Effect of oxidative stress (H_2O_2) on cell proliferation and survival of a colon cancer cell line

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INTRODUCTION

Tumor cells have highly induced antioxidant systems to be able to defend themselves against oxidative stress, and thus survive and proliferate. This project aims to demonstrate whether the elimination of the antioxidant enzyme peroxiredoxin 6 (PRDX6) in the human colon cancer cell line HCT-116 affects its survival against oxidative stress due to H_2O_2. The results may help advance the development of new cancer therapies.

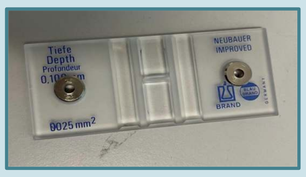
OBJECTIVES

To demonstrate that colon cancer-derived tumor cells when deleted from a key antioxidant enzyme such as PRDX6 and subjected to oxidative stress with H2O2 significantly decrease their survival and proliferation.

MATERIALS AND METHODS

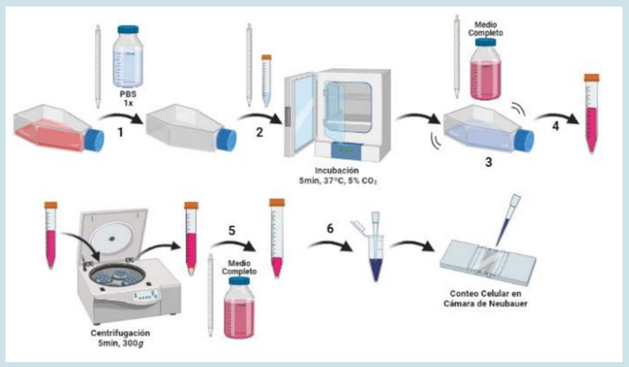
Colon cancer cell line, HCT116 (WT), and another derived from it that lacks the antioxidant enzyme PRDX6 (KO). McCoy's 5A cell culture medium, trypsin-EDTA, PBS, trypan blue, fetal bovine serum (FBS), antibiotic-antimycotic, cell culture flasks and plates, pipettes and tips, methanol, H_2O_2 and crystal violet.

Cámara de Neubauer



1.- Cell cultures.- Under sterile conditions, the cell lines were incubated at 37ºC in a 5% CO_2 atmosphere. The cells were detached with trypsin, neutralized with FBS after 3 min, and collected by centrifugation (5 min). The precipitate was disintegrated in culture medium and the cells were seeded in 6-and 24-well plates, treated with H_2O_2.

Method 1 and 2



2. Cell counting in the Neubauer chamber.- A 1:2 dilution was made with trypan blue, 10 µL was loaded into the chamber and counted using the optical microscope. The total number of cells was calculated with this formula:

No. of cells = X/4 · 10000 (Neubauer chamber factor).

Dilution factor (2) · Vmedium (mL)

3.- Cell growth curve.- 25,000 cells/well were seeded in 24-well plates. After 24 hours, they were subjected to treatment with H_2O2 (0, 10, 25 and 50 µM) for 48 hours. The cells were then detached and counted as described above.

4.- Colony-forming unit assay.- In a 6-well plate, 250 cells/well of the HCT-116 WT and KO cell line were seeded with the different H_2O_2 treatments (0, 10, 25 and 50 µM). The cells were incubated for 10 days. After this time, they were fixed with methanol (20 min) and stained with crystal violet. Excess crystal violet was removed with distilled water. It was left to dry and the plate was photographed.

5.- Statistical analysis.- The results obtained, from 3 biological replicates, were analyzed using Excel and were represented as the average ± SD (Standard deviation). To analyze the effect of H_2O_2 , a two-tailed Student's t test was performed. Tree levels of significance were established: (*) p value <0.05; (**) p value <0.01; (***) p value <0.001.

RESULTS

Effect of H_2O_2 on the growth of HCT-116 WT and KO cells for PRDX6

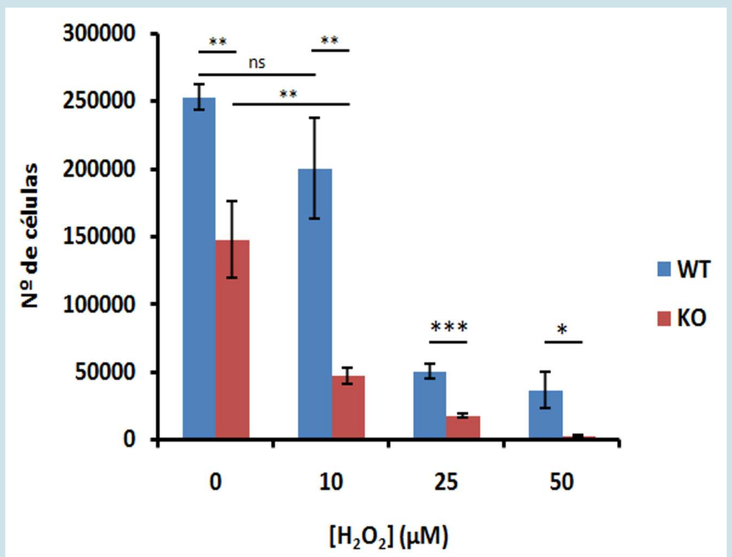


Figure 1. In the absence of H_2O_2, WT reaches a higher number of cells than KO. When adding 10 (µM) H_2O_2 there is still a great difference between the number of WT and KO cells, showing a greater effect in KO cells, which remain at 50,000 cells. Concentrations25 (µM) also decrease the number of colony forming units, the decrease being greater in WT. At concentrations 50 (µM), a large decrease in the number is again observed in KO compared to WT.

