**Carcinogenesis** vol.22 no.9 pp.1521–1526, 2001

# Quantitation of androgen receptor gene expression in sporadic breast tumors by real-time RT–PCR: evidence that *MYC* is an AR-regulated gene

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**Little is known of the function and clinical significance of the androgen receptor (AR) in human breast cancer. Paradoxically, synthetic progestins, such as medroxyprogesterone acetate, are used for second line hormone therapy of breast cancer following tamoxifen failure. A sensitive and accurate assay for AR expression in breast tumors is thus required. Here we have developed and validated a real-time RT–PCR assay to quantify *AR* gene expression at the mRNA level in a series of 131 patients with unilateral invasive primary breast tumors. *AR* expression varied widely in tumor tissues (by at least 3 orders of magnitude), being underexpressed in 24/131 (18.3%) and overexpressed in 45/131 (34.4%) relative to normal breast tissues. We observed links (or trends) between AR status and age, menopausal status, Scarff–Bloom–Richardson histopathological grade, lymph node status and estrogen receptor α and progesterone receptor status. High *AR* mRNA levels were negatively linked to *MYC* gene overexpression ($P = 8 \times 10^{-6}$), confirming previous *in vitro* studies. Our results also suggest a role of the *ARA70* gene (which encodes a major AR co-activator) in the AR pathway dysregulation observed in breast cancer. This simple, rapid and semi-automated method will be useful for screening cancer patients for altered AR expression and for predicting the response to androgen therapy in AR-related cancer patients.**

## Introduction

The role of estrogen receptor (ER) α and the progesterone receptor (PR) in human breast cancer is well established. Considerably less is known about the functional role and clinical significance of androgen receptor (AR) expression in this setting. Biochemical and immunohistochemical studies show that AR-positive tumors are more frequent (70–90%) than ERα-positive and PR-positive tumors (60–80 and 50–70%, respectively) (1–4). Although ERα, PR and AR are frequently co-expressed in breast tumors, ~10% of AR-positive tumors and, perhaps more importantly, 25% of AR-positive tumor metastases can be negative for ERα and PR (1,5). Androgens have been shown to regulate the proliferation of AR-positive breast cancer cell lines in culture (6). Synthetic progestins, such as medroxyprogesterone acetate (MPA), are used as second line hormone therapy for breast cancer following tamoxifen failure (7). Birrell *et al*. (8) suggested that the antiproliferative effect of MPA in advanced cancer is mediated by AR. *In vitro* studies confirmed that MPA inhibits the proliferation of ERα-negative and PR-negative cell lines via AR (9).

Taken together, these findings suggest that AR determination may give additional predictive information on the response to endocrine treatments in breast cancer. AR expression has mainly been studied by means of a cytosol steroid-binding assay and immunohistochemistry. Although the former measures the status and functionality of the protein, it has several methodological shortcomings (1) and is time consuming. Furthermore, it requires the use of radioactive reagents and large amounts of tumor tissue, so that it is rarely used routinely in clinical laboratories. Immunohistochemical methods suffer from a lack of inter-laboratory standardization and cannot quantify the full range of alterations. However, this method also gives information concerning the status of the protein, but above all measures alterations on an individual cell basis.

We quantified *AR* mRNA expression in a series of 131 patients with unilateral invasive primary breast tumors, using real-time quantitative RT–PCR assay. This recent method of nucleic acid quantification in homogeneous solutions has the potential to become a standard in terms of its performance, accuracy, sensitivity, wide dynamic range, high throughput capacity and inter-laboratory agreement, and also yields statistical confidence values (10).

We examined the relationship between *AR* expression status and classical clinical and pathological parameters, including patient outcome. *AR* mRNA levels were interpreted according to *ERα*, *ERβ* and *PR* transcript levels measured using the same methodology and on the same homogeneous total RNA solutions.

We also sought relationships between *AR* expression and that of genes known to be altered in breast cancer (*RB1*, *CCND1*, *MYC* and *ERBB2*), as well as several major genes involved in different steps of the AR pathway dysregulation observed in prostate cancer, i.e. the *ARA70* gene (which codes for a major AR co-activator) (11), two well-known AR-responsive genes in prostate cancer (*PAP*, coding for prostatic acid phosphatase, and *PSA*, coding for prostate-specific antigen) (12) and *DNMT1*, a DNA methyltransferase gene that is altered in tumors (13), because loss of *AR* expression is associated with methylation of the *AR* promoter in prostate cancer cells (14).

