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EFFECT OF BOVINE CuZn SUPEROXIDE DISMUTASE ON C_3 CLONE OF B-16 MOUSE MELANOME CELLS IN CULTURE

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B-16/C_3 mouse melanoma cells undergo melanogenesis and differentiation 9 days after plating under usual conditions. In our experiments the effect of exogenous bovine CuZn superoxide dismutase (CuZn SOD) on B-16/C_3 cells in cultures was studied. The exogenous CuZn SOD was added, 24 hours after cell plating, in growth medium, which either contain or not Fetal Calf Serum (FCS). B-16/C_3 melanoma cells growth was followed over 5 days. Different effect of CuZn SOD on the culture was observed in relation to FCS present in growth medium. CuZn SOD induced a dose dependent increase in melanin content of B-16/C_3 cells in serum deprived medium. Our results are discussed in respect to dismutating CuZn SOD activity, which may act to an enhanced

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level of oxidative stress, related to the higher metabolism in melanoma cells. On the other hand we supposed that CuZn SOD produced an elevated level of hydrogen peroxide. Therefore hydrogen peroxide than may play some role in cell differentiation as transmembrane messenger.

Key words: B-16 mouse melanoma, melanogenesis, differentiation, fetal calf serum (FCS), copper zinc containing superoxide dismutase (CuZn SOD).

INTRODUCTION

There are numerous experimental data suggesting that free radicals and reactive oxygen species (ROS) are mutagenic compounds known to lead to DNA damage, favor cell transformation, tumor promotion and progression (FRANK *et al.*, 2000). These oxidants may changes cellular redox state in the direction of increased oxidation. The net results of that is the loss of normal redox control of cell growth and development (SPITZ *et al.*, 2000). Enzyme copper, zinc-containing superoxide dismutase (CuZn SOD, EC 1.15.1.1) converts superoxide anion radicals (O_2^-) into hydrogen peroxide (H_2O_2), thus the activity of this enzyme might be involved in developmental regulation or differentiation (ISOHERRANEN *et al.*, 1997).

Cultures of B-16/C_3 melanoma were composed of cells, which are different in size, replicative activity and differentiation. Differentiation of these cells is coupled with synthesis of melanine starting by plating and terminating by maximal synthesis of melanine.

In this paper, we have studied the phenomenon of cancer cell differentiation with emphasis on the role of CuZn SOD in this process, using the B-16/C_3 mouse melanoma cells as a model system. We have analyzed phenotypically distinct stages of B-16/C_3 maturation using the influence of Cu Zn SOD on morphological characteristicyty of cultured cells in presence or not FCS.

MATERIAL AND METHODS

The C_3 clone of B-16 mouse melanoma was cultured in Eagle's minimal essential medium, glutamine and 0,05 M sodium bicarbonate supplemented with or not by 10% fetal calf serum (FCS). Stock cultures were maintained in logarithmic growth phase by subculture every 3 to 4 days. The cells were passed using 0.05% tripsin plus 0.02% EDTA (Gibco). Experimental cells were plated at initial density of 1500 cells/cm^2 into glass flasks (11 x 4 cm) in 10 ml of medium and cultured for 5 days at 37°C. The exogenous bovine CuZn SOD (0.5×10^{-5} M; 1×10^{-5}M) was added in culture medium, 24 hours after cell plating. On days 5 the cultures were photographed by camera-lucida fitted Opton microscope and analyzed.

RESULTS AN D DISCUSION

As showed in Fig. 1. different effect of CuZn SOD on the culture of melanoma cells was observed in relation to FCS present or not in growth medium. CuZn SOD induced a dose dependent increase in melanin content of B-16/C3 cells in serum deprived medium. The occurrence in cultured cells with FCS in the medium was not present. The synthesis of melanine led to differentiation in melanoma cells in culture. It was showed that B-16/C3 cultures did not go to melanogenesis without FCS. But under influence of MSH the melanoma cells may synthesis melanine without secreting them in culture medium. In one study MnSOD overexpression protected HeLa cervical carcinoma cells from growth suppression under condition of serum deprivation which was suggested to be related to changes in the intracellular oxidative processes of these cells (PALAZZOTTI *et al.*, 1999).

