International Immunopharmacology 73 (2019) 64–71

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Plasma membrane localization of CYP4Z1 and CYP19A1 and the detection of anti-CYP19A1 autoantibodies in humans

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ARTICLE INFO

Keywords:
Aromatase
Autoantibody
Breast cancer
CYP19A1
CYP4Z1

ABSTRACT

It is thought that autoantibody (aAb) production can be caused by (aberrant) protein targeting to the plasma surface of cells. We recently demonstrated the presence of the human cytochrome P450 enzyme CYP4Z1 on the plasma membrane of MCF-7 breast cancer cells and the detection of high titers of anti-CYP4Z1 aAbs in breast cancer patients, but not in healthy controls. In the present study we show that cells of the normal breast cell line MCF-10A do not display CYP4Z1 on their surface. By contrast, we detected CYP19A1 (aromatase) on the plasma membrane of both cell lines. Interestingly, the presence of CYPs on the cell surface did not correlate with their relative expression levels in these cell lines. Indirect ELISA experiments demonstrated the presence of anti-CYP19A1 aAbs in female breast cancer patient sera as well as in male and female controls, respectively; aAb titers in all three groups varied considerably and overall, the results obtained for each group were not significantly different from those of either of the other two groups. Based on these data we propose the hypothesis that CYP translocation to the plasma membrane, but not the intracellular expression level, is the crucial precondition for the generation of anti-CYP aAbs.

1. Introduction

Aberrant protein targeting to the plasma surface of cells is thought to be one of the factors that can stimulate the immune system to generate autoantibodies (aAbs) which are directed against these proteins. Mammalian cytochrome P450 enzymes (CYPs or P450s) are membrane bound enzymes that are either located on the cytoplasmic side of the endoplasmic reticulum or on the matrix side of the inner mitochondrial membrane [1,2]; however, it is well known that a number of microsomal CYPs can be partially localized on the plasma membrane surface of rodent or human hepatocytes [3–12]. Such plasma membrane-localized CYPs are thought to contribute to the occurrence of anti-CYP aAbs that are found in patients with some liver diseases as well as in patients with certain endocrine or autoimmune disorders [13–16]. Significantly, all CYPs for which plasma membrane localization has been shown are also known antigens. CYP4Z1 is a fatty acid hydroxylase [17,18] that is selectively expressed in mammary tissue and strongly overexpressed breast cancer and ovarian cancer cells [19,20], while in other healthy human tissues it is only found in small amounts in the tibial nerve, lung, and adipose tissue (data from the Genotype-Tissue Expression (GTEx) Project). In a recent study, we demonstrated the presence of CYP4Z1 on the outer surface of the plasma membrane of MCF-7 human breast cancer cells; moreover, we detected anti-CYP4Z1 aAbs in breast cancer patient sera and showed in an indirect CYP4Z1 ELISA that reactive aAb titers were significantly higher in breast cancer patients than in female controls [21]. However, it was not yet investigated whether there is a difference in CYP4Z1 surface expression when comparing normal breast with breast cancer cells. Also, it is unknown whether an increase in

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https://doi.org/10.1016/j.intimp.2019.05.003
Received 2 March 2019; Received in revised form 14 April 2019; Accepted 2 May 2019
Available online 10 May 2019
1567-5769/

intracellular CYP4Z1 expression levels correlates with CYP4Z1 surface expression. Another human CYP enzyme that is implicated in breast cancer is CYP19A1 (aromatase), which catalyzes the final steps of estrogen production [22]. As some breast cancer subtypes require estrogen to continue growing they are consequently treated with aromatase inhibitors in postmenopausal women [23]. However, a possible plasma membrane location of CYP19A1 has previously only been described for choriocarcinoma cells [47] but not in normal breast or breast cancer cell and in addition, nothing is known about the occurrence of anti-CYP19A1 aAbs.

Treatment of the human breast cancer cell line MCF-7 with the synthetic glucocorticoid dexamethasone, but not with progesterone, is known to strongly increase CYP4Z1 expression [24]. In addition, dexamethasone is also known to cause activation of the aromatase promoter I.4, which is predominantly used and minimally activated in normal breast adipose tissue to maintain basal levels of aromatase expression [25]. Thus, in the present study we treated MCF-7 cells and cells of the normal breast cell line MCF-10A with either dexamethasone or progesterone and assessed their effects on the expression levels of both CYPs as well as on their display on the plasma membrane. In addition, we screened sera from breast cancer patients as well as from healthy controls for the presence of anti-CYP19A1 aAbs. We show detectable plasma membrane localization of aromatase in both non-permeabilized normal breast epithelial MCF-10A and breast cancer MCF-7 cell lines by immunofluorescence with no observable variation between the untreated and treated cell lines. Interestingly, we observed localization of CYP4Z1 in the plasma membrane of MCF-7 cells but not in MCF-10A cells regardless of treatment condition. We further demonstrate aromatase aAb detection in female breast cancer patients as well as in healthy controls (male and female).

