Quantification of prolactin receptor mRNA in multiple human tissues and cancer cell lines by real time RT-PCR

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Abstract

Human prolactin (hPRL) has been reported to be involved in breast and prostate cancer development. The hPRL receptor (hPRLR) is expressed in a wide variety of tissues in at least three isoforms. In this study, a one-step real time reverse transcription PCR technique was used to determine relative expression levels of hPRLR mRNA in eleven human breast cancer cell lines, HeLa cells, three prostate cancer cell lines and nine normal human tissues. The housekeeping gene β -actin was used for internal normalization. We demonstrate that hPRLR mRNA is up-regulated in six of the eleven breast cancer cell lines tested when compared with normal breast tissue. Of the cancer cell lines tested, we found that T-47D cells have the highest level of hPRLR mRNA, followed by MDA-MB-134, BT-483, BT-474, MCF-7 and MDA-MB-453

cells. In two breast cancer cell lines (MDA-MB-468 and BT-549), the hPRLR levels were found to be comparable to that of normal breast tissue. Three breast cancer cell lines (MDA-MB-436, MDA-MB-157 and MDA-MB-231) expressed hPRLR mRNA at levels lower than that of normal tissue. In contrast, in all three commonly used prostate cancer cell lines (LNCaP, PC-3 and DU 145), the levels of hPRLR mRNA were found to be down-regulated relative to that of normal prostate tissue. Of nine normal human tissues tested, we found that the uterus and the breast have the highest levels of hPRLR mRNA, followed by the kidney, the liver, the prostate and the ovary. The levels of hPRLR mRNA were the lowest among the trachea, the brain and the lung.

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Introduction

The prolactin receptor (PRLR) belongs to the cytokine receptor superfamily. PRLR consists of three domains: the extracellular ligand binding domain, the transmembrane domain and the proline-rich cytoplasmic domain. Following PRL and PRLR interaction, signal transducers and activators of transcription (STATs) are ultimately phosphorylated prior to binding to PRL-responsive promoter elements in the nucleus resulting in PRL action (Bole-Feysot *et al.* 1998; Clevenger & Plank 1997; Das & Vonderhaar 1997). The evidence linking PRL to breast cancer development has been drawn, in part, from findings of higher PRLR levels in cancerous tissues (Laud *et al.* 2000, Ormandy *et al.* 1997, Reynolds *et al.* 1997, Touraine *et al.* 1998). Experimentally, over-expression of PRL in mice results in a high incidence of mammary tumors. In humans, there is a positive correlation between PRLR, estrogen receptor (ER) and progesterone receptor levels, and it is known that sex steroid hormones and PRL interact synergistically to initiate cancerous growth within mammary tissue (Ormandy *et al.* 1997). There is growing evidence that PRL may also play a role in early transfor-

mation events involved in prostate cancer (Costello *et al.* 1999), and that PRLR expression is altered in some neoplasms of the prostate (Leav *et al.* 1999). More importantly, the PRL antagonist hPRL-G129R, which blocks PRLR signal transduction, appears to induce breast cancer cell apoptosis (Cataldo *et al.* 2000). Therefore, we found it of interest to quantitate PRLR mRNA levels of breast and prostate human cancer cell lines and compare these directly to normal tissue levels. Ultimately, this information will be useful in the selection of cell lines for PRL-related studies based on PRLR status.

Materials and Methods

Cell lines and tissues

The following human cancer cell lines were obtained from the ATCC and maintained under the conditions recommended. We collected eleven human breast cancer cell lines (MCF-7, T-47D, MDA-MB-134, BT-483, BT-474, MDA-MB-453, MDA-MB-468, BT-549, MDA-MB-436, MDA-MB-157, and MDA-MB-231); three prostate cancer cell lines (LNCaP, PC-3, and DU 145);

and the HeLa cell line. Seven tissue total RNA preparations were obtained from Clontech Lab, Inc. (adult brain, kidney, liver, lung, trachea, uterus and prostate), and two from Stratagene, Inc. (adult breast and ovary).