## Materials and methods

*Patients and samples*

We analyzed tissue from primary breast tumors excised from 131 women treated at the Centre René Huguenin from 1977 to 1989. Tumor tissue samples of the 131 patients were collected in accordance with French regulations.

**Abbreviations:** AR, androgen receptor; ER, estrogen receptor; PR, progesterone receptor; MPA, medroxyprogesterone acetate; SBR, Scarff–Bloom–Richardson.

**Table I.** Characteristics of the 131 patients and relation to disease-free survival

| | No. of patients | Disease-free survival | |
|---|---|---|---|
| | | No. of events (%)[a] | *P* value[b] |
| Age | | | NS |
| ⩽50 | 39 | 12 (30.8) | |
| >50 | 92 | 35 (38.0) | |
| Menopausal status | | | NS |
| Pre-menopausal | 45 | 16 (35.6) | |
| Post-menopausal | 86 | 31 (36.0) | |
| Histological grade[c] | | | NS |
| I + II | 76 | 30 (39.5) | |
| III | 46 | 16 (34.8) | |
| Lymph node status | | | 0.026 |
| Node-negative | 49 | 10 (20.4) | |
| Node-positive | 82 | 37 (45.1) | |
| Macroscopic tumor size[d] | | | NS |
| ⩽30 mm | 90 | 32 (35.6) | |
| >30 mm | 34 | 13 (38.2) | |

[a]First relapses (local and/or regional recurrences and/or metastases).
[b]Log rank test.
[c]SBR classification. Information available for 122 patients.
[d]Information available for 124 patients.

The samples were examined histologically for the presence of tumor cells. A tumor sample was considered suitable for this study if the proportion of tumor cells was >60%. Immediately following surgery the tumor samples were stored in liquid nitrogen until RNA extraction.

The patients (mean age 58.2 years, range 34–91) met the following criteria: primary unilateral non-metastatic breast carcinoma on which complete clinical, histological and biological data were available; no radiotherapy or chemotherapy before surgery. The main prognostic factors are presented in Table I. The median follow-up was 8.1 years (range 1.0–15.9). Forty-seven patients relapsed (the distribution of first relapse events was as follows: 13 local and/or regional recurrences, 30 metastases and 4 both).

Specimens of adjacent normal breast tissue from nine of the breast cancer patients and normal breast tissue from three women undergoing cosmetic breast surgery were used as sources of normal RNA.

*Real time RT–PCR*

*Theoretical basis*. Quantitative values were obtained from the threshold cycle number at which the increase in the signal associated with exponential growth of PCR products begins to be detected using PE Biosystems analysis software, according to the manufacturer's manuals.

The precise amount of total RNA added to each reaction mix (based on optical density) and its quality (i.e. lack of extensive degradation) are both difficult to assess. We therefore also quantified transcripts of the *RPLP0* gene (also known as *36B4*) encoding human acidic ribosomal phosphoprotein P0 as an endogenous RNA control and each sample was normalized on the basis of its *RPLP0* content. The relative *AR* gene expression level was also normalized to a calibrator, or 1× sample, consisting of a pool of normal breast tissue specimens. Final results, expressed as *n*-fold differences in *AR* gene expression relative to the *RPLP0* gene and normal breast tissues (the calibrator), termed $n_{AR}$, were determined in exponent as follows:

$$n_{AR} = 2(\Delta Ct_{sample} - \Delta Ct_{calibrator})$$

where $\Delta Ct$ values of the sample and calibrator are determined by subtracting the average *Ct* value of the *AR* gene from the average *Ct* value of the *RPLP0* gene.

