RESISTANCE TO BLEOMYCIN INCREASES THE CHRONOLOGICAL LIFE OF CELLS

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Introduction

Studies carried out on resistant and sensitive tumor cells to antineoplastic drugs, suggest that the increase in the expression of Sirt1, which is a gene associated with an increase in longevity, may represent a phenomenon general associated with resistance to chemotherapy, independent of cell type or drug used to induce resistance.

So, if this gene is associated with aging and is also associated with the process of resistance to chemotherapy, it is possible that other genes could be involved in both processes.

Therefore, the identification of genes involved in chronological aging could have a potential utility as molecular markers in the chemotherapy treatment of cancer.

Objectives

The aim of this work is to study the relationship between the chronological aging and the resistance to Bleomycin for the purpose of establish the basic interactions between these phenomenons for further investigation of molecular markers.

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 bleomycin *Saccharomyces cerevisiae* WS8105-1CR 0.158 Bleo. 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 bleomycin. The doses used were 0, 0.001, 0.003, 0.005, 0.008, 0.01, 0.03 y 0.06 UI/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 bleomycin 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-R 0.158 Bleo.

Results and Conclusions

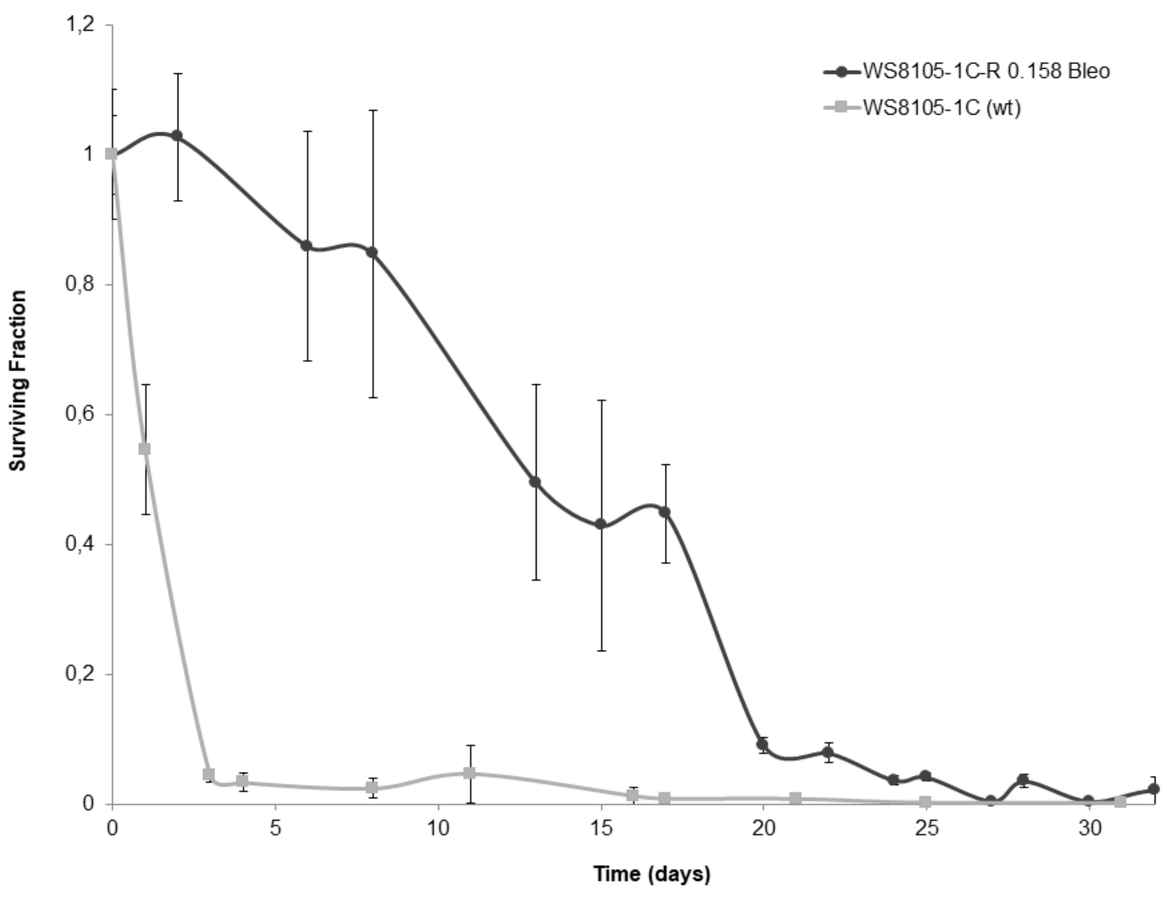
The aging test lasted 31 days. It was observed that in the strain WS8105-1C-R 0.158 Bleo (resistant to bleomycin) there was a delay of aging with respect to the wild strain. In the wild strain there was a sharp fall of the surviving fraction (FS). It was asymptotized on day 3 and with a FS value of 0.04. In contrast, in strain WS8105-1C-R300cisPt the FS fall was less sudden, and it was asymptotized on day 24 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 13.48 days for the strain WS8105-1C-R 0.158 Bleo. Therefore, there was a delay in aging in the strain WS8105-1C-R 0.158 Bleo, with respect to the wild strain, of 8.37 days.