Real-time quantitative PCR

A one-step real time reverse transcription (RT) PCR technique was used to determine relative expression levels of PRLR mRNA using the ABI Perkin Elmer Prism 7700 Sequence Detection System (Applied Biosystems). For analyses from cell cultures, total RNA was isolated from 70–90% confluent cell cultures, using the RNeasy (Qiagen) RNA isolation kit following the recommended protocol. The reaction mix included a 200 nm final concentration of both forward (derived from exon 7: 5'agaccatggatactggagta-3') and reverse (derived from exon 9: 5'ggaagatgcaggtccacat-3') PRLR-specific primers, and a 100 nm final concentration of the PRLR specific probe (5'tctgctgtcatctgtttgatta-3') labeled with FAM reporter fluorescent dye; these primers were designed for amplification of all three isoforms of PRLR. A one-step reaction mixture provided in the TaqMan Gold RT-PCR Kit (PE Applied Biosystems) was used for all amplifications (5.5 mM MgCl₂, 50 mM KCl, 0.01 mM EDTA, 10 mM Tris-HCl pH 8.3, 300 μM deoxyATP, 300 μM deoxyCTP, 300 μM deoxyGTP, 600 μM deoxyUTP, 0.025 U/ml AmpliTaq Gold DNA polymerase, 0.25 U/ml MultiScribe Reverse Transcriptase, 0.4 U/ml RNase inhibitor).

Cycle parameters for the one-step RT-PCR included a reverse transcription step at 48 °C for thirty min, followed by 40 cycles of 95 °C denaturation and 60 °C annealing/extension. Four hundred to 1500 nanograms of total RNA were used per reaction; the housekeeping gene β -actin was used for internal normalization. For analyses of PRLR in normal tissues, 100 nanograms of commercially-prepared total RNA were used per reaction. Each reaction was carried out in triplicate and repeated at least three times. Data were expressed as the means $\pm$ s.e.

Results and Discussion

Our results from RT-PCR demonstrate that T-47D cells express the highest levels of PRLR mRNA (Figures 1 and 2) among the cell lines tested. The levels of PRLR mRNA in breast cancer cell lines are much higher than those of prostate cancer cells (Fig. 3). PRLR mRNA was not detectable in HeLa cell RNA preparations (Fig. 3).

Within the panel of normal tissues, uterus and breast expressed the highest levels of PRLR mRNA (Fig. 4). We set the expression level from breast tissue to 1, to allow internal comparisons between tissues. We found that PRLR mRNA expression from the kidney was surprisingly high, suggesting an important role for PRL in this tissue.

Figure 1 Real-time RT-PCR analysis for PRLR mRNA in five breast cancer cell lines (A-E, from left to right as indicated).

Figure 2 Real-time RT-PCR analysis for PRLR mRNA in breast cancer cell lines (A-C), normal breast tissue (D) and MDA-MB-468 (E), and MDA-MB-231 (F).

Figure 3 Real-time RT-PCR analysis for PRLR mRNA in breast cancer cell lines (A, B), prostate cancer cell lines (C-E), and the HeLa cell line (F).

In order to directly compare the expression levels of PRLR between the cell lines and tissue preparations, RT-PCR reactions were carried out using 100 ng of total RNA of normal mammary or prostate tissues and 100 ng of total RNA from several breast or prostate cancer cell lines. We found that the PRLR mRNA expression level of normal breast tissue was comparable to that of the cell

Figure 4 Comparison of relative PRLR mRNA levels in nine normal human tissues. Y-axis, fold difference.

