Effects of CYP17A1 enzyme inhibition with Abiraterone Acetate in NCl-H295R, a Human Adrenocortical Cancer-Derived Cell Line

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Adrenocortical cancer (ACC) is a rare aggressive tumor with a poor prognosis mostly due to both the advanced stage of the disease at diagnosis and the limited efficacy of therapies when surgery is not curative [1]. ACC could be asymptomatic, while symptoms could be often referred to hormone excess. In approximately 60% of cases, the clinical repercussions of autonomous adrenocortical steroid excess (mostly Cushing's syndrome or rapidly worsening androgenization in women) lead to diagnosis [2]. To date, the pharmacological therapy of advanced ACC is based on mitotane and chemotherapy that have a modest efficacy, the combinantion of the 2 strategies is toxic [3]. Novel treatment strategies are urgently required. Abiraterone acetate (AA), a small molecule that irreversibly inhibits the key enzyme for steroid hormone synthesis 17alpha-hydroxylase/17,20 lyase (CYP17A1), has recently demonstrated marked efficacy in patients with metastatic castration-resistant prostate cancer [4]. CYP17A is an enzyme that catalyzes the sequential hydroxylase (required for cortisol synthesis) and lyase (required for adrenal androgen synthesis) steps that are required for conversion of C21 pregnenolone and progesterone precursors to the C19 adrenal androgens, DHEA and AED [5]. The observation that CYP17A1-dependent pathway is operating in the andrenocortical gland and that most of ACC highly produces steroids hormones [6], led us to investigate whether inhibition of CYP17A1 by AA may exerts antisecretory and antineoplastic activity in ACC cells. For this purpose, NCl-H295R cell line, established from a patient diagnosed with ACC and active secreting steroid hormones was used; SW13 cells, that likely derive from a metastatic adrenal cortex localization and do not produce steroids were used as a control.

Exposure to AA for 4 days induced a concentration-dependent decrease of cell proliferation (IC_{50} value=62.9nM [95%CI: 54.14 to 73.06]) of HCl-H295R cells, but not of SW13 cells, an effect that likely depends on CYP17A1 inhibition. By using qRT-PCR technique, indeed, we found that HCl-H259R cells express high levels of CYP17A1 mRNA while its value was not detectable in SW13 cell line. A time-course using the double staining with acridine orange and ethidium bromide was performed to visualize and quantify the number of viable, apoptotic and necrotic cells in untreated and AA-treated cells. We observed that AA deeply increased the number of apoptotic cells, suggesting that apoptosis could be the main mechanism involved in cancer cell growth inhibition induced by AA. On this line, time-course experiments using both Proteome Profiler Human Apoptosis Array (R&D System) and Western Blot technique demonstrated an increased synthesis of pro-apoptotic proteins, such as 'Death Receptors', after AA-treatment. By using Mass Spectrometry, the CYP17A1 inhibition on steroids synthesis induced by AA in HCl-H295R cells showed an increase of progesterone concentration while cortisol, DHEA, androstenedione and testosterone were drastically reduced.

Interestingly, a combinatory effect of AA and mitotane on cell proliferation was specifically observed in HCl-H295R cells. In particular, we observed a decrease of cell proliferation that reached its maximum of 65.7±3.7% after AA (10nM) and mitotane (1μM) co-treatment.

Combined together, these data indicate that AA exerts both antisecretory and antiproliferative activities, providing for the first time the potential therapeutic role of this compound, alone or in combination with mitotane, in the treatment of ACC.

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[1] Berruti et al.(2008).Ex*pert Opin Emerg Drugs*.13(3):497-509
[2] Koschker et al.(2006).*Exp Clin Endocrinol Diabetes*.114:45–51
[3] Daffara et al.(2008).*Endocr Relat Cancer*.15(4):1043-53
[4] Yang (2011).*Drugs*.71(15):2067-77
[5] Mostaghel.(2014).*Cancer Manag Res.*28(6):39-51
[6] Hecker et al.(2006).*Toxicol Appl Pharmacol.*217(1):114-24