*Primers and PCR consumables*

Primers for the *RPLP0* and target genes were chosen with the assistance of the computer programs Oligo 4.0 (National Biosciences, Plymouth, MN) and Primer Express (Perkin-Elmer Applied Biosystems, Foster City, CA). We conducted BLASTN searches against dbEST, htgs and nr (the non-redundant set of the GenBank, EMBL and DDBJ database sequences) to confirm the total gene specificity of the nucleotide sequences chosen for the primers and the absence of DNA polymorphisms. The nucleotide sequences of the primers are shown in Table II. To avoid amplification of contaminating genomic DNA, one of the two primers was placed in a different exon.

*RNA extraction*

Total RNA was extracted from breast specimens using the acid phenol/guanidium method. The quality of the RNA samples was determined by electrophoresis through agarose gels and staining with ethidium bromide and the 18S and 28S RNA bands were visualized under UV light.

*cDNA Synthesis*

RNA was reverse transcribed in a final volume of 20 µl containing 1× RT buffer (500 mM each dNTP, 3 mM $MgCl_2$, 75 mM KCl, 50 mM Tris–HCl, pH 8.3), 10 U RNasin RNase inhibitor (Promega, Madison, WI), 10 mM dithiothreitol, 50 U Superscript II RNase $H^-$ reverse transcriptase (Gibco BRL, Gaithersburg, MD), 1.5 mM random hexamers (Pharmacia, Uppsala, Sweden) and 1 µg total RNA. The samples were incubated at 20°C for 10 min and 42°C for 30 min and reverse transcriptase was inactivated by heating at 99°C for 5 min and cooling to 5°C for 5 min.

*PCR amplification*

All PCR reactions were performed using an ABI Prism 7700 Sequence Detection System (Perkin-Elmer Applied Biosystems). PCR was performed using the SYBR Green PCR Core Reagents kit (Perkin-Elmer Applied Biosystems). The thermal cycling conditions comprised an initial denaturation step at 95°C for 10 min and 50 cycles at 95°C for 15 s and 65°C for 1 min. Experiments were performed in duplicate for each data point.

*Statistical analysis*

Relapse-free survival was determined as the interval between diagnosis and detection of the first relapses (local and/or regional recurrences and/or metastases).

Clinical, histological and biological parameters were compared using the $\chi^2$ test, with Yates' correction for adjustment of the continuity of the $\chi^2$ distribution where appropriate. Differences between the two populations were judged significant at confidence levels >95% ($P < 0.05$). Survival distributions were estimated by the Kaplan–Meier method (15) and the significance of differences between survival rates was ascertained using the log rank test (16).

## Results

### *AR mRNA expression in normal breast tissues*

To determine the cut-off point for altered *AR* expression in breast cancer tissue, the $n_{AR}$ value, calculated as described in Materials and methods, was determined for 12 normal breast RNA samples. As this value consistently fell between 0.70 and 1.61 (1.15 ± 0.27, mean ± SD), values of 2 (mean + 3 SD) or more were considered to represent overexpression and values of 0.35 (mean – 3 SD) or less were considered to represent underexpression of *AR* mRNA.

### *AR mRNA expression in tumor breast tissues*

The 131 breast tumor RNA samples tested had a wide range of $n_{AR}$ values (0.008–10.3, i.e. at least 3 orders of magnitude). Compared with normal breast tissues, 69 (52.7%) tumors showed altered *AR* mRNA expression. Twenty-four tumors (18.3%) showed *AR* mRNA underexpression ($n_{AR}$ 0.008–0.31) and 45 (34.4%) showed overexpression ($n_{AR}$ 2.04–10.3). *AR* mRNA levels were similar to those observed in prostate tumor tissues (data not shown).

### *Correlation between AR mRNA levels and clinical and pathological parameters*

We sought links between *AR* mRNA expression status and standard clinical and pathological factors in breast cancer (Table III). Links (or trends) were found between *AR* gene status and age ($P = 0.063$), menopausal status ($P = 0.070$), Scarff–Bloom–Richardson (SBR) histopathological grade status ($P = 0.00083$) and lymph node status ($P = 0.049$). Patients with tumors overexpressing and/or underexpressing *AR* did not relapse more frequently (Table III) and did not have significantly shorter relapse-free survival after surgery (log rank test) compared with patients with tumors normally expressing AR.

