Dicumarol Inhibition of NADPH:Quinone Oxidoreductase Induces Growth Inhibition of Pancreatic Cancer via a Superoxide-mediated Mechanism

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INTRODUCTION

Pancreatic cancer is one of the most aggressive malignancies, the fourth leading cause of cancer death in the United States, and increasing in incidence (1). Surgical resection of the primary tumor remains the only potentially curative treatment for pancreatic cancer; however, in population-based studies the number of patients undergoing resection with curative intent can be <3% (2). Even after resection, median survival is only 12–18 months, and <20% of resected patients survive 5 years and the majority die of metastatic cancer recurrence (3). Other adjuvant treatments such as radiation therapy and chemotherapy have not improved long-term survival after resection. The rate of chemotherapeutic response is <20% (4), whereas <10% of patients benefit from radiation therapy (5, 6). Because of the lack of poor therapeutic responsiveness of pancreatic cancer to surgery, chemotherapy, and radiation therapy, survival beyond 5 years is rare with the median survival <6 months (3). Thus, effective therapies for pancreatic cancer are needed to control progression and metastatic disease.

NQO1,3 (DT-diaphorase; EC 1.6.99.2), a homodimeric, ubiquitous, cytosolic, and membrane flavoprotein, is considered to be a deactivation enzyme, because it catalyzes the two-electron reduction of quinones, including membrane ubiquinone (7). This reaction prevents the one-electron reduction of quinones by cytochrome P450 reductase and other flavoproteins that would result in oxidative cycling with generation of superoxide (O2−). NQO1 gene regulation may be up-regulated in some tumors to accommodate the needs of rapidly metabolizing cells to regenerate NAD+ (8). We hypothesized that pancreatic cancer cells would exhibit high levels of this enzyme, and inhibiting it would suppress the malignant phenotype. Reverse transcription-PCR, Western blots, and activity assays demonstrated that there is a 10-fold up-regulation of NQO1 in pancreatic cancer when compared with normal pancreas. The NQO1 properties of catalyzing bioactivation of antitumor quinones (14–16) and is expressed at high levels in many solid tumors including pancreatic cancer (17–19). A recent study by Logsdon et al. (19) using microarrays demonstrated that there is a 10-fold up-regulation of NQO1 in pancreatic cancer compared with normal pancreas. The NQO1 properties of catalyzing bioactivation of antitumor quinones and high expression in pancreatic cancer make it a principal target in therapeutic strategies to design chemotherapeutic agents.

Like other flavoenzymes, NQO1 is inhibited by DPI and the quinone analogue capsaicin (20). It differs from other quinone reductases in the cell in that it uses both NADH and NADPH as cofactors, and is selectively inhibited by low concentrations of dicumarol (21). NQO1 gene regulation is greatly up-regulated in various solid tumors compared with normal tissues of the same origin, perhaps to accommodate the needs of rapidly metabolizing cells to regenerate NAD+ (22).

Because pancreatic cancer is one of the most aggressive malignancies with rapid turnover and short doubling times, we hypothesized that pancreatic cancer cells would exhibit high levels of NQO1, and inhibiting it would suppress the malignant phenotype. Our study demonstrates that NQO1 is up-regulated in pancreatic cancer cell lines. Inhibition of NQO1 with dicumarol increased intracellular O2− production and inhibited the in vitro malignant phenotype of pancreatic cancer. The effect of inhibiting cell growth by dicumarol may be because of increased intracellular O2− production, because enforced expression of MnSOD decreased hydroethidine staining and reversed the inhibition of cell growth.