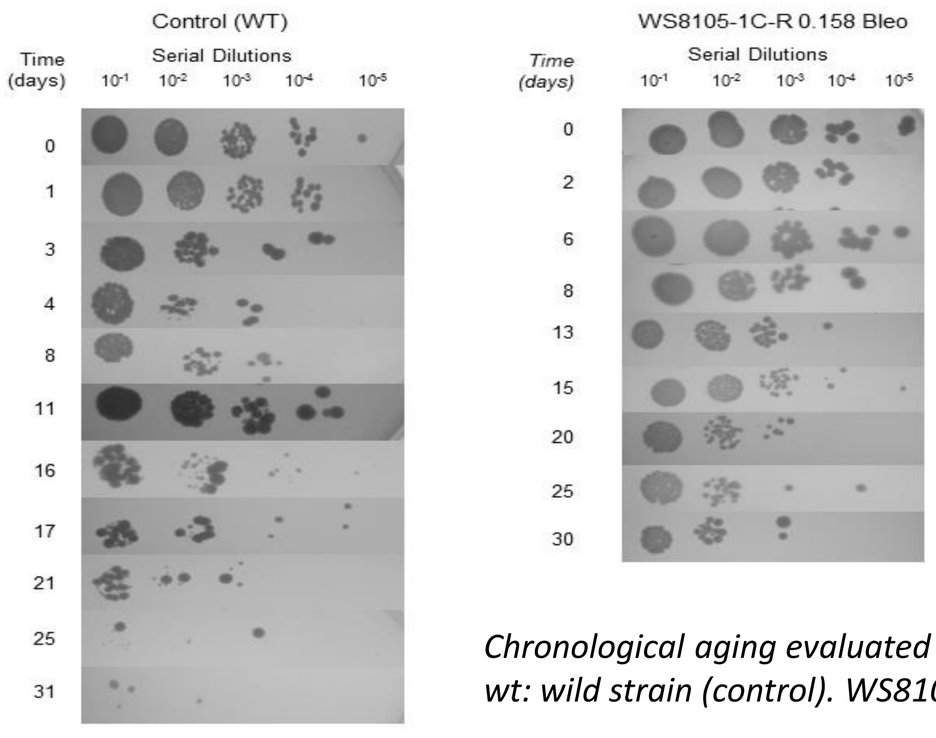
In the wild strain, the ID50 and ID90 values obtained were 0.001 UI/ml and 0.158 UI/ml, respectively. In the resistant strain WS8105-1C-R 0.158 Bleo, the ID50 and ID90 values obtained were 0.0156 UI/ml (15.6 times more resistant with respect to the wild strain) and 2.818 UI/ml (17.83 times more resistant 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-R 0.158 Bleo (resistant to bleomycin) presents an increase in the chronological life cycle, producing a delay in aging. Common cellular and molecular mechanisms between the acquisition of resistance to this drug and the delay in cellular aging could be influencing the results obtained.

Therefore, the results obtained establish that basic mechanism such as drug resistance and aging are related. Similar molecular markers could be activated which could be very useful in the diagnosis and treatment of cancer.



Yeasts chronological aging.
wt: wild strain (control). WS8105-1C-R0.158 Bleo: resistant to Bleomycin.
Mean ± SD. p = 0.0009; ANOVA



Chronological aging evaluated by drop test.
wt: wild strain (control). WS8105-1C-R 0.158 Bleo: Bleomycin resistant.