cell cycle regulated with maximum amounts in mitosis followed by a steep decline towards G1 phase. However, in contrast to normal human embryonic fibroblast, melanoma cells arrested in G0/G1 contained readily detectable levels of Aurora-A, indicating incomplete degradation of Aurora-A during mitosis. Correspondingly, immunofluorescence staining of Aurora-A revealed diffuse cytoplasmic staining of interphase melanoma cells. Centrosome amplifications and spindle aberrations were found more frequently in those melanoma cells with amplified Aurora A.
In summary we demonstrate that amplification/overexpression of Aurora-A is an almost general feature of melanoma cells derived from both early and late lesions. Our data suggest an important contribution of Aurora-A to the genomic instability of melanomas and thus to the development and progression of this devastating disease.

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Molecular mechanisms involved in the antitumoral effect of oligoelements Se, Zn and Mn plus Lachesis muta. In-vitro and in-vivo studies

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We have previously reported the antitumoral effect of the combination (O-LM) composed by Se, Zn and Mn (4µg/ml each) plus Lachesis muta (4 ng/ml) in experimental mammary carcinomas in rats and pancreatic carcinomas in mice (Int J Cancer S13:193,2002).
The objective of this study was to further investigate *in vivo* and *in vitro* the molecular mechanism involved in the action of O-LM on tumor growth and cell proliferation.
For the *in vitro* studies the human cell lines MDA-231(breast cancer),WM35 (melanoma) and PANC-1(pancreatic carcinoma) were employed. O-LM (20 µl/ml) was added to cultures and proliferation was evaluated by the clonogenic assay. The levels of superoxide (O_2^-) and hydrogen peroxide (H_2O_2) were determined by flow cytometry using fluorescence staining. The activity of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) was also determined.
Two groups of 10 nude mice, O-LMg (daily injected with 0.1 ml O-LM starting 60 days before) and Control, were inoculated with PANC-1 cells. The tumors developed were evaluated after 100 days. Besides routine histological techniques, CD38, Factor VIII, PCNA, P53, Mallory Trichromic, P.A.S. and elastic fiber coloration were performed.
O-LM produced *in vitro* a significant decrease in cell proliferation: MDA 52± 6%, PANC-1 41± 7%, WM35 44± 11%, p<0.01 vs control 100%. In addition, O-LM produced a marked increase in H_2O_2 and in SOD activity, while O_2^- levels, CAT and GSH-Px showed minor changes.
Tumors developed in the O-LMg, showed significant lower growing rate and smaller tumor mass (3,77± 0.95 cm^3 vs 9,75± 2.30 cm^3). Histological analysis indicated well vascularized tumor edge with absence of immune peritumoral reaction and vessels of all sizes including very small arteriolar and capillary vessels for Control. In contrast, O-LMg tumors showed great areas of necrosis with conserved cells located also in the tumor edge, milder vascularization with specially reduced microvessels and tumors surrounded by important limphoplasmocitic reaction. PCNA was negative and P53 was abundantly expressed by atypical cells.
In addition to the reported action on apoptosis and on MAPK pathway present results demonstrate the capability of O-LM of modulating free radicals production. The complex mechanism leading to inhibition of tumor cell proliferation is enhanced in vivo by an increased immune reaction and a decreased angiogenesis that results in a marked tumor regression.

