STUDY OF THE MOLECULAR TARGET OF NAMI-A: ANALYSIS OF THE EFFECTS ON CELL CYCLE IN KB CARCINOMA CELLS.

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Numerous studies highlighted the peculiar antimetastatic effect of the ruthenium-DMSO complex [ImH] [*trans*-$RuCl_4$(DMSO)Im] NAMI-A, in experimental models of lung metastasising solid tumours. The mechanism of this unique pharmacological property of NAMI-A is almost still unclear. The compound, tested on different cell lines *in vitro* at concentration of 0.001 to 0.1 mM, is not cytotoxic but it causes a concentration- and time-exposure dependent transitory accumulation of cells in the G_2/M phase. At the same time, an altered expression of proteins involved in the regulation of cell cycle, such as the increase of Cyclin B1, is measured.
The aim of this study is that of analysing in detail the effects of NAMI-A on the cell cycle of tumour cells after different times of exposure. The study was conducted with the human oral carcinoma KB cell line, in the light of the response rate to NAMI-A already evidenced in past experiments. KB cells were challenged with 0.1 mM NAMI-A for 1 hr in PBS-Ca^{2+}/Mg^{2+} Subsequently, cells were washed to remove the unreacted compound and cultured in complete medium for 4, 8, 12, 16, 20, 24 and 48 hrs. At each time point, cell cycle distribution was determined by flow cytometry after propidium iodide staining. At the same times, the expression of some crucial proteins [cyclin B and cdc2, that form the mitosis promoting factor MPF, their positive (cdc25C) and negative (Wee1, Myt1, p53, 14-3-3) regulators] involved in the transition from G_2- to M-phase of cell cycle was determined by Western blotting. We also examined the localisation of these proteins, distinguishing between nuclear and cytoplasmic site by means of immunocytochemistry techniques. Finally we verified the possibility of the alteration of the increase of the synthesis of cyclin B by means of RT-PCR of the relevant m-RNAs.
Flow cytometry analysis of PI stained KB cells showed that NAMI-A causes a statistically significant increase of cells in the G_2-M phase starting from 12 hrs and lasting up to 24 hrs after treatment; this effect is completely abolished by 48 hrs after treatment. The increase of cells in the G_2-M phase is paralleled by the concomitant decrease of cells in the G_0/G_1 phase and the transient increase of cells in the S phase.
The overall effect has a well defined time schedule, as shown by a significant increase of cells in the S phase at 16 hrs followed by the transition of the same cells in the G_2-M phase at 20 hrs after treatment.
Protein expression, analysed by immunoblotting techniques, showed the increase of intensity of the band corresponding to Cyclin B1 starting from 16 hrs after treatment and persisting up to 24 hrs, and this modification correlates with the observed arrest in the G_2-M phase. In the same experimental conditions, the expression of proteins, such as cdc2, cdc25C, wee1, myt1, PCNA, p53 and 14-3-3, quantified by Western blot, do not significantly vary. The analysis of the subcellular localisation (nucleus or cytoplasm) of MPF does not show any significant difference between NAMI-A treated and control cells.
In conclusion, under the experimental conditions tested, NAMI-A shows interference with the regulation mechanism of cell cycle progression, causing the transitory accumulation of the treated cells in the G_2-M phase. This effect is accompanied by a variation of cyclin B expression, presumably due to a higher synthesis of this protein rather than to an incorrect localisation of MPF. This result might be the response of cells to the alteration of the signals that bring to DNA the information for cell proliferation, as shown by G. Pintus et al., (University of Sassari) who found a pronounced reduction of the activity of ERK and of c-myc.
For this reason, my work will be focused in the future on the detailed analysis of the phosforilated status of MPF and of the other proteins that regulate its activation.