SUPPLEMENTAL MATERIAL

Figure S1. Effect of AG490 on the gene expression profile in response to R5020.

T47D-MTVL cells were serum-starved for 48 h and then pre-treated or not with AG490 1 h prior to treatment with ethanol or R5020 10 nM for 6 h, in duplicate. Cells were harvested and RNA was extracted for microarray hybridization. A customized human cDNA microarray containing 826 genes of interest to breast cancer and steroid hormone regulation was used. Full microarray data is available at GEO with accession number GSE9286 (http://www.ncbi.nlm.nih.gov/geo/index.cgi).

The figure shows the set of genes in which progesterone induction (A) or repression (B) was higher than 1.3-fold in the absence of AG. A graphical representation of the data constructed with the TM4 software package from TIGR is presented in the left panel (see Supplemental Materials and Methods).

Average R5020 / EtOH fold induction in the absence or presence of AG inhibitor, is indicated for these genes (middle columns). The right column also shows the effect of AG 490 on the basal expression of these genes, represented as fold change EtOH + AG / EtOH –AG. Fold changes higher than 1.3 are in red, lower than –1.3 in blue. Values representing the most differently expressed genes according to the Significance Analyses of Microarrays (SAM) method included in the TM4 software package, using q-values ≤0.05 as a cutoff, are green- or blue-boxed.

Fold-changes in response to hormone compared to vehicle, both in the absence or presence of AG, obtained by RT-real time PCR are also shown for selected genes.

Supplemental Material and Methods: Microarray hybridization and data analysis.

A cDNA microarray platform containing 826 cDNA clones that were selected for its involvement in breast cancer was generated (B. Miñana, L. Sumoy, M. Beato, A. Jordan, C. Ballare, M. Melia; GEO accession number GPL5953; http://www.ncbi.nlm.nih.gov/projects/geo/index.cgi). cDNA inserts were PCR amplified and spotted on Corning UltraGAPS amino-modified glass slides. mRNA samples were processed for first and second strand cDNA synthesis and in vitro transcription

with T7 RNA polymerase, basically as reported elsewhere [2, 6]. Universal reference RNA was obtained from Statagene. RNA was directly labeled with Cy3- or Cy5-dUTP (Amersham) and hybridized to spotted slides as described [4]. After washing, fluorescent images were obtained using a G2565BA Microarray Scanner System (Agilent) and TIFF images were quantified using GenePix 6.0 (Molecular Devices) software.

Raw data was processed using MMARGE, an in house developed web implementation of LIMMA, a microarray statistical analysis package of Bioconductor (http://www.bioconductor.org) that is run in the R programming environment [1, 3, 7]. Gene intensities were background subtracted (taking mean of channel intensities and background median). Spots with intensities <2 times the local background in one or both dye filter channels (Cy3 or Cy5), as well as controls, were excluded from normalization, and were referred to as "not reliable". An intensity dependent normalization algorithm (global lowess) was applied using a smoothing factor f=0.2 for all experiments. Normalized Log_2Ratios (Intensity Cy5/Intensity Cy3) were scaled so that they all had the same median absolute standard deviation across all the arrays, to give the same weight to each gene, and not only due to the magnitude of the expression ratio [8]. The computed B statistic rank value from all replicate hybridizations was used to determine the genes with significant changes. We considered genes that showed a 1.3-fold gene up or down-regulation relative to the control sample with a B-rank value above the 90th percentile worthy of further study. The value of fold change or copy number relative change was calculated as $2^{Log2Ratio}$, if the value of the ratio was >0, or $2^{-1/Log2Ratio}$, if it was <0.

In order to determine the most differentially expressed genes, we used the open-source, freely available software package for microarray data management and analysis TM4 obtained from TIGR (http://www.tigr.org/software/; [5]) that applies the Significance Analyses of Microarrays (SAM) method.

Supplemental References

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