Ah-NONRESPONSIVENESS IN MDA-MB-231 HUMAN BREAST CANCER CELLS IS RELATED TO A TRUNCATION IN THE ARNT PROTEIN

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1. Abstract

The aryl hydrocarbon (Ah)-responsiveness of MCF-7 and MDA-MB-231 human breast cancer cell lines was determined by induction of CYP1A1 mRNA levels and ethoxyresorufin-*o*-deethylase (EROD) activity with 2, 3 ,7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD). CYP1A1 mRNA levels and EROD activity were induced in the MCF-7, but not the MDA-MD-231 cell lines. Both cell lines expressed the nuclear Ah-receptor complex which bound to a ^{32}P-labelled consensus dioxin responsive element (DRE) in a gel mobility shift assay. Photoaffinity labelling indicated that the ligand binding subunit from both cell lines exhibited the same apparent molecular mass (110-kDa), whereas the Mr values calculated from the hydrodynamic properties of the nuclear Ah-receptor complex varied from 175-kDa (MDA-MB-231) to 221-kDa (MCF-7). Subsequent studies with these two cell lines indicate that differences in their Ah-responsiveness are related to the aryl hydrocarbon receptor nuclear translocator (*arnt)* gene product and in MDA-MB-231 transiently or stably transfected with the *arnt* gene Ah-responsiveness is restored. Moreover, PCR analysis has revealed a size difference in *arnt* of approximately 1 kb between the two cell lines.

2. Introduction

The apparent molecular mass of the mammalian cytosolic Ah receptor complex is 250- to 300-kDa and contains the Ah receptor ligand-binding protein and possibly two associated heat shock protein 90 subunits (1); in contrast, the molecular mass of the transformed cytosolic nuclear Ah receptor complex is approximately 180- to 200-kDa (1).

Recent studies have shown that the nuclear Ah receptor complex is a heterodimer which consists of the Ah receptor ligand binding subunit and the Arnt protein which is important for the nuclear localization of the liganded receptor complex (2,3). The genes for both the Ah receptor and Arnt proteins have recently been cloned and both proteins contain a helix-loop-helix motif which is important for interaction of the complex with DNA (4-6). It has also been reported that there is considerable variability in the apparent molecular masses of the Ah receptor ligand binding protein among different species and in strains of mice and rats. The Mr values for the photoaffinity-labelled Ah receptor varies from 95-kDa (mouse) to 124-kDa (hamster).

Although a number of studies have reported the molecular properties of the Ah receptor from several human tissues and cell lines, it has not been possible to determine whether there is a correlation between specific properties of the human Ah receptor complex and Ah-responsiveness. The trait of Ah-responsiveness and the inducibility of *CYP1A1* gene expression as characterized by aryl hydrocarbon hydroxylase or ethoxyresorufin-*o*-deethylase (EROD) activity has been correlated with an increased susceptibility to bronchogenic carcinoma in cigarette smokers compared to Ah-

nonresponsive smokers. Since CYP1A1 inducibility is dependent on the Ah receptor complex, it is possible that alterations of the proteins and genes associated with this complex may also be contributing factors in cancer risk. This paper reports one factor which influences the variability of Ah responsiveness in two different human breast cancer cell lines.

3. Materials & Methods

Cells, Chemicals and Biochemicals: The MCF-7 (breast adenocarcinoma) and MDA-MB-231 (breast adenocarcinoma) human cell lines were obtained from the American Type Cell culture collection. [^{3}H]TCDD (32 Ci/mmol) and unlabeled TCDD are routinely prepared in this laboratory (>98% pure by gas chromatographic analysis). All other chemicals and biochemicals used in this study were of the highest quality available from commerical sources.

Cell Growth: Cells were grown in minimum essential medium and supplemented with 5% fetal bovine serum plus 10 ml antibiotic-antimycotic solution, 2.2g sodium bicarbonate per liter. Cells were grown in 150 cm^2 culture flasks in an air:carbon dioxide (95:5) atmosphere at 37 °C. Radioligands in DMSO were added to the cell culture flasks so that the final concentration of DMSO in the culture medium was 0.5%. The flasks were incubated for 2 h at 37 °C.

Sucrose Density Gradient Analysis: Sucrose density analysis of nuclear extracts was performed as previously described (7).

Northern Blot Analysis: Phenol:chloroform extraction of total RNA from cells at 60% confluency was carried out according to the described procedure. RNA was separated on a 1.2% denaturing agarose gel, transferred according to (8), probed with a ^{32}P-labeled CYP1A1 cDNA probe and quantitated on a Betagen Betascope 603.

Ethoxyresorufin-O-Deethylase (EROD) Activity: EROD activity was assayed as described by (9).

RT-PCR: PCR reagents were purchased from Perkin-Elmer and all experiments were carried out using a Perkin-Elmer GeneAmp PCR System 9600. PCR primers were directed to a full length *arnt* product and were synthesized by the Gene Technologies Laboratory, TAMU.