Figure 5 Comparison of relative PRLR mRNA level in eleven breast cancer cell lines and normal breast tissue, normalized to 100 ng of total RNA. Y-axis, fold difference.

line MDA-MB-468 (Fig. 2). Therefore, PRLR mRNA expression levels in MDA-MB-468 cells were used to normalize relative expression level from all cell lines by adjusting all β -actin values to β -actin amplification levels from one μ g of MDA-MB-468 total RNA. A graphical

Table 1 Relative hPRLR mRNA levels in human cancer cell lines

Cell lines	Fold difference ($\pm$ S.E.)
T-47D	27.20 (1.24)
MDA-MB-134	12.45 (0.55)
BT483	8.62 (0.76)
BT474	8.13 (1.03)
MCF-7	3.45 (0.06)
MDA-MB-453	3.17 (0.55)
MDA-MB-468	1.0*
BT549	1.0 (0.28)
MDA-MB-436	0.69 (0.07)*
MDA-MB-157	0.62 (0.07)
LNCaP	0.006 (0.0005)
PC-3	0.002 (0.0002)
MDA-MB-231	0.0017 (0.0064)
DU145	0.00032 (0.00001)
HeLa	not detected

*Not detected by Northern blotting methods.

Table 2 Relative hPRLR mRNA levels: comparison between two studies

	Current study	Ormandy <i>et al.</i> (1997)
Cell lines		
T-47D	7.9	4.0
MDA-MB-134	3.6	5.2
BT483	2.5	4.0
BT474	2.4	2.3
MCF-7	1.0	1.0
MDA-MB-453	0.9	0.7
MDA-MB-468	0.3	ND*
BT549	0.3	0.6
MDA-MB-436	0.2	ND*
MDA-MB-157	0.2	0.4
MDA-MB-231	0.0005	ND*

*Not detected by Northern blotting methods.

representation and summary table of these findings are presented in Figure 5 and Table 1.

The results using this method were compared with those published earlier in which Northern blotting methods were used to determine relative hPRLR mRNA levels in human breast cancer cell lines (Table 2). Although the two methods generated similar values in most cases, we were able to detect PRLR mRNA expression in cell lines that had previously been noted to lack PRLR expression (MDA-MB-468 and MDA-MB-436). The finding of higher expression levels of PRLR mRNA in four cell lines (T-47D, MDA-MB-134, BT-483 and BT-474) is consistent with the findings of Ormandy *et al.* (1997). Our findings are also consistent with those of Shiu *et al.* (1987), in which PRLR numbers were directly calculated in a relatively limited panel of breast cancer cell lines.

It should be noted that although the expression level of PRLR in the normal prostate tissue is moderately high, all three commonly used prostate cancer cell lines expressed extremely low but detectable levels of PRLR mRNA (Fig. 6), ranging from approximately 165 fold lower (LNCaP), and 460 fold lower (PC-3) to 3100 fold lower (DU 145) than MDA-MB-468 levels (Table 1). We are unsure if down-regulation of PRLR is a common phenomenon of prostate cancer. In any case, one should be aware of lower PRLR levels in these cell lines relative to normal prostate tissue (Fig. 6) when choosing these prostate cancer cell lines as study models.

Real-time quantitative PCR is a method proving to be invaluable in the analysis of a number of receptors involved in breast cancer and its metastasis, including prolactin and chemokine receptors (Muller *et al.* 2001). Although normal breast tissue expressed the second highest level of PRLR mRNA of the tissue samples, this level was less than a twentieth that of the malignant cancer cell line T-47D, and well below levels of five other mammary cancer cell lines, supporting a growing body of evidence that increased PRLR expression and prolactin activity

Figure 6 Comparison of relative PRLR mRNA levels of three prostate cancer cell lines and normal prostate tissue, normalized to 100 ng of total RNA. Y-axis, fold difference.

contribute to mammary carcinoma (Clevenger & Plank 1997; Ormandy *et al.* 1997; Reynolds *et al.* 1997; Touraine *et al.* 1998; Vonderhaar 1998; Vonderhaar 1999; Wennbo & Tornell 2000).

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