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ABSTRACTS

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70 Lack of $p21^{waf1/cip1/sdi1}$ induction and of MAPK activation in senescent cells following oxidative stress

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$p21^{waf1/cip1/sdi1}$ is an inhibitor of cyclin-dependent kinases that regulates G1-> S transition and is the main effector of p53-induced growth arrest. We demonstrated that the oxidative stress induced by diethylmaleate (DEM) produces an accumulation of $p21^{waf1/cip1/sdi1}$ mRNA and protein, and in turn a significant delay of cell cycle progression. These phenomena are p53-independent, whereas they require the oxidative stress-induced activation of the *ras*-MAPK cascade. Senescent cultured cells share several characteristics with cells exposed *in vitro* to oxidative stress, as an overall increase of oxidative processes and a progressively delayed growth. The well known accumulation of $p21^{waf1/cip1/sdi1}$ mRNA and protein in senescent cells could be considered a further similarity between senescent and oxidized cells.

We observed that $p21^{waf1/cip1/sdi1}$ mRNA levels increase to the same extent in young IMR90 fibroblasts exposed to DEM and in their senescent untreated counterpart. However, whereas MAPK is strongly activated in young fibroblasts treated with DEM, and the overexpression of MEK-dominant negative mutants prevents the accumulation of $p21^{waf1/cip1/sdi1}$ following DEM treatment, MAPK is not activated in senescent cells and the exposure to DEM of these cells does not induce the activation of MAPK. These results suggest that $p21^{waf1/cip1/sdi1}$ mRNA and protein accumulation in senescent cells seems not to be related to the activation of MAPK, and that the mechanisms regulating $p21^{waf1/cip1/sdi1}$ expression following oxidative stress are not functioning in senescent cells.

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71 **PEROXIDATIVE STRESS AND CELL PROLIFERATION IN JURKAT J6 AT DIFFERENT COLTURE DENSITY**

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Lipoperoxidation products could result bioactive molecules able to modulate either the processes of cell division and induce necrotic and apoptotic cell death. In the cells of a human leukaemia cell line, Jurkat J6, we found that cellular content of glutathione, PUFA and protein decreased with the increasing of culture density. In this study we investigated if a peroxidative stress on cells at different cell density in culture could induce different effect on cell proliferation.

Cells were peroxidized with Hypoxanthine 10 μM and Xanthine Oxidase 3μg/10^6 cells, using cells cultured at low cell density (P100), medium density (P400) and confluence (P800). After the peroxidative stress, cells were seeded at 10^5cell/ml and cells were examinated during the following 72 hours for their growth and flow cytometric assays were used to determine necrosis and apoptosis, mitochondrial potential, cell cycle and BrdU incorporation. P100 and P400 were characterized by an increased doubling time (over 30 h) at every time tested (from 24 to 72 h after treatment) while P800 from the 48th hour reached a value of DT comparable to controls (25-28 h). Moreover in P100 the percentage of apoptotic and necrotic cells was always higher than in P400, in P800 and in controls. However P400 and P800 showed a slightly higher percentage of apoptotic cells than the controls. The mitochondrial potential was not affected, showing that an ATP impairment is not involved in this induction of apoptosis. The cell cycle analysis showed that, while P200 and P400 had patterns similar to controls, in P800 there was an increase in the fraction of cells in S phase with respect to C800. A slightly higher DT however, and a minor incorporation of BrdU with respect to C800 seems to indicate a slowdown in the progression along this phase.

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72 MyoD can enhance the activity of retinoblastoma gene promoter through a CREB site.

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We have previously demonstrated that the muscle-specific transcription factor MyoD induces the retinoblastoma gene (Rb) promoter. Here we show that the MyoD-mediated induction of the Rb promoter does not require new protein synthesis. Moreover, we identified the minimal Rb promoter region targeted by MyoD as a 50 bp sequence containing the binding sites of three families of transcription factors: E2F, ATF/CREB and E4TF1. Site-directed mutagenesis of these sites indicated that: 1. the E4TF1 binding site is required for the promoter basal activity; 2. the ATF/CREB site is important for both the basal and MyoD-induced promoter activity; 3. the E2F site modulates the basal promoter activity.

By means of band shift experiments, we determined that the transcription factor CREB binds the ATF/CREB site and that both the CBP and p300 transcriptional coactivators participate to the DNA-bound complex. Moreover, we have been able to identify MyoD as a further component of this CREB-DNA complex. These results together with our previous finding that MyoD and p300 can physically and functionally interact (Yuan et al., 1996, J. Biol. Chem., 271, 9009-9013), suggest that MyoD might induce the activity of CREB through binding to the p300 transcriptional coactivator. Based on this hypothesis we asked whether the MyoD-dependent activation of a Rb promoter CAT construct could be potentiated by coexpression of exogenous p300. A three fold increase of Rb-CAT expression was observed upon cotransfection of MyoD and p300 relative to the induction by MyoD alone. Moreover, we found that MyoD and p300 can cooperate to induce a canonical CREB responsive CAT construct (-71SRIF-CAT).

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