4. Results and Discussion

The Ah-responsiveness of the two human breast cancer cell lines was evaluated by determining the induction of *CYP1A1* gene expression by TCDD. Minimal or no induction was observed for the MDA-MB-231 cells, whereas an ED_{50} value for the MCF-7 cells was 0.5±0.02 nM, as determined by log probit analysis of TCDD induced EROD activity. CYP1A1 mRNA levels induced by 10 nM TCDD were determined by Northern blot analysis. Increased gene expression was observed only in the MCF-7 cell line (8.3-fold induction), whereas no induction was observed in the MDA-MB-231 cells.

Velocity sedimentation analysis of the nuclear Ah receptor complexes gave sedimentation coefficients of 6.40 and 7.23 S for the MDA-MB-231 and MCF-7 human breast cancer cell lines, respectively. There was significant variability in the calculated Mr values for the nuclear Ah receptor complexes which varied from 175 to 221-kDa for the MDA-MB-231 and MCF-7 cells, respectively.

The results in Figure 2 summarize the Northern analysis of arnt mRNA derived from MCF-7 and MDA-MB-231. These data illustrate that a single lower molecular weight transcript is observed for MDA-MB-231 cells (lane B) whereas an additional higher molecular weight mRNA is observed in Ah-responsive MCF-7 cells (lane A).

Figure 1 illustrates a ~1 kb size difference between the PCR-amplified region of the arnt transcript from wild-type MDA-MB-231 cells (lane A) and MCF-7 cells (lane B). Primers (21-mers) were designed using a previously published arnt mRNA sequence (4) and were directed at amplification of ~2-kb transcript; 1 µg total RNA (untreated) was used in reverse transcription, followed by 35 cycles of PCR.

These results indicate that the nuclear Ah receptor complex from both human cell lines contain the Arnt and ligand binding Ah receptor proteins, and therefore differences in the Mr values for the nuclear AhR complex in the MCF-7 and MDA-MB-231 cell lines appear to be associated with variability in the Arnt protein. In order to determine the deleted regions within the *arnt* gene, we have begun sequencing of the products from PCR.

Assay	MDA-MB-231 cells	MCF-7 cells
EROD Inducibility	no activity	0.5±0.02 nm ED_{50}
Gel Mobility Shift	binds DRE	binds DRE
Hydrodynamic Properties	6.40S, 175-kDa	7.23S, 221-kDa
Northern, arnt mRNA	low MW mRNA	wt MW mRNA
RT-PCR	~1-kb product	~2-kb product
Photoaffinity labeling (ligand-binding subunit)	Mr = 110-kDa	Mr = 110-kDa

Table I. Summary of assays and results in both human cell lines.

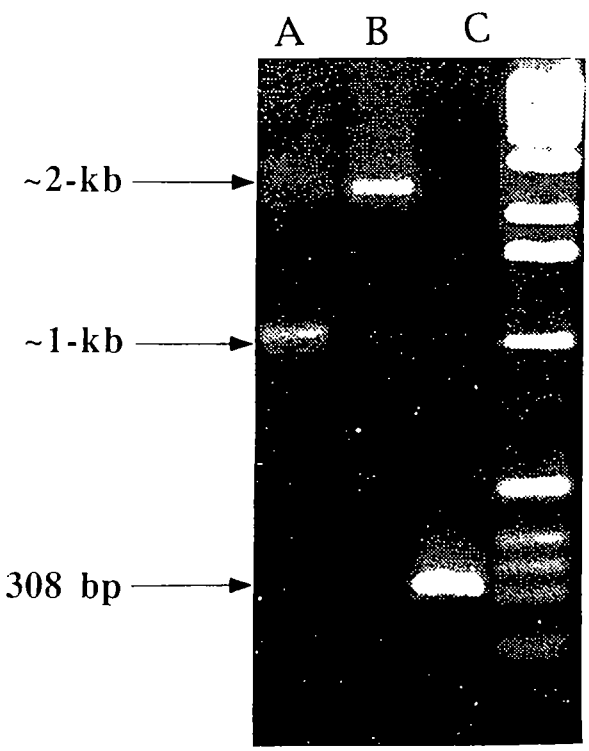
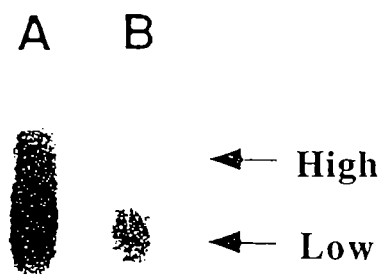


Figure 1. RT-PCR of total RNA from MDA-MB-231 (lane A) and MCF-7 (lane B) cells. Lane C is pAW109 positive control.

Figure 2. Northern analysis of hArnt mRNA from MCF-7 (lane A), and MDA-MB-231 cells (lane B).



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