PIK3CA and MAPK molecular analysis in breast cancer cell lines after everolimus treatment.

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The PI3K/AKT/mTOR signaling pathway leads to alteration in multiple cell processes including cell cycle, survival, adhesion, motility and angiogenesis [1]. Since this pathway is frequently deregulated in human cancer [2], the mTOR inhibitor Everolmus (EVE), represents an interesting therapeutic option in breast cancer. In particular, EVE is already employed for the treatment of postmenopausal women with advanced hormone receptor-positive, HER-2 negative breast cancer in association with exemestane, after failure of treatment with letrozole or anastrozole [3].
In order to identify the molecular determinants of sensitivity to EVE, this study is aimed to identify the molecular profile that may predict resistance or sensitivity to mTOR inhibition, evaluating the growth inhibitory effect of EVE at 72h, 96h, 120h and 144h in breast cancer cell lines MCF-7, T-47D, ZR-75-1, CAMA-1, HCC-1500 and MCF-10A non-tumorigenic human epithelial cell line. The different sensitivity of cell lines has been correlated first to the cell line genetic profile of PI3K/mTOR and RAS/ERK/MAPK pathways, then to phosphorylation of mTOR, AKT and ERK after treatment with EVE.
EVE showed different growth inhibitory effects depending on cell line: in non-tumorigenic MCF-10A, cell growth was inhibited with a 'transient' and non-concentration dependent effect. As concerns tumorigenic cell lines, EVE inhibited the growth proliferation in a concentration-dependent manner: in terms of potency and efficacy, ZR-75-1 and CAMA-1 cell lines resulted to be good-responders. Differently, T47D and MCF-7 seemed to be less sensitive to EVE. Instead, HCC1500 showed a '*sui generis*' profile, since EVE inhibited cell growth with an efficacy comparable to T47D, but with a high potency comparable to CAMA-1.
Regarding the molecular analysis on cell lines, MCF-7, T47D and HCC1500 had activating mutations in PIK3CA gene (E545K in MCF-7, H1047R in T47D, and T1025T in HCC1500), while, interestingly, PTEN mutations associated with loss of PTEN have been characterized in EVE responsive cell lines (D92H in CAMA-1, L108R in ZR-75-1).
Concerning the kinase phosphorylation status, basal phosphorylation of mTOR in T47D, a low-responder cell line, resulted to be about 2-fold higher compared to ZR-75-1, a good-responder cell line. EVE 1nM (120h) showed a higher inhibitory effect in ZR-75-1 with a reduction of about 50%. Instead, in T47D mTOR phosphorylation was reduced of about 30%. p-AKT(Ser473) and p-AKT(Thr308) have been also evaluated: basal phosphorylation of AKT both on Ser473 and Thr308 resulted to be significantly higher in PIK3CA mutant T47D cell line, compared to PTEN mutant ZR-75-1. 120h-EVE treatment did not affect phosphorylation level of AKT Ser473 and Thr308 in ZR-75-1. In contrast, in T47D, the EVE concentrations evoked a significant increase of AKT phosphorylation on both aminoacidic residues in a concentration-dependent manner. Due the cross activation of PI3K/AKT/mTOR via MAPK pathway, p-ERK has been assessed: basal level was significantly higher in T47D compared to ZR-75-1. EVE induced increasing ERK phosphorylation in a concentration-dependent manner in T47D. Looking at the clinical practice, detection of activating mutations in PIK3CA and inactivating mutations of PTEN is likely to be a useful biomarker in order to predict response or resistance to EVE in breast cancer patients. Furthermore, monitoring AKT and ERK increasing phosphorylation may early predict development of resistance to treatment in order to switch the therapy.

1. Garcia Z, Kumar A, Marques M, Cortes I, Carrera AC. Phosphoinositide 3-kinase controls early and late events in mammalian cell division. EMBO J 2006;25: 655-661.
2. Parsons DW, Wang TL, Samuels Y, Bardelli A, Cummins JM, DeLong L, et al. Colorectal cancer: mutations in a signalling pathway. Nature 2005;436: 792.
3.Atkins MB, Yasothan U, Kirkpatrick P. Everolimus. Nat Rev Drug Discov. 2009;8:535-536