MATERIALS AND METHODS

Human Pancreas

Human pancreatic specimens were retrieved from neurologically devastated, heart-beating patients that were transplant donors where the pancreas was considered unsuitable for transplantation or no recipient was available. Pancreatic specimens were discarded and not used in this study if the donor had any history of pancreatic disease. All of the specimens were retrieved at the University of Iowa Hospitals and Clinics. The protocol to use the human pancreatic specimens was approved by the University of Iowa Institutional Review Board for Human Subjects on February 12, 2001.
In Vitro Growth Characteristics

Cell Growth. Cells (1 × 10^4) were plated in triplicate in 1.5 ml complete medium in 24-well plates. Cells were trypsinized and then counted on alternate days for 2 weeks using a hemocytometer. Cell population doubling time in hours (DT) was determined in triplicate using the following equation:

\[ DT(b) = 0.693(t - t_i)/\ln(N_f/N_i) \]

where \( t_f \) = time at which exponential growth began, \( t_f = \) time in hours, \( N_f = \) cell number at time \( t_f \), and \( N_i = \) initial cell number (26). To determine the role of NQO1 inhibition, dicumarol (50–250 \( \mu \)M) was added to MIA PaCa-2 cell cultures. A 50 \( \mu \)M concentration of dicumarol was dissolved in water by drop-wise addition of 0.1 \( \mu \)l NaOH. Addition of up to 2.5 \( \mu \)l of this solution per ml (250 \( \mu \)M highest final concentration) did not change the pH of complete medium.

Plating Efficiency. Control and dicumarol-treated cells (2 × 10^3) were plated in triplicate into 60-mm dishes in complete medium. The dishes were maintained in the incubator for 6 days to allow colony formation. The colonies were then fixed and stained with 0.1% crystal violet and 2.1% citric acid, and those colonies containing >50 cells were scored.

Anchorage-independent Growth in Soft Agar. Cells (5 × 10^3) were suspended in 3 ml of complete medium containing dicumarol (0–250 \( \mu \)M) in a solution of 6% agar in double-distilled H_2O so that the final concentration of the agar was 0.3%. This suspension was then plated over 3 ml of complete medium made using a 6% agar solution in double-distilled H_2O so that the final concentration of the bottom agar was 0.5%. After 16 days, colonies of >0.1 mm in diameter were scored. The clonogenic fraction was determined using the following equation:

\[ \text{Soft Agar Plating Efficiency (PE) = (colonies formed/cells seeded) × 100} \]

Determination of Reactive Oxygen Species

To dissect potential sources of \( O_2^- \) production the following inhibitors of major oxidases were added: the NQO1 inhibitor dicumarol (50–250 \( \mu \)M), the flavoprotein inhibitor DPI (100 \( \mu \)M), and an AdMnSOD. \( O_2^- \) production was determined using three different assays.

Lucigenin Assay. Production of \( O_2^- \) was measured by lucigenin-enhanced chemiluminescence. Cells were plated in a microplate well containing PBS and placed in a microplate lumimeter. Lucigenin (5 \( \mu \)M to avoid superoxide production) was added by injector, mixed by orbital shaker, and luminescence measurements obtained every 30 s for 10 min at 37°C. The lumimeter reports relative light units emitted, which are proportional to superoxide levels. Dark current readings (photomultiplier background signal) were subtracted. The maximum rate of superoxide generation and the integrated relative light units for 10 min was determined and normalized to mg of protein. An advantage of using the microplate system for measurement of superoxide levels in cells is that the measurement can be made in attached cells (which may have differences from cells in suspension), and there is no need for digestion from the culture plate to obtain a cell suspension (which may alter cell function).

