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# Investigating the role of estrogen receptor beta (ER $\beta$ ) and estrogen-related receptor alpha (ERR $\alpha$ ) in combinatorial drug treatment of triple-negative breast cancer

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Triple-negative breast cancer (TNBC) lacks expression of estrogen receptor alpha (ER $\alpha$ ), progesterone receptor (PgR) and HER2, accounts for ~15% of breast cancer cases, is treated with chemotherapy, has poor prognosis, and is considered non-responsive to targeted hormonal therapy with tamoxifen. Recent studies have shown, however, that TNBC expresses three isoforms of ER $\beta$ , the hormone-binding ER $\beta$ 1 and the orphan ER $\beta$ 2 and ER $\beta$ 5 as well as estrogen-related receptor alpha (ERR $\alpha$ ); and that tamoxifen treatment of ER $\beta$ 1-expressing TNBC is associated with better disease outcome.<sup>1</sup> Tissue microarray IHC assessment of 306 invasive and 40 non-invasive TNBC cases along with 91 samples of normal breast for levels of expression of 5 biomarkers of potential therapeutic response, namely total ER $\beta$ , ER $\beta$ 1, ER $\beta$ 2, p65NFkB and pcJun, revealed association of ER $\beta$ 2 with ER $\beta$ 1 and pcJun in invasive TNBC on top of the normal association of ER $\beta$ 1 with p65NFkB and pcJun. The therapeutic implications for ER $\beta$ 1-positive TNBC are discussed.

TNBC-derived cell line MDA-MB-231 (ER $\alpha$ -/PgR-/HER2-/EGFR+) was found to express ER $\beta$ 2, ER $\beta$ 5 and ERR $\alpha$  but not ER $\beta$ 1 and therefore is a model of ER $\beta$ 1-negative TNBC. Hence, it was used to investigate the role of ER $\beta$ 2/5 and ERR $\alpha$  in the ability of hydroxy-tamoxifen (OHT), potential low-affinity ligand of both ER $\beta$ 2/5 and ERR $\alpha$ , to act synergistically with other targeted drugs in repressing cancer cell growth. Gefitinib (GEF), an EGFR inhibitor, Genistein (GEN) and Trichostatin A (TSA), HDAC inhibitors reportedly restorative of ER $\alpha$  expression, and XCT790, an ERR $\alpha$  inverse agonist, were tested for synergy with OHT against MDA-MB-231 cells. GEN and TSA failed to restore ER $\alpha$  or ER $\beta$ 1 expression. Interestingly, GEF(10 $\mu$ M) and XCT790(10 $\mu$ M) displayed synergy with OHT(1 $\mu$ M). An ER $\beta$ 2/5 knock-down mutant of MDA-MB-231 cells exhibited higher sensitivity to OHT alone or in combination with GEF compared to the mock knock-down mutant, suggesting that ER $\beta$ 2/5 is not the target of OHT. Similarly, the ERR $\alpha$  shRNA knock-down mutant displayed higher sensitivity to GEF and OHT compared to the mock knock-down cells, suggesting that ERR $\alpha$  is also not the target of OHT. Effective synergy of OHT with either of GEF and XCT790 was observed at 7  $\mu$ M OHT, consistent with off-target effect(s) of OHT. Microarray gene expression analysis of MDA-MB-231 cells treated with GEF(10 $\mu$ M) and/or OHT(1 $\mu$ M) provided an insight into the molecular determinants of sensitivity of MDA-MB-231 cells to OHT and GEF when acting alone and in combination.

<sup>1</sup> Honma et al. (2008) *J Clin Oncol* 26: 3727-34

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