**Table II.** Oligonucleotide primer sequences used

| Gene | Oligonucleotide | Sequence | PCR product size (bp) |
|---|---|---|---|
| *RPLP0* | Upper primer | 5′-GGC GAC CTG GAA GTC CAA CT-3′ | 149 |
| | Lower primer | 5′-CCA TCA GCA CCA CAG CCT TC-3′ | |
| *AR* | Upper primer | 5′-CCT GGC TTC CGC AAC TTA CAC-3′ | 168 |
| | Lower primer | 5′-GGA CTT GTG CAT GCG GTA CTC A-3′ | |
| *ERα* | Upper primer | 5′-CCA CCA ACC AGT GCA CCA TT-3′ | 108 |
| | Lower primer | 5′-GGT CTT TTC GTA TCC CAC CTT TC-3′ | |
| *ERβ* | Upper primer | 5′-AGA GTC CCT GGT GTG AAG CAA G-3′ | 143 |
| | Lower primer | 5′-GAC AGC GCA GAA GTG AGC ATC-3′ | |
| *PR* | Upper primer | 5′-CGC GCT CTA CCC TGC ACT C-3′ | 121 |
| | Lower primer | 5′-TGA ATC CGG CCT CAG GTA GTT-3′ | |
| *DNMT1* | Upper primer | 5′-TAC CTG GAC GAC CCT GAC CTC-3′ | 103 |
| | Lower primer | 5′-CGT TGG CAT CAA AGA TGG ACA-3′ | |
| *ARA70* | Upper primer | 5′-ACA ATT ACT CTG CGC CAG ACC A-3′ | 89 |
| | Lower primer | 5′-GCT GAA CTA GCA TGA GCC ATC AA-3′ | |
| *PSA* | Upper primer | 5′-ACC AGA GGA GTT CTT GAC CCC AAA-3′ | 161 |
| | Lower primer | 5′-CCC CAG AAT CAC CCG AGC AG-3′ | |
| *PAP* | Upper primer | 5′-CAT CTG GAA TCC TAT CCT ACT CTG-3′ | 111 |
| | Lower primer | 5′-AGT TCT TGA AAA CGA GGG CA-3′ | |

**Table III.** Relationship between *AR* mRNA level and the standard clinical and pathological factors

| | Total population (%) | *AR* mRNA level [no. of patients (%)] | | | *P* value[a] |
|---|---|---|---|---|---|
| | | Underexpression | Normal | Overexpression | |
| Total | 131 (100.0) | 24 (18.3) | 62 (47.3) | 45 (34.4) | |
| Age | | | | | NS (0.063) |
| ⩽50 | 39 (29.8) | 7 (29.2) | 24 (38.7) | 8 (17.8) | |
| >50 | 92 (70.2) | 17 (70.8) | 38 (61.3) | 37 (82.2) | |
| Menopausal status | | | | | NS (0.070) |
| Pre-menopausal | 45 (34.3) | 8 (33.3) | 27 (43.5) | 10 (22.2) | |
| Post-menopausal | 86 (65.7) | 16 (66.7) | 35 (56.5) | 35 (77.8) | |
| Histological grade[b] | | | | | 0.00083 |
| I+II | 76 (62.3) | 5 (25.0) | 41 (67.2) | 30 (73.2) | |
| III | 46 (37.7) | 15 (75.0) | 20 (32.8) | 11 (26.8) | |
| Lymph node status | | | | | 0.049 |
| Node-negative | 49 (37.4) | 13 (54.2) | 17 (27.4) | 19 (42.2) | |
| Node-positive | 82 (62.6) | 11 (45.8) | 45 (72.6) | 26 (57.8) | |
| Macroscopic tumor size[c] | | | | | NS |
| ⩽30 mm | 90 (72.6) | 16 (72.7) | 39 (66.1) | 35 (81.4) | |
| >30 mm | 34 (27.4) | 6 (27.3) | 20 (33.9) | 8 (18.6) | |
| Relapses | | | | | NS |
| + | 47 (35.9) | 7 (29.2) | 21 (33.9) | 19 (42.2) | |
| − | 84 (64.1) | 17 (70.8) | 41 (66.1) | 26 (57.8) | |

[a]$\chi^2$ test.
[b]SBR classification. Information available for 122 patients.
[c]Information available for 124 patients.