2. Materials and methods

2.1. Cell lines culture and conditional treatment

The human breast cancer cell line MCF-7 [26] and normal control breast cell line MCF-10A [27] were obtained from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (CBTCCCAS), Shanghai Institute of Cell Biology, Chinese Academy of Sciences, Shanghai, China. MCF-7 cells were maintained in Dulbecco's modified Eagle's medium (Thermo Fisher Scientific; Waltham, MA, USA) supplemented with 10% Fetal Bovine Serum (Thermo Fisher Scientific) and 1% Penicillin-Streptomycin (Thermo Fisher Scientific). MCF-10A cells were cultured in Mammary Epithelial Cell Growth Medium (Lonza; Basel, Switzerland) supplemented with 100 ng/mL cholera toxin (Thermo Fisher Scientific). The cell lines were subjected to no more than eight passages in culture when used in experiments and cultivated under standard conditions at 37 °C in an atmosphere of 95% air and 5% CO_2. For CYP induction cells were treated with progesterone (2 μM) or dexamethasone (1 μM) for 21 h as described previously [24]; control cells were treated with solvent (DMSO) only.

2.2. RNA extraction, reverse transcription and quantitative real-time PCR analysis

RNA extraction, reverse transcription and quantitative real-time PCR were done as described previously [28]. The primers used were as follows (forward and reverse, respectively): CYP4Z1 (5′-ATCTCCAAC CAGCGCATGAA-3′ and 5′-CCCAGCGCCTTTTCTGAGTA-3′); CYP19A1 (5′-CAAGAAGAGCGTGTTAGAGG-3′ and 5′-CTGGACAGGTTGGAG GAG-3′). YWHAZ (5′-GATGAAGCCATTGCTGAACTTG-3′ and 5′-CTAT TTGTGGGACAGCATGGA-3′). The determined CT values of the CYP genes were normalized to the relative mean of the expression of the housekeeping gene YWHAZ, which codes for the 14-3-3 protein zeta (14-3-3ζ) and has previously been shown to be a very reliable housekeeping gene for qPCR studies [29]. Primers were purchased from GENEWIZ (Tianjin, China). Amplification and detection were performed using a QuantStudio 6 Flex Real Time PCR System (Thermo Fisher Scientific).

2.3. Immunofluorescence

For immunofluorescence staining, cells were plated on glass coverslips in 6 well culture plates (Merck; Darmstadt, Germany) under standard conditions and treated with dexamethasone or progesterone as indicated. The cells were subsequently washed three times in phosphate-buffered saline (PBS) (Solarbio; Beijing, China) for 5 min each and fixed with 4% para-formaldehyde (Beijing Leagene Biotechnology, Beijing, China) at room temperature for 10 min. Cells were then washed again in PBS, blocked with 5% skimmed milk in PBS for 30 min at room temperature, washed in PBS for three times as before and probed with primary antibodies (rabbit anti-CYP4Z1 (DF3590), Affinity biosciences; Zhenjiang, China) or mouse anti-CYP19A1 (sc-374176), Santa Cruz Biotechnology; Dallas, TX, USA) at 1:1000 dilution in PBS and incubated overnight at 4 °C and then washed three times in PBS. The corresponding secondary antibodies (Alexa Fluor 488 goat anti-rabbit IgG (A11008), Thermo Fisher Scientific, or Alexa Fluor 488 goat anti-mouse IgG (A11001), Thermo Fisher Scientific) were used at 1:1000 dilution in PBS with subsequent incubation in the dark for 2 h at room temperature and three washes in PBS. Then cells were incubated with Deep Red plasma membrane stain (C10046, Thermo Fisher Scientific) for 25 min followed by three washes in PBS. Slides were mounted with 4′,6-diamidin-2-phenylindol (DAPI) Fluoromount-G (0100-20; SouthernBiotech; Birmingham, AL, USA) to counterstain nuclei. Fluorescence microscopy was performed using an Eclipse E600 microscope (Nikon; Tokyo, Japan) and NIS-Elements F Version 3.0 software (Nikon) equipped with a digital sight DS-5Mc camera (Nikon) using exposure times of 100 ms for DAPI and 2 s for both FITC and deep red staining, respectively.

