Abstract P3-05-19: A new cell panel to study oestrogen receptor loss in acquired endocrine resistant breast cancer

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Abstract

Background: Oestrogen receptor positive (ER+) breast cancer patients can acquire endocrine resistance and 10-20% tumours have lost ER at relapse. While growth factor pathway hyperactivity and ER promoter methylation contribute to de novo ER negativity, ER loss in acquired resistance is largely unexplored. We have recently developed 11 lines from MCF7, T47D, BT474 & MDAMB361 cells to model acquired resistance emerging with prolonged (3 year) endocrine treatment both in ER+/HER2- and ER+/HER2+ disease. Here we establish prevalence of acquired ER loss in the panel, determine any association with aggressiveness, and explore ER loss mechanisms in acquired endocrine resistance.

Methods: Authenticated acquired resistant models derived from endocrine responsive lines cultured for 3 years with 10^{-7}M tamoxifen (TamR), 10^{-7}M fulvestrant (FasR) or oestrogen deprivation (5% charcoal-stripped foetal calf serum SFCSR) were profiled for ER & PR by PCR, immunocytochemistry and Western blotting (+/- 1-2wk antihormone withdrawal), for 2nd-line endocrine responsiveness and for migration using Boyden chamber assays vs. time-matched controls. Src kinase, EGFR, HER2, MAPK & AKT activity were examined and whether their respective inhibition using saracatinib or gefitinib (1μM), trastuzumab (100nM), U0126 or LY294002 (5μM) for 1wk restored ER. ESR1 promoter methylation was examined by bisulfite modification & MethyLight PCR.

Results: Substantial ER mRNA & protein loss occurred in 7/11 long-term acquired endocrine resistant lines. This was irreversible by antihormone withdrawal and paralleled by complete PR loss and endocrine growth-insensitivity. While seen in all fulvestrant resistant lines, ER loss was less frequent with tamoxifen (in MCF7TamR & MDATamR) and only seen in oestrogen deprived resistant T47DSFCSR cells. Increased migration accompanied acquired ER loss in ER+/HER2- derived MCF7TamR, MCF7FasR & T47DSFCSR cells and was saracatinib-sensitive. Src and further growth factor pathway activity increased in several acquired resistant models, and ER loss associated with increased EGFR/HER2 in the MCF7- & MDA-derived cells and with increased MAPK activity in all lines. Weak ER recovery was seen in antioestrogen resistant models treated with saracatinib (MDATamR, MCF7FasR), gefitinib (MDATamR, BT474FasR, MCF7FasR/TamR) or trastuzumab (MCF7TamR). ESR1 DNA methylation was only prominent in MDATamR and MCF7TamR cells. No inhibitor restored ER in the T47D-derived cells, including T47DSFCSR which also lacked ESR1 methylation.

Conclusions: Although ER loss is very prominent in this acquired resistant cell panel, it demonstrates there is capacity of prolonged antihormones, chiefly antioestrogens, to promote receptor loss independent of initial HER2 status. Acquired ER loss clinically would be expected to confer endocrine insensitivity and poorer prognosis given the panel findings. Where ER loss emerged with antioestrogens there was some mechanistic-overlap with de novo ER negativity, including ER promoter methylation for acquired tamoxifen resistance.

Our future studies will use the panel to address if targeting these mechanisms can be optimized for ER recovery or if further mechanisms also drive ER loss in acquired resistance, notably for prolonged oestrogen deprivation.