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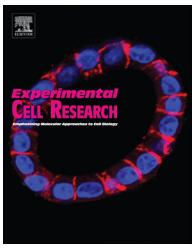

Research Article

# Dichloroacetate attenuates hypoxia-induced resistance to 5-fluorouracil in gastric cancer through the regulation of glucose metabolism

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ABSTRACT

In this study, we investigated whether gastric cancer with hypoxia-induced resistance to 5-fluorouracil (5-FU) could be re-sensitized following treatment with low-dose dichloroacetate (DCA), an inhibitor of the glycolytic pathway. The expression profiles of hypoxia-inducible factor-1α (HIF-1α) and pyruvate dehydrogenase kinase-1 (PDK-1) were analyzed in tissues from 10 patients with gastric cancer who had different responses to adjuvant 5-FU treatment. For the *in vitro* assays, cell viability and apoptosis were evaluated with and without treatment with 20 mM DCA in the AGS and MKN45 cell lines, as well as in *PDK1* knockdown cell lines. The expression levels of HIF-1α and PDK-1 were both elevated in the tumor tissues relative to the normal gastric tissues of most patients who showed recurrence after adjuvant 5-FU treatment. Cellular viability tests showed that these cell lines had a lower sensitivity to 5-FU under hypoxic conditions compared to normoxic conditions. Moreover, the addition of 20 mM DCA only increased the sensitivity of these cells to 5-FU under hypoxic conditions, and the resistance to 5-FU under hypoxia was also attenuated in *PDK1* knockdown cell lines. In conclusion, DCA treatment was able to re-sensitize gastric cancer cells with hypoxia-induced resistance to 5-FU through the alteration of glucose metabolism.



## Introduction

Although the incidence of gastric cancer has declined in recent decades, it remains one of the most common malignant tumors worldwide and is the second leading cause of cancer deaths after lung cancer [1]. Radical resection of the primary tumor and local lymph nodes can increase the cure rate for gastric cancer [2]. However, patients with metastatic or recurrent gastric cancer

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demonstrate a poor prognosis despite systemic treatment with various chemotherapeutic agents [3,4]. 5-Fluorouracil (5-FU) is an important agent used for the systemic treatment of gastric cancer. In patients with advanced gastric cancer, as well as those who have undergone curative resection, single or combined regimens based on 5-FU have shown survival benefits [5–7]. Nevertheless, a number of patients are resistant to treatment with 5-FU, and this is a main obstacle that must be overcome in order to improve the survival rate of patients with gastric cancer. Blocking the incorporation of 5-FU into nucleic acids may play an important role in the development of resistance to 5-FU [8]. In addition, the abnormal expression of target enzymes, such as thymidylate synthase and dihydropyrimidine dehydrogenase, can also contribute to 5-FU resistance. However, the clinical options available for overcoming 5-FU resistance are limited [9].

A hypoxic microenvironment is frequently induced in the center of solid tumors, and cancers with hypoxia-induced phenotypes are often resistant to chemotherapy and radiotherapy and have a poor prognosis [10]. In particular, the cancer cells with lower levels of oxygenation are more resistant to several chemotherapeutic agents, including 5-FU [11]. Hypoxia inducible factor-1 (HIF-1) may have a crucial role in the development of hypoxia-induced resistance to chemotherapeutic agents, as this factor governs the hypoxic response in cancer cells. HIF-1 is composed of dimers of the alpha and beta subunits and is a well-known regulator of expression of a number of genes related to hypoxia-induced angiogenesis, migration, proliferation and altered glycolysis. Of these phenotypes, the activation of the aerobic glycolytic pathway is a unique feature that enables malignant tumor cells to survive under hypoxic conditions. Even under normoxic conditions, cancer cells with HIF-1-induced phenotypes show an increased rate cytoplasm-based glycolysis, rather than mitochondrial-based oxidative phosphorylation, and use glycolysis as their energy source to generate large amounts of ATP. In addition, this phenotype is significantly correlated with resistance to apoptosis [12–14]. Therefore, the aerobic glycolytic pathway represents a potential target for the reversal of hypoxia-induced resistance to chemotherapy.