2.4. Serum samples and participants

Breast cancer serum samples from 12 female patients (age range: 32–70 with a mean of 46.2) and control serum samples from healthy adults with no malignancies from 12 males (age range: 21–54 years with a mean of 41.6) and 12 females (age range: 37–55 with a mean of 46) were used in this study. All participants gave written, informed consent under a study protocol approved by the institutional ethics committees. The participants were recruited from Wuhan Xiehe Hospital, Hubei, China. For the cohort of patients recruited, eight had pathological diagnosis as invasive ductal carcinoma. The other four had varying tumor classification ranging from medullary to fibrous, ductal carcinoma, and malignant breast tumor, respectively. For receptor proteins expression, three patients had a progesterone receptor (PR)/estrogen receptor (ER)/human epidermal growth factor receptor (HER2/neu) positive status, one was ER^+, one was $PR/HER2/neu^+$, two were ER/PR^+, one was $HER2/neu^+$ and the remaining four were triple-negative breast cancer.

2.5. Quantification of aAb titers by ELISA

Recombinant CYP19A1 protein was expressed and purified as described previously [30]. Since initial experiments indicated some cross reactions of human sera with traces of bacterial GroEL present in the recombinant protein preparations (data not shown), GroEL was removed using immunoprecipitation (see below). Measurement of anti-CYP19A1 aAbs by indirect ELISA was essentially done as described previously for CYP4Z1 [21] with a single modification: Polystyrene flat-round bottom high protein-binding capacity 96 well plates (Guangzhou Jet BioFiltration; Guangzhou, China) were coated by overnight incubation at 4 °C with 10 ng of recombinant CYP19A1 full-length protein per well in 50 mM carbonate/bicarbonate buffer pH 9.6. All other steps

were done as before.

2.6. Immunoprecipitation (IP)

1 μL (2.47 μg) of recombinant CYP19 protein and 1.5 μL (1.5 μg) of anti-GroEL antibody (ab82592, Abcam) were added to 50 μL PBS and incubated with shaking at 4 °C for 2 h; then 100 μL of a 50% protein A-Sepharose (Protein A CL-4B; 17-0780-01, GE Healthcare) were added and the mixture was incubated with shaking at 4 °C overnight. The protein A-Sepharose-antibody-GroEL complexes were then collected by centrifugation (10.000 *g*, 4 °C, 10 s) and the supernatant was used for ELISA as described above.

2.7. Western blot analysis

Use of serum samples as primary antibodies in Western blot analysis for CYP19A1 detection was essentially done as described previously for CYP4Z1 [21]. Briefly, recombinant CYP19A1 protein was mixed with Laemmli sample buffer and resolved on a 10% SDS PAGE loading 1.5 ng of protein per well. For Western blot analysis serum samples were then applied as primary antibody in 1:500 dilution in Tris-buffered saline with 0.05% Tween-20 (TBST) or with mouse anti-CYP19A1 (as above) in 1:1,000 dilution in TBST as positive control; membranes were incubated overnight at 4 °C followed by five washes in TBST buffer. The membranes were subsequently incubated with horseradish peroxidase (HRP) conjugated secondary antibodies (goat anti-human IgG (ab6858, abcam) in 1:50,000 dilution in TBST or goat anti-mouse IgG (as above) in 1:10,000 dilution in TBST) at room temperature for 1 h, then washed again with TBST. Signals were detected by enhanced chemiluminescence using the SuperSignal West Pico Chemiluminescent Substrate (34080, Thermo Scientific).

2.8. Statistical analysis

Data collected are expressed as means ± standard deviations. Data were analyzed by a paired student's *t*-test or one way analysis of variance (ANOVA) using GraphPad Prism software. P values < 0.05 were considered statistically significant.

