The histones as possible epigenetic targets of the anticancer ruthenium and osmium derivative compounds (RDCs and ODCs)

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In the last decades, several anticancer metal-based agents have emerged as promising alternative to the platinum drugs for their better pharmacological profile[1]. Thus, a large number of chemical and biological investigations have been carried out in order to individuate their specific targets, showing that, beside the DNA, nuclear and cytosolic proteins could have a central role in their mechanism of action[2]. Among these, the proteins binding the DNA, the histones, have emerged[3-4]. These proteins, allowing the different chromatin condensation state, have a crucial role in the DNA replication, transcription and repair[5] and their post-translational modifications are considered among the most common epigenetic changes[6]. Recently, also extra-nuclear functions of histones have emerged, since they can act as endogenous danger signals[7].

Therefore, the present work was focused on the role of histones, and more in general of the epigenetics, in the mechanism of action of the N-C heterocyclic compounds ruthenium- (RDC11) and osmium- (ODC2, ODC16, ODC20) based[8-10].

To evaluate the direct interaction of metal compounds with the histones, we did time-course experiments (1, 6, 24, 48 h) on purified histones treated with different concentrations of the compounds and analyzed by gel electrophoresis. To study the epigenetic changes induced by the organometallics in the cellular environment, we treated the colorectal carcinoma HCT116 and the gastric adenocarcinoma MKN45 cell lines with the IC_{50} and the IC_{75} concentrations of RDC11, ODC2, Cisplatin, Oxaliplatin. In particular, we evaluated: the DNA damage by Comet Assay; the histones expression and the post-translational modifications by immunoprecipitation and Western blot analysis; the histones cellular localization using different protein extraction buffers. In order to consider the results in the light of apoptotic cell levels we analyzed also the caspase-3 activation. In addition, to correlate the data with the oxidative stress induced by the metal compounds, some experiments were carried out pre-treating the cells with the antioxidant agent N-acetylcysteine (NAC).

RDC11 and ODC2 have shown to induce DNA damage and to bind the histones, as well as they provoked H2A and H2B polymerization and H3 degradation, events that could strengthen the chromatin perturbation. The hypothesis that the interaction with the histones could participate to the DNA damage is supported by the different behavior of ODC16 and ODC20, for which the lack of histones binding corresponds with a poor induction of the DNA damage. Moreover, the different profiles of activity demonstrated the importance of the structure-activity relationship. RDC11 and ODC2 induced both H3 acetylation, which can lead to the induction of specific gene expression, such as the pro-apoptotic genes[6] and H3 translocation, event that could be upstream of the cell death pathways[7]. In addition, the pre-treatment with NAC reduces the DNA damage and prevents the H3 translocation, suggesting that the interaction of organometallic*s* with the histones is strictly correlated with their potential to induce oxidative stress. All together these data demonstrate that the histones could be crucial targets to mediate the cellular response to the organometallics and confirm also an important role of the epigenetics changes in their mechanism of action.

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