Molecular profile of tumor response to everolimus in different breast cancer cell lines

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Dysregulation of the PI3K/Akt/mTOR pathway has been recognized in many human tumors and is implicated in the promotion of cancer cell growth and survival. Accordingly, molecules that block the function of mTOR, such as rapamycin and its analogues, including everolimus (EVE), yield antiproliferative activity in a variety of malignances [1].
The purpose of this study is to examine thecorrelationbetween the growth inhibitory effect of EVE in different breast cancer cell lines and the phosphorylation of ERK which could represent a possible mechanism of secondary resistance. Furthermore genetic variants of kinases involved in PI3K/Akt/mTOR pathway have been analyzed in order to find possible genetic biomarkers which could predict the response to EVE.
The effect of EVE on cell proliferation was evaluated using WST-1(Roche) in T47D, ZR-75-1 and HCC-1500 from ductal carcinoma, MCF-7 and CAMA-1 from adenocarcinoma; non-tumorigenic epithelial MCF-10A has been used as reference cell line. Cells were cultured overnight in 96-well plates (3×10^3cells/well). Cells were then treated for 72h, 96h, 120h and 144h with EVE (100nM, 10nM, 1nM and 0,1nM), vehicle (DMSO 0,01%) or control (no-treatment), and their viability was finally assessed. The number of surviving cells in each sample was determined by a spectrophotometer, recording the absorbance at 450nm, after 1h of WST-1 incubation.
The expression of kinases was measured by the Alpha*SureFire* technology (PerkinElmer) accordingly to manufacture's instructions. Molecular analysis on DNA evaluated the mutational status of PI3K, Akt, PTEN, TSC1, TSC2 and mTOR genes.
EVE showed different growth inhibitory effect depending on the cell line. In particular, on CAMA-1 and ZR-75-1 cell lines, EVE inhibited cell proliferation with highest potency (about 1nM) and highest efficacy in a clear concentration-related manner. In contrast, on T47D, HCC-1500 and MCF-7 cell lines, EVE inhibited cell proliferation in a concentration-dependent manner, but with a lower grade of efficacy, despite of a potency of about 1nM. As concern the non-tumorigenic epithelial cell line (MCF-10A), EVE inhibited cell proliferation with a 'transient' and non-concentration dependent effect, which, at 144h, resulted in a full recovery of viability, irrespective of the pharmacological treatment.
Interestingly the most responsive cell lines (ZR-75-1 and CAMA-1) and the less responsive ones (T47D, HCC1500 and MCF-7) show distinct mutational profile. PTEN polymorphisms, that compromise the phosphatase activity of the protein, have been detected in ZR-75-1 (c.323TG) and in CAMA-1(c.274GT) cell lines, while mutations in PIK3CA kinase domain have been detected in MCF-7 (c.1633CA), in T47D (c.3140AT) and in HCC-1500 cell lines (c.3075CT) suggesting a possible correlation between genetic profile and response to treatment.
Beside the genetic data, great attention has been focused on the phosphorylation of ERK which could represent a secondary mechanism of resistance.
Low basal levels of p-ERK have been detected in ZR-75-1 and in CAMA-1 while in T47D, HCC-1500 and MCF-7 p-ERK levels was significantly higher. The 120h-treatment with EVE 0,1 and 1 nM in ZR-75-1, the most responsive cell line, increased of about two-fold p-EKR levels with respect to vehicle. The ZR-75-1 increased p-ERK level was comparable to basal level of p-ERK in T47D, the less responsive cell line; in this cell line, the 120h-treatment with EVE 0,1 and 1nM had no significant effect on p-ERK level.
These preliminary data strongly suggest that, besides the genetic profile, also p-ERK may be viewed as a valuable immunohistochemical biomarker that could predict the response to EVE.
Further studies are needed to validate the reliability of these biomarkers in the clinical practice, in order to specifically identify patients affected by EVE-responsive types of breast cancer, and thus to optimize a highly effective an 'personalized' use of EVE.

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