Hydroethidine. Intracellular generation of \( O_2^- \) was assessed using hydroethidine fluorescence. \( O_2^- \) reacts with hydroethidine to produce ethidium bromide, which binds to nuclear DNA and fluoresces red. Hydroethidine is one of the best reagents now available for measuring intracellular \( O_2^- \). Cells were incubated for 30 min with hydroethidine (5 \( \mu \)M) and after rinsing to remove extracellular dye, fluorescence was detected in the cells with a Bio-Rad MRC-1024 laser scanning confocal microscope equipped with a krypton-argon laser. Excitation and emission wavelengths were 488 and 610 nm. Fluorescence was detected with a 585-nm long pass filter. Control and treatment groups were always imaged in parallel to ensure that the processing techniques and laser settings were identical. All of the images were collected using a 512 × 512 pixel format and archived for subsequent analysis. The fluorescence of hydroethidine was quantitated using flow cytometry. Cells were grown to subconfluence in 60-mm dishes, and initially treated with or without dicumarol (50–250 \( \mu \)M) for 4 h, washed, and incubated with hydroethidine (10 \( \mu \)M) for 40 min. The cells were then removed by trypsinization, which was neutralized with PBS containing 10% FCS and then analyzed by flow cytometry (Becton Dickinson FACSscan). To determine the specificity of \( O_2^- \) changes with...
dicumarol treatment, subsequent experiments were performed with cells receiving no treatment (controls), dicumarol 100 μM, or dicumarol 100 μM + pretreatment with an adeno-viral construct containing the cDNA for MnSOD.

Cytochrome c. Unfortunately, in live cells, the cytochrome c assay can only measure extracellular O$_2^-$ and that is why the above assays are useful, even if they may not be as specific. The cytochrome c assay relies on the use of the addition of SOD to make it specific for O$_2^-$.

Adenovirus Gene Transfer. To determine the specificity of the above assays in measuring O$_2^-$ generation and to delineate the mechanisms of NQO1 inhibition on pancreatic tumor cell growth, additional experiments were performed on MIA PaCa-2 cells by increasing expression of MnSOD using an adeno-viral vector containing the cDNA for MnSOD. The adenovirus construct used was a replication-defective, E1- and partial E3-deleted recombinant adenoviral vector containing the cDNA for MnSOD. The adenovirus construct was obtained from the University of Iowa Gene Transfer Vector Core. Approximately 10⁶ MIA PaCa-2 were plated in 10 ml of complete medium in a 90-cm² plastic dish and allowed to attach for 24 h. Cells were then washed three times in serum- and antibiotic-free medium. The AdMnSOD, suspended in 3% sucrose, was then applied to cells suspended in 4 ml of serum- and antibiotic-free medium at 100 MOI. Cells were incubated with the adenovirus constructs for 24 h. Medium was then replaced with 4 ml of complete medium for an additional 24 h before cells were harvested. Three days later, intracellular generation of O$_2^-$ was assessed using hydroethidine fluorescence and cell growth curves determined in MIA PaCa-2 cells (controls), MIA PaCa-2 cells treated with dicumarol (100 μM) for 4 h, and MIA PaCa-2 cells infected with AdMnSOD and then treated with dicumarol (100 μM) for 4 h. Also, cells were harvested to determine changes in MnSOD protein and activity after infections with and without AdMnSOD. Immunoreactive protein corresponding to MnSOD was identified and quantitated from total cell protein by the specific reaction of the immobilized protein with its antibody. Total protein was electrophoresed in a 12.5% SDS-polyacrylamide running gel and a 5% stacking gel. The proteins were then electrotransferred to nitrocellulose sheets. After blocking in 20% fetal bovine serum for 1 h, the sheets were washed and then treated with antisera to MnSOD (1:1000) for 1 h. Polyclonal rabbit-antihuman antibodies to MnSOD has been prepared and characterized in our laboratory (27). This antibody has been shown to react with the appropriate protein in a variety of species, including hamster (28) and human (23, 26). The blot was incubated with horseradish peroxidase-conjugated goat-antirabbit

