Karyotype of MCF-7 cells

The established breast cancer cell line MCF-7 was obtained from the American Type Culture Collection (ATCC HTB-22, ATCC, Manassas, VA). Cells were cultured in Modified IMEM (Gibco/Invitrogen, Carlsbad, CA.) with 10% FBS (Atlanta Biologicals, Lawrenceville, GA.) and Pen-Strep (Gibco/Invitrogen). Harvesting of cells, fixation, and preparation of metaphase spreads and G-banding was performed using standard procedures (1, 2). Karyotypes were described according to the current ISCN (3).

Supplementary Figure 1 shows the G-banding pattern and karyotype of the MCF-7 cells used in these experiments. The modal number of chromosomes in the MCF-7 cells = 67; range = 55 to 68. Chromosome numbers were in the near-triploid range. There were multiple marker chromosomes including one large submetacentric chromosome and two large subtelocentric chromosomes. Chromosome 9 was monosomic and chromosome 20 was monosomic or nullisomic in all cells. Only one cytogenetically identifiable X chromosome was present per cell. One derivative X chromosome was present clonally, add(X)(q27~28). One derivative chromosome 19 was also present clonally, add(19)(p13). Many additional rearrangements appear to be present which could not be verified by G-banding alone.

The karyotype could not be completely characterized by G-banding. The composite G-band karyotype is described with reference to triploidy, according to ISCN convention: 55-69,add(X)(q27~28)[2],-X[5],-X[5],?add(3)(q21)[4],-9[5],-9[5],-12[5],-13[5],-13[3],-14[5],-15[5],-16[5],-16[3],-17[5],-18[5],-19[3],add(19)(p13)[2],-20[5],-20[5],-21[5],-22[5],+12~19mar[5] [cp5],inc.

These observations are consistent with the cytogenetic and molecular cytogenetic studies for MCF-7 cells reported by other investigators (4-7).

ERα and ERβ expression in MCF-7 cells

Our previous demonstration of ERβ protein in MCF-7 cells was based on detection by western blot with ERβ antibodies H150 and N19 (from Santa Cruz Biotechnology) (8). We reported that ERβ expression in MCF-7 was 4.1 ± 0.4 fmol/μg whole cell extract protein (WCE) based on 13 different western blots quantified against rhERβ (baculovirus expressed and quantitated by specific [^{3}H]E_2 assay) standards. Concomitantly, using monoclonal ERα antibody AER320 (Neomarkers) in western analyses, the estimated ERα protein expression was about half the level of ERβ = 2.0 ± 0.4 fmol/μg WCE (8). In that publication we noted that lower specific [^{3}H]E_2 binding, *i.e.*, 0.11-0.16 fmol ER/μg WCE was detected in HAP assays, indicating, as established long ago by many investigators (for example (9, 10)), a loss of E_2 binding ability of ER proteins in extracts from cells.

Although numerous investigators have reported ERβ expression in MCF-7 cells (11-51), the specificity of ERβ antibodies remains a concern. We previously reported that H150 and AER320 recognized ERβ and ERα, but not the other ER subtype, respectively demonstrating antibody specificity (52). The specificity of the Santa Cruz N19 ERβ antibody was demonstrated in work from another lab (53). We note that we have obtained consistent values for ERβ expression as estimated by quantitative western blot using MCF-7 purchased directly from ATCC at various times from Sept. 2004-Nov. 2006.

SFig2A and B shows western blots for ERβ using 2 other ERβ antibodies: PA1-311 and MA1-23217 (Affinity Bioreagents). In SFig2A, we also compare ERβ in MCF-7 obtained from ATCC *versus* MCF-7 obtained directly from the Karmanos Cancer Center in 2004. The calculated concentration of ERβ in the 55 kDa band is 4.7 and 2.2 fmol/μg WCE protein for MCF-7 ATCC and MCF-7 Karmanos, respectively. SFig2B shows that monoclonal ERβ antibody MA1-23217 recognized ERβ in MCF-7 WCE, but did not recognize rhERα. We note the protein degradation of the WCE used in the right panel of SFig2B.

Absolute quantification of ERα and ERβ1 mRNA by quantitative real-time PCR.

To address the issue of mRNA expression of ERα and ERβ, quantitative real-time RT-PCR was performed starting with 100 ng of RNA from MCF-7 cells obtained from ATCC and using pCDNA3.1 expression plasmids for hERα and hERβ1 (54) for absolute quantification. A standard curve was generated from a dilution series of template DNA of pCDNA 3.1- ERα and pCDNA3.1-ERβ plasmids of known concentrations (Figure 2D). The standard curve was represented graphically as a plot of threshold cycle, C(t) against the logarithm of the amount of DNA (Figure 2D). The slopes of -3.105 and -2.77 indicates 100 % efficiency of the PCR with ERα and ERβ primers, respectively (http://www.finnzymes.fi/pdf/dynamo_cdna_synthesis_kit_f470_1_0.pdf.).

$$\text{PCR efficiency} = ((10^{\frac{-1}{\text{slope}}}) - 1) \times 100\ \%$$

The amount of cDNA for ERα and ERβ from RNA samples prepared from the MCF-7 cells was calculated by linear regression analysis. All of the samples were amplified in triplicate and real-time PCR amplifications were repeated with two separate samples of the MCF-7 cells harvested at different time and normalized to 18S. These data indicate that the cDNA contained 0.12 pg of ERα and 0.24 pg of ERβ, indicating similar levels of the ERα and ERβ mRNA in the MCF-7 cells obtained from ATCC and grown in IMEM supplemented with 10% FBS and pen-strep.

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