3. Results

3.1. Comparison of expression levels of CYP4Z1 and CYP19A1 in MCF-7 and MCF-10A cells

Induction of CYP4Z1 expression in MCF-7 cells upon treatment with 1 μM dexamethasone, but not with 2 μM progesterone, has been reported previously [24]. In the present study we wanted to compare the MCF-7 results with those of MCF-10A, and moreover, we wanted to investigate the effect of both compounds on CYP19A1 expression in both cell lines as well. As expected, treatment of MCF-7 cells with dexamethasone resulted in a 4.3-fold increase in CYP4Z1 expression compared to untreated cells (Fig. 1a), while the effect of progesterone was much weaker (1.5-fold increase), but still significant. Surprisingly, untreated MCF-10A cells displayed the same CYP4Z1 relative expression level as MCF-7 cells (0.000032). Treatment with either dexamethasone or progesterone also had a positive effect on CYP4Z1 expression in MCF-10A cells, leading to an increase by a factor of 1.6 in both cases. By contrast, the overall expression pattern of CYP19A1 is very different (Fig. 1b). First of all, there is a much stronger CYP19A1 expression in MCF-7 than in MCF-10A, regardless of treatment: In comparison to CYP4Z1, CYP19A1 expression in untreated MCF-7 cells is 124-fold higher while in MCF-10A cells it is only 5.8-fold higher. In MCF-7 cells, treatment with either dexamethasone or progesterone did not have a significant effect on CYP19A1 expression. In MCF-10A cells, progesterone treatment lead to a small (twofold) but significant increase in CYP19A1 expression, while dexamethasone treatment had no effect.

3.2. Plasma membrane expression of CYP4Z1 and CYP19A1 in MCF-7 and MCF-10A cells

Recently we reported the detection of CYP4Z1 on the outer surface of non-permeabilized MCF-7 cells [21]. Here we wanted to extend this observation by testing whether treatment of MCF-7 cells with 1 μM dexamethasone or 2 μM progesterone has an influence on this plasma membrane localization of CYP4Z1; moreover, we wanted to compare our findings in MCF-7 cells with those of MCF-10A cells. As expected, untreated MCF-7 cells displayed a clear CYP4Z1 signal on the plasma membrane (Fig. 2a); MCF-7 cells treated with progesterone showed the same signal (Fig. 2b), while in the case of cells treated with dexamethasone the signal was even enhanced (Fig. 2c). By contrast, untreated cells of the normal breast epithelial cell line MCF-10A did not express detectable levels of CYP4Z1 (Fig. 2d), and the same was true for MCF-10A cells treated either with dexamethasone or progesterone (data not shown). The results from immunofluorescence detection of aromatase in non-permeabilized MCF-7 and MCF-10A cell lines are shown in Fig. 3. Both cell lines show clear plasma membrane signals for CYP19A1 regardless of treatment. To the best of our knowledge, this is the first report of CYP19A1 protein being detected at the plasma membrane of breast cells.

3.3. Detection of anti-CYP19A1 aAbs in sera from breast cancer patients and healthy controls

The next aim of this study was to search for anti-CYP19A1 aAbs in breast cancer patients sera and to compare them to controls. For this purpose we set up an indirect ELISA using human recombinant CYP19A1 full-length protein as the target as well as female breast cancer patient sera or (male and female) controls, respectively, as probes. Under these conditions female control sera gave signals between OD_{415} 0.2 and 0.7, while results from sera of male controls as well as those of breast cancer patients were not significantly different (Fig. 4a). In order to confirm the specificity of the ELISA signals, and moreover, to determine whether linear epitopes are involved in the aAb detection as in the case of CYP4Z1 [21], we performed Western blot analysis of recombinant CYP19A1 protein using three sera with high ELISA signals as primary antibody. For comparison, a positive control anti-CYP19A1 primary antibody was also tested. This experiment showed clear signals in all three test samples as well as in the control (Fig. 4b). It was found that the test sera indeed recognize CYP19A1 and, moreover, that at least one linear epitope within CYP19A1 is recognized by the aAbs. These experiments demonstrate for the first time the presence of anti-CYP19A1 aAbs in humans.