Fig. 1. A, NQO1 is expressed to high levels in pancreatic cancer cell lines but not in normal human pancreas. RNA was harvested and semiquantitative RT-PCR performed for NQO1. Primers used were sense: 5’-CAGGCGCCCCGGACTGCAAGAGCC and antisense: 3’-GGGAAGCCT-GGAAAGA-TACCCAGA. Reaction products were resolved and visualized for RNA input. B, NQO1 protein is expressed at elevated levels in pancreatic carcinoma cells compared with normal pancreas. Total protein extracts were prepared and protein concentrations determined. Protein (20 μg/lane) was separated by 12% SDS-PAGE and electrotransferred to nitrocellulose membranes. After transfer, membranes were blocked and then incubated overnight with anti-NQO1 monoclonal antibody. The area of the bands relative to the human pancreas cells as measured by densitometric analysis is shown at the bottom of the lanes; these numbers indicate the relative amount of immunoreactive protein. C, NQO1 enzymatic activities are increased in pancreatic cancer cell lines compared with normal pancreas. Activity was assayed spectrophotometrically by measuring cytochrome c reduction at 550 nm in the presence of NADH. Bars, ±SE; n = 3. D, correlation plots of NQO1 immunoreactive protein densitometry versus NQO1 enzymatic activity. Enzymatic activity and the Western blot were determined from the same sample. Immunoreactivity for NQO1 protein correlated well (r² = 0.96; P < 0.01) with the NQO1 activity assay.

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RESULTS

RT-PCR for Detection of NQO1 mRNA. RT-PCR demonstrated that NQO1 is expressed in four human pancreatic cancer cell lines. Fig. 1A shows that the NQO1 PCR bands are much more intense in the pancreatic cancer cell lines than in normal human pancreas as reported in other solid tumors when compared with normal cells of the same origin (25).

Western Analysis for NQO1. The levels of NQO1 immunoreactive protein expressed in human pancreas and pancreatic cancer cell lines were assessed by Western blot analysis. Fig. 1B demonstrates immunoreactive protein for NQO1. Normal human pancreas had minimal amounts of immunoreactive protein when compared with the four cell lines. In normal pancreas and the AsPC-1 cell line, two distinct bands could be seen in the Western analysis. Previous studies have suggested that the lower molecular weight band (M_r 500–1000 smaller than wild-type NQO1) observed may be a result of proteolytic processing or chemical degradation and was included in the densitometric analysis (29). It should be noted that the increases in NQO1 seem to be greater than the increases in NQO1 protein shown in Fig. 1B. This could be because the RT-PCR reaction was only semiquantitative and was saturated in the carcinoma samples after 30 cycles. The significance of the different migrations in the Western blots is unknown, but suggests that the proteins in the cancer cell lines may be different because of mutations or some other protein modification.

NQO1 Enzyme Activity. Fig. 1C demonstrates NQO1 enzyme activity, which was significantly higher in the pancreatic cancer cell lines compared with normal pancreas. Normal pancreas had NQO1 enzyme activity of 1.7 ± 0.3 nmol/min/mg compared with NQO1 enzyme activity in the pancreatic cancer cell lines, which ranged from 60 ± 4 nmol/min/mg in the MIA PaCa-2 cell line up to 133 ± 12 nmol/min/mg in the Capan-1 cell line. To determine the clonogenic capacity of NQO1-treated cells, we performed a plating efficiency assay. Mean plating efficiency of dicumarol (0–250 μM) significantly decreased the plating efficiency to 10.0 ± 0.6%, and to nondetectable levels, respectively (P < 0.01 versus control). Growth in Soft Agar. To examine anchorage-dependent growth, we performed a soft agar assay. Whereas malignant MIA PaCa-2 cells form colonies in soft agar, normal cells do so in far smaller numbers. As seen with plating efficiency, dicumarol significantly reduced colony formation (Fig. 2C) with the higher doses of dicumarol. Soft agar plating efficiency was 1.1 ± 0.1% in the parental cells and significantly reduced to 0.9 ± 0.1% in cells treated with dicumarol 100 μM.
Maximal reduction in colony formation was observed at a dicumarol dose of 250 μM, where the colony formation decreased to 0.3 ± 0.03% (Means ± SE; \( P < 0.05 \) vs. 50, and 100 μM dicumarol-treated cells versus 0 μM dicumarol).

