

In vivo model of xenograft in zebrafish embryos as a model to study the cytotoxicity of abiraterone acetate in adrenocortical carcinoma cells



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Biography

Born in Darfo (Brescia, Italy) on November 14, 1962. 1987: Medical School Degree Summa cum Laude, University of Brescia 1992: PhD in Experimental Pharmacology, University of Milan, 1995: Assistant Professor of Pharmacology, University of Brescia Medical School 1996: Specialist in Biochemistry and Clinical Chemistry 2010: Associate Professor of Pharmacology, University of Brescia Sandra Sigala is an author or co-author of over 90 scientific papers, of which 63 are published in peer-reviewed international journals, 9 in international books and over 20 in Italian journals and books.



Publications

Adrenocortical Carcinoma and CT Assessment of Therapy Response: The Value of Combining Multiple Criteria

Hyperthermic Intraperitoneal Chemotherapy for Primary or Recurrent Adrenocortical Carcinoma. A Single Center Study

Efficacy of the EDP-M Scheme Plus Adjunctive Surgery in the Management of Patients with Advanced Adrenocortical Carcinoma

Cytotoxic Effect of Trabectedin In Human Adrenocortical Carcinoma Cell Lines and Primary Cells

Activity and safety of temozolomide in advanced adrenocortical carcinoma patients

Antineoplastic activity of artemisinin in adrenocortical carcinoma.

Background: Adrenocortical Cancer (ACC) is a rare tumor with an estimated incidence between 0.7 and 2.0 per million population per year (1). Surgery is the only potentially curative treatment modality. Systemic therapies have a limited efficacy and the prognosis of locally advanced or metastatic ACC patients is often dismal. Mitotane is the only drug approved to treat ACC both in adjuvant setting and metastatic disease (2, 3); the drug pharmacokinetics and safety profile, however, limit its efficacy (4). Mitotane can be administered either alone or in association with etoposide, doxorubicin and cisplatin (EDP-M) (5, 6). In this scenario, the introduction of new potentially effective drugs, or the demonstration of efficacy in ACC of already available drugs is of paramount importance. The aim of this study is to provide the first evidence that the zebrafish embryo model is a useful tool to evaluate in vivo cytotoxicity of drugs potentially efficacious on ACC. To do so, we validated in zebrafish embryo the results already obtained in immunodeficient mice xenografted with the NCI-H295R cells treated with abiraterone acetate (AbiAc) (7).

Methods: The experimental conditions for AbiAc absorption in AB zebrafish embryos as embryos number, AbiAc concentrations and absorption time-curve by LC-MS/MS were set up. The AbiAc effect on steroid production in AB zebrafish embryos was as well measured. ACC cells (the cortisol-secreting NCI-H295R cell line and the ACC29 primary cells belonging from a cortisol-secreting ACC patient, and the negative control cells SW13) were treated with the Dili fluorescent dye and about 240 cells/4 nl were injected in the subperidermal space of the yolk sac of AB zebrafish embryos (n=80±10). Cell area was measured with Noldus DanioScope™ software.

Results: AbiAc absorption in AB zebrafish embryos was stage-dependent. Abiraterone (Abi) concentration decreased while its main metabolite, namely $\Delta 4A$, increased. Accordingly, we demonstrated that zebrafish expressed the enzyme 3β -hydroxysteroid dehydrogenase mRNA, that converts Abi in $\Delta 4A$. Furthermore, AbiAc reduced zebrafish embryos cortisol production and increased progesterone. Three days after cell injection (T3), the area of cortisol-secreting ACC cells in solvent-treated embryos was significantly higher compared to 1 μ M AbiAc-treated embryos, while no AbiAc effect was observed in SW13, that lacks the Abi target enzyme CYP17A1. In particular, the inhibition of NCI-H295R cell area growth in AB zebrafish embryos was about 60% after 3 days, which is even higher than that we observed in immune incompetent mice, where it reached the 34% inhibition about 60 days after the cell injection and 15 days after the end of 16 days treatment (7).

Conclusions: Zebrafish embryo xenografted with ACC tumor cells could be a useful, fast and reproducible experimental model to preclinically test the activity of new drugs potentially active in human ACC. We were able indeed to confirm the in vivo cytotoxicity of AbiAc using an animal model which offers several advantages over other models, like mice (8-12). It should be underlined that AbiAc was directly added into the fish water and was significantly adsorbed by embryos, reaching concentrations able to exert the cytotoxic effect, thus simplifying the treatment procedure. Furthermore, the ACC xenograft in zebrafish was fast, as 3 days of AbiAc treatment were sufficient to demonstrate a significant difference in the NCI-H295R and ACC29 cell proliferation rate. The AbiAc effect was due to the direct binding on its target enzyme as the tumor area of xenografts with non steroidogenic (13), CYP17A1 negative (7) SW13 cells was not modified after 3 days exposure to 1 μ M AbiAc, confirming the insensitivity of these cells to the cytotoxic effect of AbiAc treatment (7). In the personalized medicine era, the low number of cells needed and the lower length of the experiments make the zebrafish model potentially candidate to prepare ACC patient-derived xenografts in order to perform a real-time selection of the most appropriate cytotoxic drugs for each patient. On these bases, we reproduced results obtained with the NCI-H295R cell line in a patient-derived xenograft, obtained from primary cells established from a cortisol-secreting ACC, thus giving support to the possibility to develop this in vivo method to screen available therapeutic options for a cancer such as ACC, with a poor prognosis and a scarcity of therapeutic options.

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