*Relationship between AR mRNA levels and ERα, PR and ERβ expression status*

Patients were subdivided into three equal groups with low ($n = 43$), intermediate ($n = 44$) and high ($n = 44$) *ERα*, *PR* and *ERβ* mRNA levels. As shown in Table IV, we found a strong positive association between *AR* gene status and *ERα* ($P < 10^{-7}$) and *PR* gene ($P = 3\times10^{-7}$) status and a negative association with *ERβ* gene status ($P = 0.0026$). Seven (5.3%) 'ERα-negative' (low *ERα* mRNA expressed) tumors overexpressed *AR* and one (0.8%) *AR*-underexpressing tumor had a high *ERα* mRNA level. The *AR* and *ERα* mRNA status of these tumors was confirmed by repeat RT–PCR.

*Relationship between AR mRNA levels and RB1, CCND1, MYC and ERBB2 expression status*

The 131 tumors studied for *AR* expression had previously been tested for *RB1*, *CCND1*, *MYC* and *ERBB2* mRNA expression (17–19; manuscript in preparation). We found a significant positive link between *AR* underexpression and *RB1* underexpression ($P = 0.0046$) and a significant negative link between *AR* overexpression and *MYC* overexpression ($P = 8\times10^{-6}$), but no link between *AR* and *CCND1* or *ERBB2* mRNA status (Table V).

*Relationship between AR mRNA levels and ARA70, DNMT1, PAP and PSA expression status*

*ARA70*, *DNMT1*, *PAP* and *PSA* mRNA levels were analyzed in 10 *AR*-underexpressing and 10 *AR*-overexpressing breast tumors (Table VI). For the *ARA70* and *DNMT1* genes patients were subdivided into two equal groups of tumors with low ($n = 10$) and high ($n = 10$) mRNA levels. For the *PAP* and *PSA* genes, which were very weakly expressed, patients were subdivided into tumors with detectable and no detectable mRNA molecules. We found a significant positive association

**Table IV.** Relationship between *AR* mRNA levels and *ERα*, *PR* and *ERβ* mRNA levels

| | Total population (%) | *AR* mRNA level [no. of patients (%)] | | | *P* value[a] |
|---|---|---|---|---|---|
| | | Underexpression | Normal | Overexpression | |
| Total | 131 (100.0) | 24 (18.3) | 62 (47.3) | 45 (34.4) | |
| *ERα* RNA status | | | | | $<10^{-7}$ |
| Low | 44 (33.6) | 23 (95.8) | 14 (22.6) | 7 (15.6) | |
| Intermediate | 44 (33.6) | 0 | 33 (53.2) | 11 (24.4) | |
| High | 43 (32.8) | 1 (4.2) | 15 (24.2) | 27 (60.0) | |
| *PR* RNA status | | | | | $3\times10^{-7}$ |
| Low | 44 (33.6) | 20 (83.3) | 15 (24.2) | 9 (20.0) | |
| Intermediate | 44 (33.6) | 3 (12.5) | 28 (45.2) | 13 (28.9) | |
| High | 43 (32.8) | 1 (4.2) | 19 (30.7) | 23 (51.1) | |
| *ERβ* RNA status | | | | | 0.0026 |
| Low | 44 (33.6) | 3 (12.5) | 26 (41.9) | 15 (33.3) | |
| Intermediate | 44 (33.6) | 5 (20.8) | 22 (35.5) | 17 (37.8) | |
| High | 43 (32.8) | 16 (66.7) | 14 (22.6) | 13 (28.9) | |

[a]$\chi^2$ test.