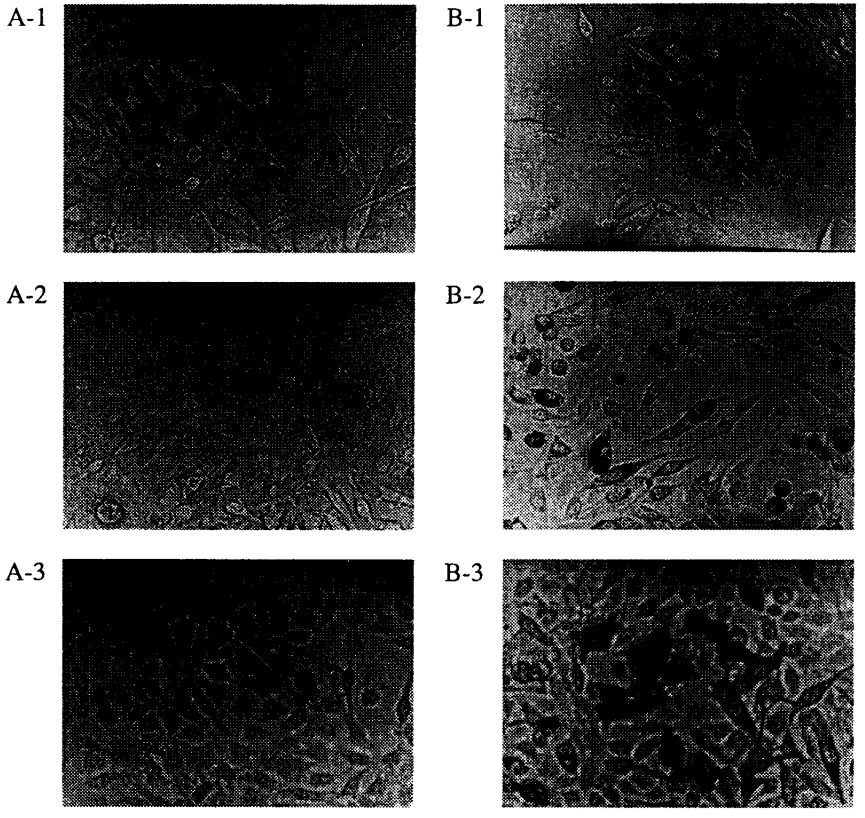

Fig. 1. B-16 mouse melanoma cells growth in culture 5 days. A. growth medium with FCS. B. growth medium without FCS. 1 - control; 2 - control + 0.5 x 10^{-5} M bovine CuZn SOD; 3- control + 1 x 10^{-5} M bovine CuZn SOD.

Treatment with SOD may act as scavenging of O2-. mostly outside of cells. Malignant cells may also haw elevated level of SOD what is response to an enhanced level of oxidative stress related to the higher metabolism. These exogenous but also inducted activity of SOD reduce but not obviously eliminate the oxidant stimulus, which produced malignant phenotype. Namely, melanoma cell lines expressing high CuZn SOD levels changed their ability to form colonies and had a more differentiated phenotype *in vitro*.

Our results are also discussed in respect to dismutating CuZn SOD activity and overproducing of hydrogen peroxide which, than, may play some role in cell differentiation as transmembrane messenger (Barnouin *et al.*, 2002). Modulating not only superoxide but also H_2O_2 scavenging enzymes can significantly decrease tumor mass (OBERLEY *et al.*, 1981).

Although the role of CuZn SOD has not been carefully evaluated in regard to carcinogenesis it plays a role in intracellular signaling and regulation of extracellular matrix remodeling. The influence of SOD enzymes on melanoma cells may also be dependent on the growth phase of the cells, culture medium, plating density and oxygen atmosphere.

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UTICAJ GOVEDJE BAKAR-CINK SUPEROKSID DISMUTAZE NA KULTURE ĆELIJA C_3 KLONA B-16 MIŠJEG MELANOMA

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Izvod

Ćelije B-16/C_3 melanoma miša melaniziraju i diferenciraju devetog dana posle zasadjivanja pod uobičajenim uslovima. U našim eksperimentima smo proučavali uticaj egzogeno dodavane CuZn superoksid-dismutaze (CuZn SOD) na ćelije B-16/C_3 u kulturi. Egzogena CuZn SOD je dodavana 24 časa posle zasadjivanja ćelija u medijume za rast koji su sadržavai ili ne govedji fetalni serum. Rast ćelija B-16/C_3 je praćen pet dana. Zapažen je različit uticaj CuZn SOD na ćelijske kulture gajene sa serumom ili bez njega. CuZn SOD izaziva dozno zavisnu sintezu i nakupljanje melanina u ćelijama koje su rasle u medijumu koji nije sadržavao govedji fetalni serum. Naši rezultatu se mogu diskutovati u svetlu dismutacione aktivnosti CuZn SOD koja deluje na povećani nivo oksidacionog stresa izazvanog visokim metabolizmom melanoma ćelija. S druge strane, pretpostavili smo da CuZn SOD svojom aktivnošću proizvodi više vodonik peroksida. Vodonik peroksid, nadalje, može da ima izvesnu ulogu u diferencijaciji ćelija kao transmembranski signalni molekul.

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