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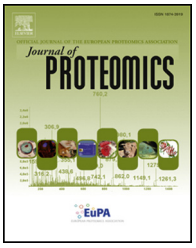

Proteomic analysis of A2780/S ovarian cancer cell response to the cytotoxic organogold(III) compound Aubipy$_c$

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ABSTRACT

Aubipy$_c$ is an organogold(III) compound endowed with encouraging anti-proliferative properties in vitro that is being evaluated pre-clinically as a prospective anticancer agent. A classical proteomic approach is exploited here to elucidate the mechanisms of its biological actions in A2780 human ovarian cancer cells. Based on 2-D gel electrophoresis separation and subsequent mass spectrometry identification, a considerable number of differentially expressed proteins were highlighted in A2780 cancer cells treated with Aubipy$_c$. Bioinformatic analysis of the groups of up-regulated and down-regulated proteins pointed out that Aubipy$_c$ primarily perturbs mitochondrial processes and the glycolytic pathway. Notably, some major alterations in the glycolytic pathway were validated through Western blot and metabolic investigations.

Biological significance
This is the first proteomic analysis regarding Aubipy$_c$ cytotoxicity in A2780/S ovarian cancer cell line. Aubipy$_c$ is a promising gold(III) compound which manifests an appreciable cytotoxicity toward the cell line A2780, being able to overcome resistance to platinum. The proteomic study revealed for Aubipy$_c$ different cellular alterations with respect to cisplatin as well as to other gold compound such as auranofin. Remarkably, the bioinformatic analysis of proteomic data pointed out that Aubipy$_c$ treatment affected, directly or indirectly, several glycolytic enzymes. These data suggest a new mechanism of action for this gold drug and might have an impact on the use of gold-based drug in cancer treatment.

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1. Introduction

Metal-based agents, and more specifically platinum drugs, play a major role in current chemotherapeutic regimens for cancer treatment [1]. Nonetheless, the clinical use of platinum drugs is still heavily limited by a high systemic toxicity and by the frequent occurrence of intrinsic or acquired resistance [2]. This triggered the search of other families of anticancer drugs based on metals different from platinum, *e.g.* ruthenium, tin and copper [3]. Nowadays, gold compounds offer an effective and innovative alternative to platinum drugs and are therefore intensively investigated [4]. Interest in gold compounds is also fuelled by the observation that they usually manifest a very different pharmacological profile compared to established anticancer platinum drugs implying the occurrence of original and innovative modes of action and the chance to overcome platinum resistance [5,6]. Hence, over the last two decades, several promising families of Au-based drug candidates, with the gold center in the oxidation state +3 or +1, featuring diverse structural motifs, were prepared, characterized and their biological and pharmacological profiles initially assessed [7,8]. Relevant examples are offered by a few classical mononuclear gold(III) complexes [9] such as gold(III) dithiocarbamates [10] and gold(III) porphyries [11]; by some organogold(III) compounds [12,13]; a few binuclear gold(III) complexes [14]; various neutral, two-coordinate gold(I) complexes [15], inspired to auranofin; a number of lyophilise cationic gold(I) complexes such as [Au(dope)2]+, and others.

Compounds were mainly driven by their chemical analogy with platinum(II)-based drugs [6]. It was postulated that the biological effects of gold(III) compounds might be a consequence of direct DNA damage as for cisplatin and its analogs. However, in contrast to general expectations, a number of subsequent studies suggested that gold(III) compounds exert their cytotoxicity and antiproliferative effects mainly through DNA independent mechanisms [16]. Notably, it has been shown that these gold(III) compounds are able to trigger apoptosis by a direct mitochondrial damage [1,12,17,18]. Also, there are suggestions that a few peculiar proteins such as thioredoxin reductase, the proteasome or the nuclear factor kb (NF-kb) system may constitute major targets for these gold compounds [1,18–20].

Aubipy$_c$ is a promising gold(III) compound that was characterized chemically and biologically through a few recent studies [21,22]. It consists of a square planar gold(III) center receiving three donors – i.e. C, N, N – from the tridentate bipyridyl ligand while the fourth coordination position is occupied by a hydroxide group (Fig. 1); the latter is the preferential site for ligand replacement reaction and for protein biding. Aubipy$_c$ is acceptably stable under physiological conditions even in the presence of reducing agents. Previous studies revealed that Aubipy$_c$ manifests an appreciable cytotoxicity toward the human ovarian cancer cell line A2780, being able to overcome resistance to platinum. Moreover, it was found that Aubipy$_c$ induces apoptotic cell death [21]. Since this gold compound is a strong inhibitor of thioredoxin reductase, it was hypothesized that cell death proceeds through apoptosis possibly as a consequence of a direct mitochondrial damage [23]. However, details of the molecular mechanism leading to mitochondrial damage and consequent induction of apoptosis remain unexplored and warrant further studies.

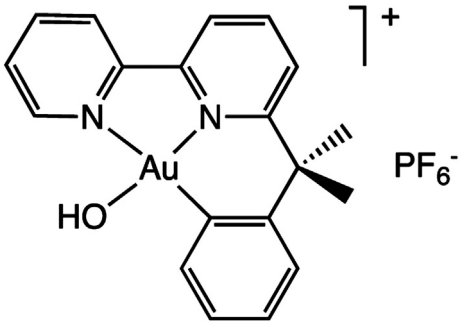

Fig. 1 – Chemical structure of Aubipy$_c$.

Here we have adopted a proteomic approach to better characterize the mechanism of action of Aubipy$_c$. Proteomics is today a very powerful investigation tool of cellular processes providing detailed and direct insight into the fine alterations in cell homeostasis induced by exposure to drugs and xenobiotics. Such alterations occurring at the level of several intracellular signaling and metabolic pathways may tell us which parts of the complex cellular machinery are mainly affected by treatment, also suggesting which parts are the most probable biomolecular targets for a specific compound. Previous studies well documented the application of proteomic approaches to the study of the mechanism of various anticancer metal-based drugs [24]. Studies on platinum compounds revealed cellular responses induced by cisplatin treatment and also disclosed the molecular basis for platinum resistance [25,26]. On the other hand, a number of studies on the proteomic effects of selected gold compounds illustrated the complex responses elicited in cancer cells, highlighting the role of specific defense systems. Some interesting hypotheses were made concerning the likely antiproliferative mechanisms of some gold compounds. Relevant changes in the expression of a number of proteins engaged in redox metabolism, stress response, in mitochondrial functions as well as in apoptosis pathways were detected [11,27]. In previous works we obtained proteomic results for the gold(III) compound Auoxo6 and Auranofin on A2780 cell line, either sensitive (A2780/S) or resistance (A2780/R) to cisplatin. Concerning the A2780/S, the results pointed out that the mode of action of Auoxo6 is strictly related to that of auranofin. Both gold compounds trigger caspase 3 activation and apoptosis and the affected proteins are involved in cell redox homeostasis and stress response [28]. Conversely, in the A2780/R cell line, Auranofin affected the expression of proteasome apparatus proteins whereas Auoxo6 modified the expression of proteins related to mRNA trafficking and stability [29]. More recently, we performed a proteomic study on two additional gold compounds, Au$_2$phen and Aul12 in A2780/S cells. The obtained results were basically in line with those of Auoxo6 and Auranofin in A2780/S cells. We observed few protein expression variations. Moreover, both compounds affected proteins involved in protein synthesis, mRNA splicing and proteins involved in proteasome-mediated protein degradation [30]. However, in all these studies, no underlying mechanism or pathway could be clearly identified.