4. Discussion

Breast cancer is by far the most frequently diagnosed cancer and cause of cancer death in women with an estimated 1.7 million new cases (25% of all cancers in women) and 0.5 million cancer deaths (15% of all cancer deaths in women) per year worldwide [31]. Despite significant improvements in treatment, early detection remains the most important prognostic factor for better outcome [32]. However, the currently employed methods of breast cancer screening, such as mammography, breast exams, genetic screening, ultrasound, and magnetic resonance imaging, all have shortcomings in terms of sensitivity, specificity, complexity, cost, and compliance, respectively [33]. Therefore, there is an urgent need to develop new diagnostic tools that allow for a sufficiently early diagnosis of breast cancer before it progresses to an often incurable metastatic stage. Serum aAbs against tumor-associated antigens (TAAs) are a group of biomarkers which are of great interest in this respect, since they are easily accessible in blood samples and have a long half-life [34,35]. Cancer-associated aAbs have been proposed for

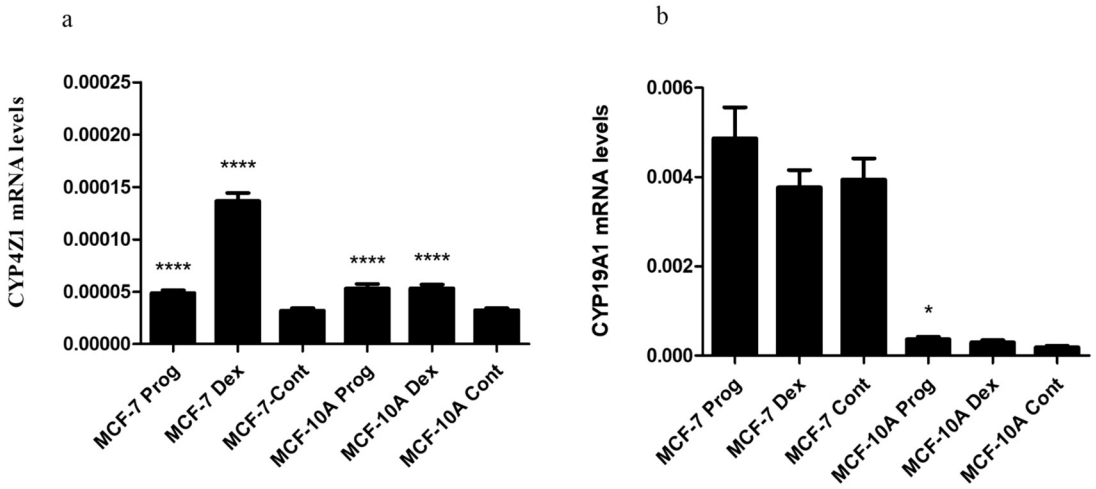

Fig. 1. CYP4Z1 and CYP19A1 mRNA levels in the human breast cancer cell line MCF-7 and normal breast cell line MCF-10A. Cells were treated either with 2 μM progesterone (Prog), 1 μM dexamethasone (Dex), or mock-treated (control) as indicated and expression of CYP4Z1 and CYP19A1 was measured by real-time qPCR. Data shown represent the mean of triplicate measurements from each sample. The error bars indicate the standard deviation. $^{*}P < 0.05$; $^{****}P < 0.001$ vs. control.