Effect of H_2O_2 on the survival of HCT-116 WT and PRDX6 KO cells

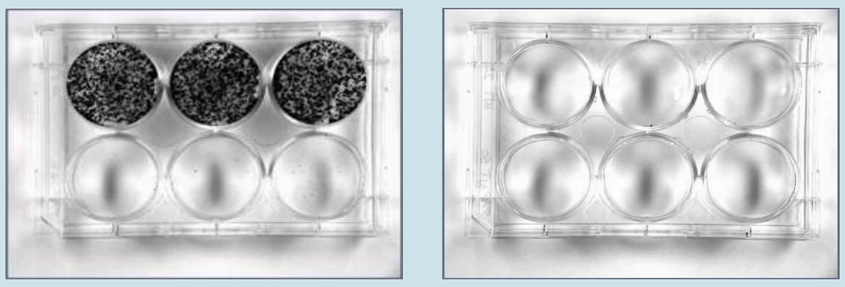


Figura 2. *KO with different treatments of H_2O_2 (0, 10, 25 and 50 µM).*

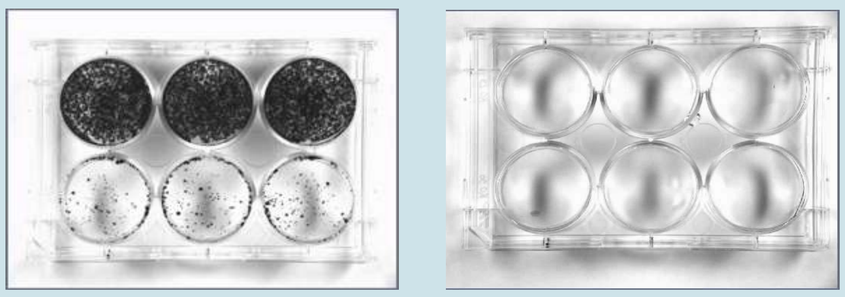


Figura 3. *KO with different treatments of H_2O_2 (0, 10, 25 and 50 µM).*

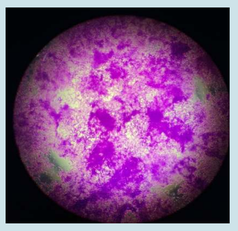


Figure 4. *Detail of WT colony forming units treated with 0 µM of H_2O_2*

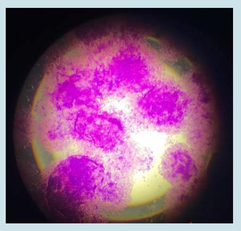


Figure 5. *Detail of KO colony forming units treated with 0 µM of H_2O_2*

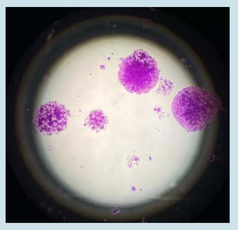


Figure 6. *Detail of WT colony forming units treated with 10 µM of H_2O_2*

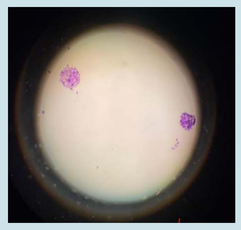


Figure 7. *Detail of KO colony forming units treated with 10 µM of H_2O_2*

Figures 2 and 3 show the effect of the absence (KO) or not (WT) of peroxiredoxin 6 (0 μM H_2O_2) on cell survival. The same can be seen at higher magnification in the light microscope images shown in Figures 4 and 5, where WT and KO cells can be seen to survive in the absence of H_2O_2. The HCT116 WT cell line shows a higher number of colony-forming units compared to KO, which indicates a higher survival of these cells, since each of these colonies comes from an original cell that is dividing.

Figures 2 and 3 of colony forming units also show the effect of 10, 25 and 50 µM concentrations of H_2O2 on the survival of KO and WT cells, respectively. It can be seen that hydrogen peroxide at concentration 10 μM decreases cell survival in both HCT116 KO and WT cells, a fact that is also observed in Figures 6 (WT) and 7 (KO), but the effect is greater in KO cells.

In addition, it is clearly seen that WT line also shows higher proliferation, as the size of WT colonies is higher than the colonies of KO cells due to a higher division rate of the surviving cells

CONCLUSIONS

1. HCT-116 cells KO for PRDX6 proliferate significantly less than WT cells.
2. HCT-116 KO for PRDX6 cells are more sensitive to oxidative stress (H_2O_2).
3. High doses of hydrogen peroxide are lethal to both cell types

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