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Mechanisms of cross resistance between Cisplatin and Bleomycin in Saccharomyces cerevisiae.

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Introduction

About 90% of all cancer deaths are influenced by the appearance, at some point in the evolution of this disease, of tumor resistance to cytostatic treatments, so the magnitude of this problem is considerable, since cancer is one of the leading causes of mortality.

There are tumors with natural resistance to cytostatics, and others that despite being sensitive to the start of treatment, progressively become refractory, that is to say, they acquire resistance.

In addition, tumor cells can simultaneously acquire resistance against a diverse group of drugs. This phenomenon is called pleotropic resistance or multidrug resistance. This mechanism could explain the tumor resistance observed in chemotherapy treatments with multiple agents.

Objetives

The aim of this work is to study possible mechanisms of cross resistance between Cisplatin and Bleomycin in *Saccharomyces cerevisiae.*

Material and methods

Yeast strain and culture medium

The experiments were carried out with the haploid yeast strain *Saccharomyces cerevisiae* WS8105-1C (genotype: *MATalpha, ade2, arg4-17, trp1-289, ura3-52*), with the resistant haploid yeast strain to cisplatin *Saccharomyces cerevisiae* WS8105-1C-R300cisPt and with the resistant haploid yeast strain to bleomycin *Saccharomyces cerevisiae* WS8105-1C-R 0.158 Bleo. Yeast cells were grown in a solid medium of YPD (1 % Bacto-yeast extract, 2 % Bacto-peptone, 2 % dextrose and 2 % Bacto-agar) for the cytotoxicity assay.

Chemicals

The antineoplastic drugs used were cisplatin and bleomycin. The doses used were 0, 1, 10, 50, 100, 300, 500, 700 and 900 µg/ml of cisplatin and, 0, 0.001, 0.003, 0.005, 0.008, 0.01, 0.03, 0.05 and 0.06 UI/ml of bleomycin.

Experimental protocol

Cytotoxicity test: Prior to exposures, wild yeast cells were cultured during five days on YPD-agar plates at 30°C and then a loop was suspended in 1000 µl of sterile water at a titer of 2E+7 cells/ml. This quantity of cells was added to test tubes with different doses of cisplatin and they were completed with sterile water until 1000 µl. Then, the tubes were cultured during 24 hours at 30°C and cells washed twice with sterile water. For drop test assay, six 10-fold serial dilutions from each sample were prepared and five-microliter aliquots of each dilution were spotted onto YPD plates. The same cytotoxicity test was carried out but using bleomycin.

In order to evaluate if there were mechanisms of cross-resistance, the following protocol was performed.

On the one hand, 2E+7 cells/ml of resistant yeast strain to cisplatin WS8105-1C-R300cisPt were added to test tubes with the different doses of bleomycin; and on the other hand, 2E+7 cells/ml of resistant yeast strain to bleomycin WS8105-1C-R 0.158 Bleo were added to test tubes with the different doses of cisplatin. The test tubes were completed with sterile water until 1000 µl. Then, the tubes were cultured during 24 hours at 30°C and cells washed twice with

sterile water. For drop test assay, six 10-fold serial dilutions from each sample were prepared and five-microliter aliquots of each dilution were spotted onto YPD plates.

Results and conclusions

On the one hand, the cytotoxicity curve for bleomycin obtained by drop test showed that the decrease of the surviving fraction in strain WS8105-1C-R300cisPt was clearly greater than in the wild strain after exposure to Bleomycin. The ID50 and ID90 values obtained were 0.001 UI/ml and 0.158 UI/ml, respectively, in the wild strain and, 0.00054 IU/ml of Bleomycin (1.85 times more sensitive with respect to the wild strain) and 0.00097 IU/ml of Bleomycin (162.89 times more sensitive with respect to the wild strain).

On the other hand, the cytotoxicity curve for cisplatin obtained by drop test allowed to calculate the ID50 and ID90 values, obtaining 90 µg/ml and 300 µg /ml, respectively, in the wild strain and, 42 µg /ml of cisplatin (2,14 times more sensitive with respect to the wild strain) and 1384 µg /ml of cisplatin (5,24 times more resistant with respect to the wild strain), in the WS8105-1C-R 0.158 Bleo strain.

There were not statistically significant differences between the strain studies and doses of drugs.

In conclusion, no cross resistance between cisplatin and bleomycin was obtained in strains resistant to these cytostatic agents in *S. cerevisiae*. Further studies are needed to clarify the absent of cross resistance in tumoral cells.

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