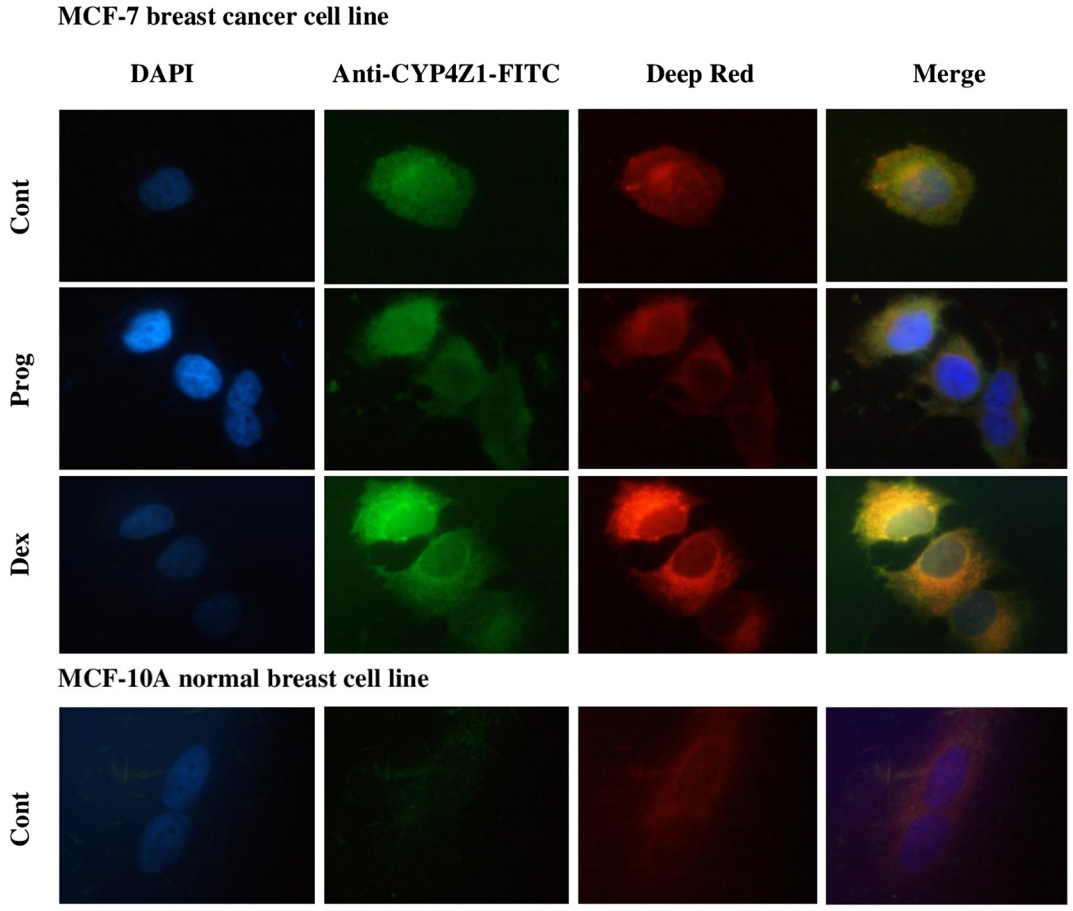

Fig. 2. Detection of CYP4Z1 on the outer surface of non-permeabilized MCF-7 or MCF-10A cells by rabbit anti-CYP4Z1 IgG and goat anti-rabbit IgG conjugated to Alexa fluor 488. The non-permeabilized cells were probed with antibodies and stained with DAPI and Deep Red plasma membrane stain as indicated. Magnification is ×1,000 for all images. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

various clinical aspects, such as biomarkers for early cancer detection, as tools to monitor therapy, and to predict disease progression; moreover, evidence for a specific humoral response against a number of intracellular and surface TAAs is now well-established in breast cancer patients [36]. However, the role of aAbs against TAAs in immunosurveillance or tumorigenicity is not well understood, as some studies suggest that they could contribute to cancer progression while others report antitumor effects. For instance, human Fab fragments specific for breast tumor driven antigens promote the growth of breast cancer cells [37]; on the other hand, anti-HER2 aAbs effectively block breast carcinoma colony formation by attenuating downstream signal transduction [38]. More recently, immune responses against TAAs were