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Analysis of induced expression of human cyclin D1 alternative transcripts in a mouse fibroblast gene knockout cell line

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Human cyclin D1 (*CCND1*) mRNA is alternatively spliced to produce transcripts *a* and *b*. Splicing has been shown to be modulated by a common polymorphism located in the conserved splice donor region of exon 4 of the gene. Patient genotype has been associated with tumour progression and clinical outcome in a range of cancers. Currently few studies have investigated the biological function of *CCND1* transcript *b in vitro*. Using an inducible expression system we investigated the expression of human *CCND1* transcript *b* (*CCND1*^{ex4} (in comparison with human cyclin D1 transcript *a* (*CCND1*^{ex4} (in a mouse fibroblast knock out for cyclin D1 (MEF- *Cyl1*$^-$ (.
Inducible expression was examined using the LacSwitch™ system in stable clones isolated from MEF-*Cyl1*$^-$ transfectants. Induced expression of *CCND1*^{ex5}, produced a 31kDa protein located predominantly in the cell nucleus. Induction of *CCND1*^{ex5} resulted in no significant difference in DNA synthesis (detected by BrdU incorporation), or the ability of cells to grow in serum-deprived conditions. In contrast induction of *CCND1*^{ex4} produced a 36kDa protein and led to a significant increase in DNA synthesis after 32 hours compared to non-induced cells (p=0.012). Further, clones expressing *CCND1*^{ex4} exhibited a significantly increased ability to grow in serum deprived (2% FCS) medium compared to non-induced clones (p=0.0004). However induction of *CCND1*^{ex5} significantly enhanced the ability of MEF$^{Cyl1-/-}$ cells to form colonies in soft agar, (average 30-fold increase) compared to non-induced and *CCND1*^{ex4} expressing cells.
Our data support the emerging view that *CCND1* alternate transcripts encode proteins with differing, independent, biological functions. We suggest that

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Protective effect of oligolements and Lachesis muta to high doses of ionizing radiation on normal tissue. In vitro and in vivo studies

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In previous studies we demonstrated the antitumoral action of oligoelements (Selenium, Zinc, Manganese, 4μ g/ml each) plus Lachesis *muta* 4ng/ml (O-LM) in experimental and in human carcinomas producing a significant increase in survival rates (Int J Cancer S13:245,2002). Also, we reported the protective effect of O-LM to high doses of chemotherapy on normal tissue (J Trace Elem Exp Med 16:39,2003).

The objective of the present paper was to study the *in vitro* and *in vivo* effect of O-LM on the modulation of cellular damage produced by ionizing radiation.

Transformed (MDA-231 and MCF-7) and normal (HBL-100) mammary cells were irradiated with dose ranging from 0 to 10 Gray (Gy) employing a ^{137}Cs calibrate source; in parallel experiments, O-LM was added to cell culture (20 ul/ml) for 24 h previous and up to 24 h post-irradiation. The Survival Curves and the radiobiological parameters (Survival Fraction at 2 Gy, SF_{2Gy}) were analysed.

The protective effect of O-LM was evaluated on small intestine and bone marrow of twenty *nude* mice whole-body irradiated with 10 Gy, divided in two groups: a) O-LM, receiving 0.1 ml sc O-LM daily starting 90 days before irradiation; b) Control, receiving daily injection of saline solution. All animals were sacrificed 5 days after irradiation. In addition to routine histological techniques, apoptotic cells (Apoptag kit) and Bax, Bcl2 and p53 protein expression (immunofluorescence) were determined on epithelial cells of small intestine.

The results obtained indicate that O-LM produced a significant decrease in survival of irradiated malignant cells ($p<0.001$) showing enhancement of radiosensitivity. The SF_{2Gy} was 0.24± 0.03 for non-treated and 0.14± 0.02 ($p<0.001$) for O-LM-treated cells, without changes on normal HBL-100.

Intestinal mucosae of O-LM group showed mild atrophy and conservation of villous projections and the number of crypts was 35% higher than in Controls; apoptosis was detected only in the top of villous prolongation while in Control a high number of apoptotic cells were observed along all the mucosae. Bone marrow of O-LM-treated animals showed lower grade of aplasia (I-II) compared to control ones (III).

Our results indicate a clear protective effect on healthy tissue produced by O-LM, as well as a significant increase in radiosensitivity on transformed but not in normal cells. This effect can be produce by the selective modulation of antioxidant enzymes and open an important perspective of clinical application.