Integrative molecular profiling of triple negative breast cancers identifies FGFR2 as a potential therapeutic target

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Supplementary figure legends

Supplementary figure 1

a) arrayCGH profiles of MFM223 and SUM52PE with black arrow indicating amplification of *FGFR2*. b) *FGFR2* expression assessed by quantitative RT-PCR in a panel of 40 breast cancer cell lines, relative to median expression on non-amplified cell lines. c) Western blot of cell line lysates probed with antibodies against ERα, PR, HER2 with βACTIN loading control.

Supplementary figure 2

a) Hybridisation of *FGFR2* FISH probe (green) on normal human metaphase demonstrating hybridisation to a single chromosomal region on the long arm of chromosome 10.

b) *FGFR2* copy number per cell assessed by real time copy number PCR expressed relative to LINE-1 DNA content (MCF7 2, HCC1143 3.1, MFM223 199, SUM52PE 140). Error bars SEM.

Supplementary figure 3

a) Sensitivity of breast cancer cell lines to FGFR2 siRNA, demonstrating sensitivity of MFM223 (red) to FGFR2 silencing. Cell lines were transfected with FGFR2 siRNA, PLK1 siRNA as a positive toxicity control to assess transfection efficiency, or siCON non-targeting control, and survival assessed at 5-7 days post transfection with Cell Titre-Glo® cell viability assay (Promega). Survival of FGFR2 siRNA and PLK1 siRNA

transfected cells was expressed relative to that of siCON transfected. b) Western blot of lysates from MFM223 and CAL51 transfected 72 hours earlier with siFGFR2 or siCON, demonstrating knockdown of FGFR2 in both cell lines. Two different exposures of FGFR2 are shown, with β ACTIN loading control.

Supplementary figure 4.

a) MFM223 were grown in 10% serum or in serum free conditions, and growth assessed after 72hrs. b) MFM223 were grown for 72 hrs in 10% serum or in serum free conditions, and media was supplemented with a range of concentrations of PD173074 pan FGFR tyrosine kinase inhibitor, and survival expressed relative to that of untreated cells. c) Fraction of MFM223 cells grown in serum free conditions in subG1, as assessed by PI FACS, after 24 hours exposure to 1μM PD173074. Error bars SEM. $P<0.01$ (Student's T test).

Supplementary figure 5

a) MFM223 cells were treated for 1 hour prior to lysis with 1μM PD173074, 250nM BEZ235, or both inhibitors. Lysates were subject to SDS-PAGE and western blotting with indicated antibodies. b) PI cell cycle profiles of MFM223 after 24 hours exposure to 1μM PD173074, BEZ235 250nM, or combination of both inhibitors. c) Combination index (CI) assessed using Median Effect Model of MFM223 treated with PD173074 and BEZ235, plotted against fractional effect of the combination. Combined CI of 1.02 indicating additive effect, Error bars SEM. d) Sequencing of *PI3KCA* exon 20 indicating heterozygous 3140A>G mutation in MFM223 (kinase domain H1047R mutation). e) Western blot of indicated cell line lysates probed with antibodies against PTEN (138G6, 9559 Cell Signaling) with β ACTIN loading control. SUM52PE do not express PTEN.

Supplementary Methods

Quantitative RT-PCR

Quantitative RT-PCR: cDNA was synthesised from RNA using Superscript III and random hexamers (Invitrogen) as per manufacturers instructions. Quantitative PCR was performed on the ABI Prism 7900T system (Applied biosystems) using standard curve method. Expression of *FGFR2* was assessed with QuantiTect SYBR Green PCR Kits (Qiagen) and Forwards primer 5'-TGATGATGAGGGACTGTTGG-3' and Reverse 5'-TTCTTGTGTCAGGGTAACTAGGTG-3'. *FGFR2* was expressed relative to the levels of endogenous controls S18 (Hs02387368_g1), MRPL19 (Hs00608519_m1) and β-ACTIN (4310881E) (All Taqman chemistry, Applied biosystems). All reactions were performed individually, with the exception of MRPL19 and β-ACTIN which were performed in multiplex.

Serum free culture

MFM223 were plated in routine 10% serum medium, the following day washed X2 with PBS, and media replaced with DMEM advanced (Invitrogen) with no serum supplemented and 2mM L-glutmaine (Sigma). Following 24 hours, media was replaced a further time and cells grown in a range of doses of PD173074 for 72 hours.

FGFR2 real-time copy number PCR

5ng of Genomic DNA was amplified using Quantitect SYBR green (Qiagen) in a 20 ul reaction by absolute quantification 7900HT Fast Real-Time PCR System (Applied biosystems). Primers *FGFR2* (Forwards 5'- TCCTTCGGGGTGTTAATGTG -3', Reverse 5'- GTTGGCTGGCTTATCCATTC -3' corresponding to exon 16) and *LINE*-

1 DNA concentration control (For 5'-TGGCACATATACACCATGGAA-3', Rev TGAGAATGATGGTTTCCAATTTC-3'). Diploid breast cancer cell lines CAL51 DNA was used derive standard curves. Amplification was defined as a relative copy number >2 *FGFR2/LINE-1* relative to the copy number of CAL51.

PIK3CA sequencing

DNA was amplified with AmpliTaq Gold (Invitrogen) (Exon 9 For 5'-GGGAAAATGACAAAGAACAGC-3' Rev 5'-ACATGCTGAGATCAGCCAAA-3', Exon 20 For 5'-TATTCGACAGCATGCCAATC-3' Rev 5'- TGTGTGGAAGATCCAATCCA-3') and PCR products subject to direct sequencing with amplification primers.