In most malignant cells, pyruvate dehydrogenase kinase (PDK), which is up regulated by HIF-1, is one of the crucial enzymes responsible for enhancing glycolysis, rather than mitochondrial oxidative phosphorylation. This is because PDK can inhibit pyruvate dehydrogenase (PHD), which is a key regulator of cellular glucose utilization and mitochondrial oxidation. In our previous study, we reported that the overexpression of PDK-1, a representative isoenzyme of PDKs, in gastric cancers was correlated with the expression of HIF-1α. Additionally, in patients who underwent curative resection for gastric cancers, PDK-1 served as an independent biomarker that was predictive of survival after adjuvant treatment using 5-FU [15]. Therefore, the functional inhibition of PDK-1 may constitute a good strategy for reversing hypoxia-induced resistance to 5-FU in gastric cancers. Dichloroacetate (DCA) is a well-known inhibitor of PDK that has been used clinically for more than 30 years for the treatment for lactic acidosis due to genetic abnormalities. Although several studies have suggested the possibility of using DCA as a therapeutic agent for treating malignant tumors [12,16,17], its clinical application has been limited due to a lack of evidence.

Based on these previous results, we hypothesized that DCA may present synergic effects with 5-FU in gastric cancers demonstrating hypoxia-induced resistance. In the present study, we sought to identify differences in the expression pattern of proteins involved in the hypoxia-PDK-PDH axis in human tissues from gastric cancer patients according to their responsiveness to adjuvant 5-FU. In addition, we investigated the *in vitro* cytotoxicity of combining DCA treatment with 5-FU in gastric cancer cell lines and explored the underlying mechanisms related to changes in glycolytic metabolism induced by DCA.

## Materials and methods

### Human tissue collection and immunohistochemical staining

Gastric cancer and adjacent normal gastric tissues were obtained from 12 patients who were diagnosed with gastric cancer and underwent curative resection at the Department of Surgery, Ajou University Hospital, Suwon, Korea between January 2004 and December 2006. The protocol was approved by the Institutional Review Board of Ajou University Hospital (AJOU-MED-KSP-12-323), and we obtained informed consent from the patients for tissue collection. Immediately after surgical resection, a portion of the tissue was frozen in liquid nitrogen and saved for Western blotting analysis, while the rest of the tissue was fixed in formalin and paraffin-embedded for histologic diagnosis and staining for immunohistochemical examination.

The patients were treated with 5-FU throughout the year following surgical resection and were followed up over 5 years. Six patients presented with recurrence within 3 years of the operation, while the other 6 patients survived for more than 3 years without recurrence.

Immunohistochemical staining was performed on the 10% buffered neutral formalin-fixed, paraffin-embedded tissue microarray slides using a rabbit polyclonal antibody specific for HIF-α and PDK. Briefly, after deparaffinization, the sections were rehydrated, washed and subjected to microwave antigen retrieval in 10 mM citrate buffer, pH 6.0. The sections were then immersed in 3% $H_2O_2$ for 15 min to block endogenous peroxidase activity and incubated for 30 min at room temperature with the primary antibodies for HIF-1α (1:100 dilution, Novus Biologicals, Littleton, CO) and PDK-1 (1:100 dilution, Santa Cruz Biotechnology, Santa Cruz, CA). The expression of these proteins was detected using the Ultravison LP Detection kit and 3,3′ diaminobenzidine (Thermo Scientific, Cheshire, WA), and the section was counterstained with Harris hematoxylin. The proportion of tumor cells showing positive staining was semiquantitatively evaluated as negative (less than 5%), 1+ (5–0%), 2+ (30–60%) and 3+ (more than 60%) by a pathologist who did not have any knowledge of the patients′ clinical outcome.

For Western blotting, the frozen tissues were homogenized using a Biovoltexer Mixer (Chemglass Life Science, Vineland, NJ). The total protein extracted from tissues was estimated using the Bradford method according to the manufacturer′s protocol (Bio-Rad, Hercules, CA). Western blotting was carried out as described below for the gastric cancer cell lines.

### Reagent and cells

5-FU and sodium DCA were purchased from Sigma Chemical (St. Louis, MO). The human gastric cancer cell lines AGS and