**Superoxide Production in Dicumarol-treated Cells.** To investigate whether inhibition of NQO1 with dicumarol would alter generation of \( \text{O}_2^- \), MIA PaCa-2 cells were treated with an inhibitor of flavoenzymes, DPI, and a selective inhibitor of NQO1, dicumarol (0–250 μM), for 4 h. Cells were washed, and lucigenin-enhanced chemiluminescence was performed for detection of \( \text{O}_2^- \) as measured by lucigenin-enhanced chemiluminescence. Additionally, dicumarol (100 and 250 μM) had no effect on \( \text{O}_2^- \) levels measured by the cytochrome c assay (Fig. 3B). DPI (100 μM) caused a slight decrease in \( \text{O}_2^- \) levels as measured by cytochrome c (MIA PaCa-2: 3.43 ± 0.08 nmol/10^6 cells/h versus DPI 100 μM: 2.81 ± 0.15 nmol/10^6 cells/h; \( P < 0.05 \) versus control; \( n = 3 \)). Thus, DPI, which is a nonspecific inhibitor of NADPH oxidase, may have affected membrane oxidases that the cytochrome c could detect, whereas dicumarol did not.

Next, cells were treated with DPI (100 μM) and with dicumarol (50–250 μM) for 4 h, washed and incubated with hydroethidine, and examined with flow cytometry (Fig. 3C) and confocal laser scanning microscopy (Fig. 3D). Treatment with DPI resulted in a 2.2-fold increase in hydroethidine fluorescence as measured by flow cytometry. Treatment with dicumarol (50 and 100 μM) resulted in a 2.2- and 2.5-fold increase in hydroethidine fluorescence as measured by flow cytometry (\( P < 0.05 \) versus dicumarol μM; \( n = 3 \)). Dicumarol (250 μM) had little effect on hydroethidine fluorescence when compared with no treatment. These differences may be explained in that higher doses of dicumarol are extremely toxic to the MIA PaCa-2 cell line leading to overwhelming cell death as evidence by the effects of this dose of dicumarol in the growth curves, soft agar, and plating efficiencies.

To determine whether the signal measured by hydroethidine fluorescence resulted from \( \text{O}_2^- \), we used an AdMnSOD. MIA PaCa-2 cells were incubated with the adenovirus constructs at 100 MOI for 24 h. Three days later, cells (infected and noninfected) were treated with dicumarol for 4 h, incubated with hydroethidine, and examined by flow cytometry and confocal laser scanning microscopy. An increase in MnSOD immunoreactivity was observed in cells infected with the AdMnSOD construct and then treated with dicumarol (Fig. 4A). MnSOD immunoreactivity was low in the parental cells and in the dicumarol-treated cells. MIA PaCa-2 cells infected with AdMnSOD blocked the increase in hydroethidine fluorescence seen with treatment by dicumarol (100 μM) when examined with flow cytometry (Fig. 4B) and confocal laser scanning microscopy (Fig. 4C).

To determine whether the increased \( \text{O}_2^- \) levels from dicumarol treatment was leading to inhibition of cell growth in the MIA PaCa-2 cells, we performed growth curves on the parental cell line, MIA PaCa-2 cells without treatment with dicumarol (0 μM), treated with...
cells treated with dicumarol (100 \mu M) demonstrated growth similar to the parental cell line. Each point was determined in triplicate from the same culture.

**Discussion**

Our study demonstrates a potential role of NQO1 in the growth of pancreatic cancer. NQO1 was present in only very small amounts in normal pancreas and up-regulated in the pancreatic cancer cell lines. NQO1, a flavoenzyme, catalyzes the two-electron reduction of quinones. This reaction then prevents the one-electron reduction of quinones by cytochrome P450 reductase and other flavoproteins that would result in redox cycling with generation of \( \text{O}_2^{--} \). This oxidoreductase system is greatly up-regulated to support cell growth (30). Previous investigations have hypothesized that NQO1 is up-regulated in various tumors to accommodate the needs of rapidly metabolizing cells to regenerate \( \text{NAD}^{+} \) (31). NQO1 has attracted interest over the years as an enzyme involved in detoxification of xenobiotics such as quinones and quinone-imines (14–16), and an enzyme associated with protection against mutagenesis and carcinogenesis (11–13). NQO1 has also been characterized as being capable of generating antioxidant forms of ubiquinone and vitamin E during oxidative stress, providing evidence that this enzyme may function as an antioxidant (9, 10).