**Table V.** Relationship between *AR* mRNA levels and *RB1*, *CCND1*, *MYC* and *ERBB2* mRNA levels

| | Total population (%) | *AR* mRNA level [no. of patients (%)] | | | *P* value[a] |
|---|---|---|---|---|---|
| | | Underexpression | Normal | Overexpression | |
| Total | 131 (100.0) | 24 (18.3) | 62 (47.3) | 45 (34.4) | |
| *RB1* RNA status[b] | | | | | 0.0046 |
| Underexpressed | 27 (21.9) | 10 (47.6) | 12 (20.3) | 5 (11.6) | |
| Normal | 96 (78.1) | 11 (52.4) | 47 (79.7) | 38 (88.4) | |
| *CCND1* RNA status[c] | | | | | NS |
| Overexpressed | 43 (32.8) | 4 (16.7) | 22 (35.5) | 17 (37.8) | |
| Normal | 88 (67.2) | 20 (83.3) | 40 (64.5) | 28 (62.2) | |
| *MYC* RNA status[d] | | | | | $8\times10^{-6}$ |
| Overexpressed | 28 (21.4) | 13 (54.2) | 14 (22.6) | 1 (2.2) | |
| Normal | 103 (78.6) | 11 (45.8) | 48 (77.4) | 44 (97.8) | |
| *ERBB2* RNA status[e] | | | | | NS |
| Overexpressed | 22 (16.8) | 1 (4.2) | 12 (19.4) | 9 (20.0) | |
| Normal | 109 (83.2) | 23 (95.8) | 50 (80.6) | 36 (80.0) | |

[a]$\chi^2$ test.
[b]Bièche *et al*. (17). Information available for 123 patients.
[c]Bièche *et al*., in preparation.
[d]Bièche *et al*. (18).
[e]Bièche *et al*. (19).

**Table VI.** Relationship between *AR* mRNA levels and *ARA70*, *DNMT1*, *PAP* and *PSA* mRNA levels

| | Total population (%) | *AR* mRNA level [no. of patients (%)] | | *P* value[a] |
|---|---|---|---|---|
| | | Underexpression | Overexpression | |
| Total | 20 (100.0) | 10 (50.0) | 10 (50.0) | |
| *ARA70* RNA status | | | | 0.0073 |
| High | 10 (50.0) | 2 (20.0) | 8 (80.0) | |
| Low | 10 (50.0) | 8 (80.0) | 2 (20.0) | |
| *DNMT1* RNA status | | | | NS |
| High | 10 (50.0) | 5 (50.0) | 5 (50.0) | |
| Low | 10 (50.0) | 5 (50.0) | 5 (50.0) | |
| *PAP* RNA status | | | | NS |
| Detectable | 13 (65.0) | 6 (60.0) | 7 (70.0) | |
| Not detectable | 7 (35.0) | 4 (40.0) | 3 (30.0) | |
| *PSA* RNA status | | | | NS |
| Detectable | 10 (50.0) | 6 (60.0) | 4 (40.0) | |
| Not detectable | 10 (50.0) | 4 (40.0) | 6 (60.0) | |

[a]$\chi^2$ test, with Yates' correction where appropriate.
[b]Number of patients (%).

between *AR* and *ARA70* expression ($P = 0.0073$), but no link between *AR* status and *DNMT1*, *PAP* or *PSA* mRNA levels. Moreover, the highest levels of *PAP* and *PSA* gene expression in this breast tumor series were far lower that those observed in prostate tumor tissues (data not shown).

## Discussion

In this study we applied a recent RT–PCR method (10) to the quantification of *AR* gene expression. We tested 12 normal breast tissue and 131 unilateral invasive primary breast tumor RNAs. *AR* mRNA was detected in all breast tumor samples and also in all normal breast tissues. These results confirm the higher sensitivity of RT–PCR compared with steroid-binding and immunohistochemical assays. Another major advantage of real-time RT–PCR is the large linear dynamic range, suited to analyzing genes, such as *AR*, associated with wide ranges of mRNA expression in tumor tissues (0.008–10.3 times normal in this series). It is noteworthy that this range (~3 orders of magnitude) is smaller than those of *ERα* and *PR* (at least 4 orders of magnitude; data not shown), suggesting that AR levels are more tightly controlled than those of other sex hormone receptors.

