (for 0.5–8 h treatments) - cells/ml using a mixture of conditioned and fresh medium, and then remained undisturbed until the time of drug administration. To check the possible influence of cell culture conditions, in some experiments the culture medium was re-supplemented with 2 mM glutamine and 1 mM pyruvate, or the serum concentration was decreased (ranging from 0 to 5%). For glucose deprivation, the cells were extensively washed with phosphate-buffered saline (PBS) and then seeded at the appropriate concentration in glucose-lacking RPMI medium supplemented with 10% (v/v) serum. For experiments with IGF-1, 16 h before treatments the cells were washed and seeded in standard RPMI medium supplemented with 1% (v/v) serum. Human peripheral blood lymphocytes (PBLs)

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