Previous studies have demonstrated a specific growth-inhibitory effect of dicumarol in some tumor cell lines. Inhibition of NQO1 by dicumarol is not from interference with vitamin K epoxide oxidoreductase or a previously unrecognized aspect of vitamin K metabolism, because addition of vitamin K does not impair the growth-inhibitory effect of dicumarol in melanoma cells (31). The growth-inhibitory effect of dicumarol may also be relatively specific for tumor cells, because proliferation of normal human airway myocytes was not affected (32). Indeed, our studies demonstrate that normal pancreas has little NQO1 immunoreactive protein compared with the abundant amount of protein in the pancreatic cancer cell lines studied. Thus, the growth-inhibitory effect of dicumarol may not affect normal pancreas, whereas inhibition of NQO1 by dicumarol leads to increased intracellular \( \text{O}_2^{--} \) levels as detected by hydroethidine fluorescence and flow cytometry. Cells received no treatment, treatment with dicumarol (100 \mu M) for 4 h, and transfected with the AdMnSOD vector and treatment with dicumarol (100 \mu M) for 4 h. The cells were then incubated with hydroethidine (10 \mu M) and fluorescence quantitated by flow cytometry. Bars, \pm SE; \( P < 0.05 \) versus the parental cell lines (controls), \( n = 3 \). C, after incubation with hydroethidine, cells were also examined by confocal laser scanning microscopy demonstrating that MIA PaCa-2 cells transfected with AdMnSOD blocked the increase in hydroethidine fluorescence seen with treatment by dicumarol. D, cell growth. MIA PaCa-2 cells treated with dicumarol (100 \mu M) demonstrate reductions in cell growth compared with MIA PaCa-2 cells with no treatment. MIA PaCa-2 cells that had increased expression of MnSOD and were treated with dicumarol demonstrated growth similar to the parental cell line. Each point was determined in triplicate from the same culture. *, \( P < 0.001 \) versus MIA PaCa-2 cells (controls).

dicumarol (100 \mu M), and MIA PaCa-2 cells infected with AdMnSOD and then treated with dicumarol (100 \mu M). Enforced expression of MnSOD with AdMnSOD partially blocked the inhibition of cell growth from dicumarol treatment at 24 h (Fig. 4D).
changes the malignant phenotype of pancreatic cancer by decreasing cell growth, plating efficiency, and growth in soft agar.

The role of NQO1 in biological systems has been determined by the use of the competitive inhibitor dicumarol, which binds reversibly to the pyridine nucleotide binding site on NQO1 and is competitive against pyridine nucleotide (33, 34). Dicumarol has been used as a component of the standard activity assay for NQO1 for many years (20); however, it can inhibit many enzymes in addition to NQO1 (35). Dicumarol is also extensively protein bound, which can complicate its use in cellular systems (36). Furthermore, the effective concentration of dicumarol that is required to inhibit NQO1 depends on the efficiency of the second substrate or electron acceptor because of the competitive nature of dicumarol inhibition and the “ping-pong” kinetic mechanism of NQO1 (37).

Our study also compliments the recent study from Li et al. (38) that demonstrated inhibition of NAPDH oxidase with iodonium compounds including DPI, resulting in increased mitochondrial O$_2^{\cdot-}$ production leading to apoptosis. DPI, which inhibits flavoenzymes like NQO1, increased ethidium fluorescence in HL-60 cells, and the DPI-induced generation of O$_2^{\cdot-}$ was reduced by overexpression of MnSOD. In our study, inhibition of NQO1 increased hydroethidine fluorescence, and the dicumarol-induced generation of O$_2^{\cdot-}$ was also reduced by overexpression of MnSOD. Although previous studies in rat liver have demonstrated that the bulk of NQO1 is located in the cytoplasm, lesser amounts are present in the mitochondria (39). The cellular distribution of NQO1 in rapidly dividing tumor cells is variable (40). However, increasing mitochondrial superoxide production may prove to be a useful mechanism in treating cells that overexpress NQO1.