We observed both underexpression (18% of samples) and overexpression (34%) of *AR* mRNA in this breast tumor series. The 24 *AR*-underexpressing tumors had very low levels of *AR* mRNA (mean of the $n_{AR}$ values $0.07 \pm 0.06$) compared with the 62 tumors with normal *AR* expression ($1.12 \pm 0.49$), suggesting a bimodal distribution of *AR* expression and allowing us to use an unequivocal cut-off ($n_{AR} = 0.35$) to distinguish the two tumor groups. As a strong correlation has been reported between *AR* mRNA copy number and *AR* protein abundance (20), the 24 *AR*-underexpressing tumors would correspond to 'AR-negative' tumors in steroid-binding and immunohistochemical assays.

Overall, the results for AR-negative tumors in this study agree with those reported in the literature. The frequency (18%) of AR-negative tumors in our breast tumor series is similar to that obtained with steroid-binding and immunohistochemical assays (1,4). *AR*, *PR* and *ERα* expression were strongly intercorrelated, but we observed one AR-negative tumor that contained *ERα* (0.8% of our tumor series) and several *AR*-overexpressing tumours that did not contain *ERα* (5.3%), in keeping with others reports (1,4,21). The negative association between *AR* and *ERβ* was probably due to the negative link between ERβ and ERα (data not shown). In addition to PR and ERα negativity, we found that *AR* gene underexpression was associated with SBR histopathological grade III but not with a poor prognosis, confirming that AR is more a marker of tumor aggressiveness (poorly differentiated tumors) than a predictor of patient outcome in breast cancer. As expected, we also found a correlation between *AR* underexpression and *RB1* underexpression; indeed, in the same tumor series *RB1* underexpression was also associated with poorly differentiated tumors (correlation with SBR histopathological grade III and PR and ERα negativity) (17).

The 107 AR-positive tumors fell into two groups: those with normal *AR* expression ($n = 62$) and those with *AR* overexpression ($n = 45$). The amount of *AR* mRNA increased in tumors from both elderly and post-menopausal patients (Table III), in agreement with Lea *et al*. (1). This may be due to *AR* up-regulation to compensate for the decline in circulating sex steroids.

We observed a strong negative link between *AR* overexpression and *MYC* gene overexpression. No such link was observed between *ERα* (or *ERβ*) and *MYC* expression (data not shown). This study confirms the direct regulation of *AR* transcription by the c-myc transcriptor factor via a myc consensus site in an *AR* exonic region (22) and the down-regulation of *MYC* mRNA associated with androgen-induced suppression of the transformed phenotype in the human prostate carcinoma cell line LNCaP (23). No correlation was observed between *AR* overexpression and altered expression of the *RB1*, *CCND1* and *ERBB2* genes. This is in disagreement with previous data (24–26), indicating that retinoblastoma protein, cyclin D1 and c-erbB2 control the transcriptional activity of *AR*, the latter regulating its own trancription.

We observed a positive correlation between *AR* and *ARA70* expression, confirming the specificity of ARA70 in controlling transcription activity of *AR* in breast cancer, as in prostate cancer (27). We did not observe a correlation between *AR* and *DNMT1* expression, suggesting that the loss of *AR* expression in breast tumors is not due to up-regulation of *DMNT1* via hypermethylation of the *AR* promoter CpG island. It is noteworthy that this finding does not exclude *AR* promoter methylation as a possible cause of *AR* down-regulation, due to modified expression of a DNA methyltransferase gene other than *DMNT1*. Finally, we found no link between *AR* status and *PAP* or *PSA* status. These two genes showed far lower expression levels than in prostate tissue, confirming the high specificity of *PAP* and *PSA* expression for prostate tissue.

In conclusion, our data suggest the involvement of several AR-mediated pathways in the regulation of breast tumor growth. Further characterization of these pathways may lead to new androgenic therapies for breast cancer.

Accurate determination of *AR* status, combined with *ERα* status, could help to select optimal endocrine therapies for breast cancer. The rapid, cost-effective, highly sensitive high throughput RT–PCR assay used here to determine *AR* status should be useful as a routine tool in AR-based clinical applications in breast cancer and other AR-related cancers.

## Acknowledgements

We thank the Centre René Huguenin staff for assistance in specimen collection and patient care. This work was supported by the Association pour la Recherche sur le Cancer and the Ministère de l'Enseignement Supérieur et de la Recherche.

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*Received February 9, 2001; revised May 17, 2001; accepted June 4, 2001*