RELATIONSHIP BETWEEN CHRONOLOGICAL AGING AND ACQUIRED RESISTANCE TO CISPLATIN IN THE YEAST *SACCHAROMYCES CEREVISIAE*

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Introduction

Cellular senescence, which has traditionally been associated with organism aging, has recently emerged as a determinant key related to cancer chemotherapy. The Sir2 gene is associated with an increase in longevity in yeasts, worms, flies and rodents. The human homolog, Sirt1, is also involved in longevity, by inhibiting cellular senescence.

Studies carry out in tumor cells of neuroblastoma, osteosarcoma, breast and ovary, resistant and sensitive to antineoplastic drugs, suggest that the increase of Sirt1 expression may represent a general phenomenon associated with resistance to chemotherapy, independent of cell type or drug used to induce resistance. Thus, after analyzing tumor biopsies after treating patients with chemotherapy, high amounts of Sirt1 have been observed.

In addition, studies carried out on cisplatin-resistant cells show that Sirt1 is altered, suggesting that it might represent a molecule that responds to stress, and might be related to chemotherapy.

Therefore, the identification of genes involved in senescence could have a potential therapeutic utility for the prediction and/or prevention of resistance to antineoplastic drugs.

Objectives

The aim of this work is to study the relationship between chronological aging and acquired resistance to Cisplatin in the yeast *Saccharomyces cerevisiae*.

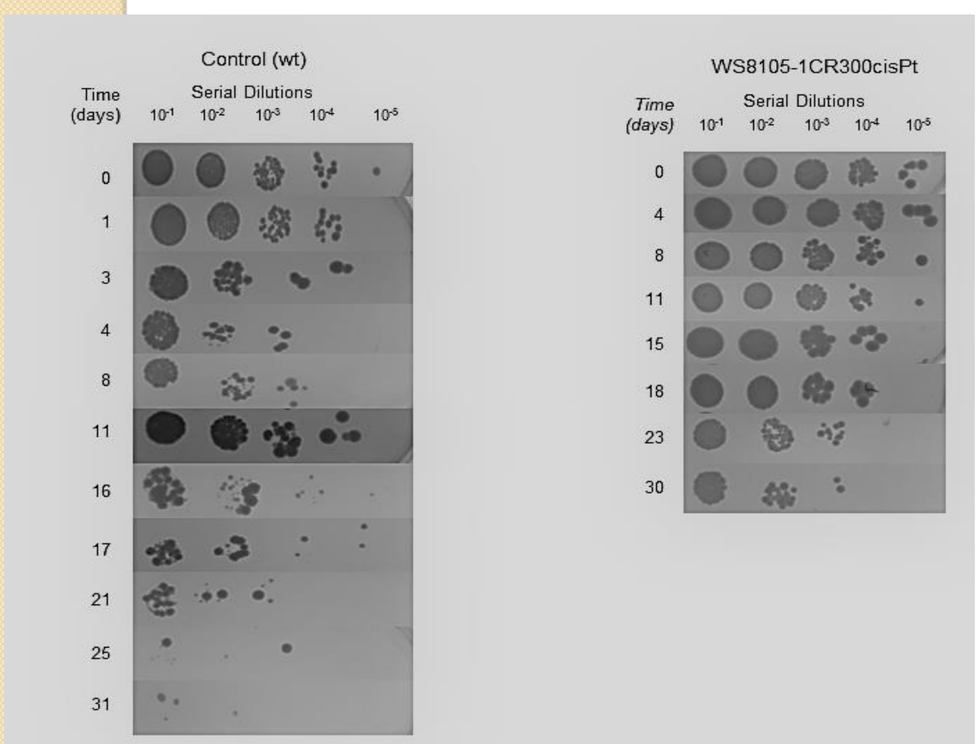
Results and conclusion

The aging test lasted 31 days. It was observed that in the strain WS8105-1C-R300cisPt (resistant to Cisplatin) there was a delay of aging with respect to the wild type strain. In the wild strain there was a sharp fall of the surviving fraction (FS). It was asymptotized on day 3 and with a FS value of 0.04. In contrast, in strain WS8105-1C-R300cisPt the FS fall was less sudden, and it was asymptotized on day 15 with a FS value of 0.04.

The FS50 was calculated obtaining a result of 1.61 days for the wild strain and a result of 5.22 days for the strain WS8105-1C-R300cisPt. Therefore, there was a delay in aging in the strain WS8105-1C-R300cisPt, with respect to the wild strain, of 3.24 days.

In the wild strain, the ID50 and ID90 values obtained were 90 µg/ml and 300 µg/ml, respectively. In the resistant strain WS8105-1C-R300cisPt, the ID50 and ID90 values obtained were 224 µg/ml (2.5 times more resistant with respect to the wild strain) and 735 µg/ml (2.78 times more resistant with respect to the wild strain), respectively. Thus, it was observed that for the ID50 and ID90 values there was an increase in resistance associated with an increase in the aging delay, with respect to the wild strain.

In conclusion, the strain WS8105-1C-R300cisPt (resistant to Cisplatin) presents an increase in the chronological life cycle, producing a delay in aging. This fact suggests that it is possible the expression of common cellular and molecular mechanisms between the acquisition of resistance to this drug and the delay in cellular aging. These results could have important implications in tumor response to treatments.



Chronological aging evaluated by drop test.
wt: wild strain (control). WS8105-1CR300cisPt: Cisplatin-resistant strain.

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) and with the resistant haploid yeast strain to cisplatin *Saccharomyces cerevisiae* WS8105-1C-R300cisPt. Yeast cells were grown in a liquid medium of SDC (2% dextrose, 0,17% nitrogen base, 0,5% ammonium sulphate [(NH4)2SO4], 0,15 % four-fold excess of the supplements ade, arg, trp and ura) for the aging assay, and 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 drug used was cisplatin. The doses used were 0, 50, 100, 300, 500, 700 and 900 µg/ml for the cytotoxicity assay.

Experimental protocol

Aging assay: Yeast cells were cultured during five days on YPD-agar plates at 30°C. Next, 1.5E+6 cells/ml were added to test flask with 20 ml of SDC medium. They were cultured during 4 days at 30°C, shaking at 300 rpm until they reached the stationary phase. From that moment, the chronological aging phase began and it was evaluated every two or three days by droptest, so six 10-fold serial dilutions from each sample were prepared and five-microliter aliquots of each dilution were spotted onto YPD plates. Finally, the surviving fraction was calculated.

Cytotoxicity test: Prior to exposures, 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 test was carried out to the resistant strain WS8105-1C-R300cisPt.

Yeasts chronological aging.
wt: wild strain (control). WS8105-1CR300cisPt: Cisplatin-resistant strain.
Mean ± SD. p = 0.0009; ANOVA