The mechanisms involved in the growth-inhibitory effects of dicumarol may be different depending on the cell line studied. In our present study, dicumarol resulted in an increased intracellular burst of O$_2^{\cdot-}$ as measured by hydroethidine. In cells transiently transfected with the antioxidant MnSOD and then treated with dicumarol, O$_2^{\cdot-}$ levels were decreased compared with treatment with dicumarol alone, whereas cell growth was similar to the parental cell line suggesting that the increased intracellular generation of O$_2^{\cdot-}$ may be involved in the growth-inhibitory effects of dicumarol in pancreatic cancer cells. Brar et al. (31) have demonstrated a similar reduction in cell growth in a melanoma cell line treated with dicumarol. Malignant melanoma M1619 cells also demonstrate expression of NQO1 by RT-PCR, and growth inhibition with dicumarol and other flavoenzyme inhibitors. However, in the melanoma cell line, measurement of O$_2^{\cdot-}$ by the cytochrome c assay suggested that the highest dose of dicumarol (250 μM) decreased O$_2^{\cdot-}$ generation. Our present study demonstrated that dicumarol (250 μM) did not affect cytochrome c reduction, lucigenin-enhanced chemiluminescence, or hydroethidine staining when compared with the parental cell line, but had the greatest effect in inhibition of in vitro cell growth. These differences may be explained in that the higher dose of dicumarol is extremely toxic to both the pancreatic and melanoma cell lines leading to overwhelming cell death. Also, there may be a different mechanism involved depending on the cellular compartment, which is affected by NQO1 inhibition. Lower doses of dicumarol (100 μM) also did not change cytochrome c reduction or lucigenin-enhanced chemiluminescence, but increased hydroethidine staining, in a similar fashion as the nonspecific flavoenzyme inhibitor, DPI, which was also demonstrated by the study of Li et al. (38). Increased levels of hydroethidine staining (intracellular O$_2^{\cdot-}$), but not lucigenin-enhanced chemiluminescence (total O$_2^{\cdot-}$) or cytochrome c (extracellular O$_2^{\cdot-}$), suggests that in MIA PaCa-2 pancreatic cancer cells, inhibition of NQO1 results in intracellular generation of O$_2^{\cdot-}$.

Adenocarcinoma of the pancreas is resistant to almost all classes of chemotherapeutic drugs. Currently, the only active agent appears to be the DNA chain terminator gemcitabine (2’,2’-difluorodeoxycytidine), which results in an response rate of <20% (4). Even after curative resection, the 5-year survival rates achieved at specialized centers are <20%, and the majority of patients die of metastatic cancer recurrence (3). Other adjuvant treatments such as radiation therapy and chemotherapy have not improved long-term survival after resection. Thus, novel treatment strategies directed against this devastating malignancy are greatly needed.

In summary, NQO1 is up-regulated in pancreatic cancer cell lines but absent in the normal human pancreas. Selective inhibition of NQO1 with dicumarol alters the malignant phenotype MIA PaCa-2 pancreatic cancer cells by inhibiting cell growth, plating efficiency, and growth in soft agar. The mechanism involved in growth inhibition of pancreatic cancer by selective inhibition of NQO1 appears to be an increased intracellular production of O$_2^{\cdot-}$. Both the increased intracellular production of O$_2^{\cdot-}$ and the growth inhibition by dicumarol are blunted with transfection of an adenoviral vector containing the cDNA for MnSOD. These mechanisms suggest that altering the intracellular redox environment of pancreatic cancer cells may be an effective strategy directed against pancreatic cancer.

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