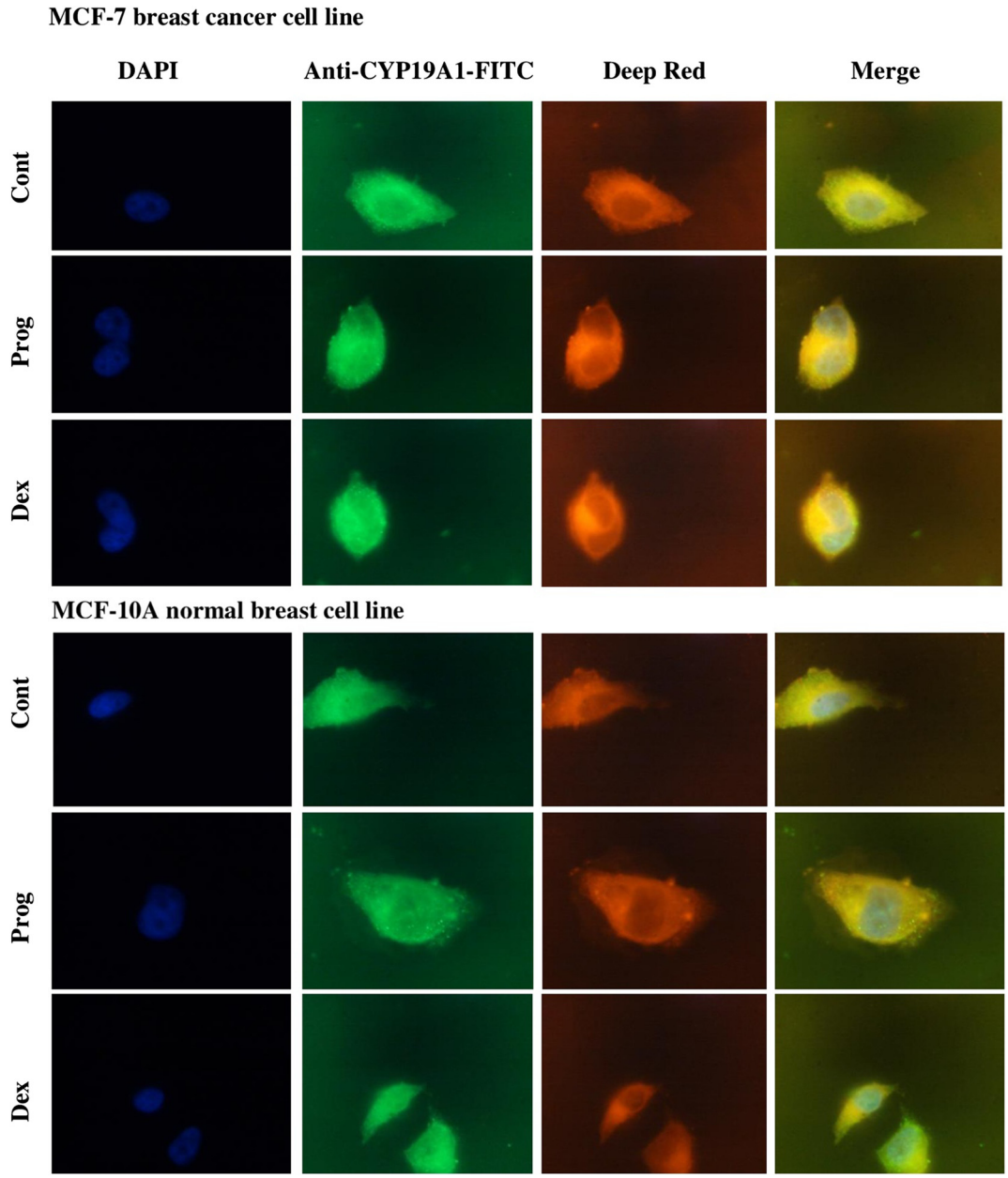

Fig. 3. Detection of CYP19A1 on the outer surface of non-permeabilized MCF-7 or MCF-10A cells by mouse anti-CYP19A1 IgG and goat anti-mouse IgG conjugated to Alexa fluor 488. The non-permeabilized cells were probed with antibodies and stained with DAPI and Deep Red plasma membrane stain as indicated. Magnification is ×1,000 for all images. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

reported to play an important role in cancer immunoediting and possibly also in immunotherapy [39]. We have recently shown that CYP4Z1 is a TAA in breast cancer patients [21]. Since earlier studies found a strong correlation of CYP4Z1 expression and tumor progression in breast cancer patients [40], we wanted to investigate the interplay between CYP expression levels in normal and cancerous cells, CYP translocation to the plasma membrane, and the occurrence of anti-CYP autoantibodies that might serve as biomarkers.

For this purpose, we first monitored the expression levels of both CYPs under study in MCF-7 and MCF-10A cells by real-time qPCR. In addition, we also treated cells for 21 h with either 1 μM dexamethasone or 2 μM progesterone because these are the same conditions as those previously used by the group of Eric Johnson when they studied the induction of CYP4Z1 expression by a variety of compounds [24]. As expected, dexamethasone treatment of MCF-7 cells caused a 4.3-fold increase in CYP4Z1 expression compared to untreated cells (Fig. 1a), while the effect of progesterone was much weaker (1.5-fold increase). In view of the fact that a different housekeeping gene (actin) was used in the earlier investigation as compared to ours (YWHAZ), we conclude that the results obtained in both studies are basically identical. Untreated MCF-10A cells displayed the same CYP4Z1 expression level as MCF-7 cells, which was rather unexpected, as a higher expression level of CYP4Z1 in breast cancer cells as opposed to normal breast cells (if not cell lines) has been reported by a number of studies [41]. Treatment with either dexamethasone or progesterone increased CYP4Z1 expression in MCF-10A, but with dexamethasone this increase was much weaker as in MCF-7 cells. This means that the effect of progesterone on MCF-10A and MCF-7 was basically identical, while the effect of dexamethasone was 2.6-fold more pronounced in MCF-7 than in MCF-10A. In this context it is interesting to note that dexamethasone is used as a supportive care comedication for cancer patients undergoing chemotherapy to counteract certain side effects of their antitumor

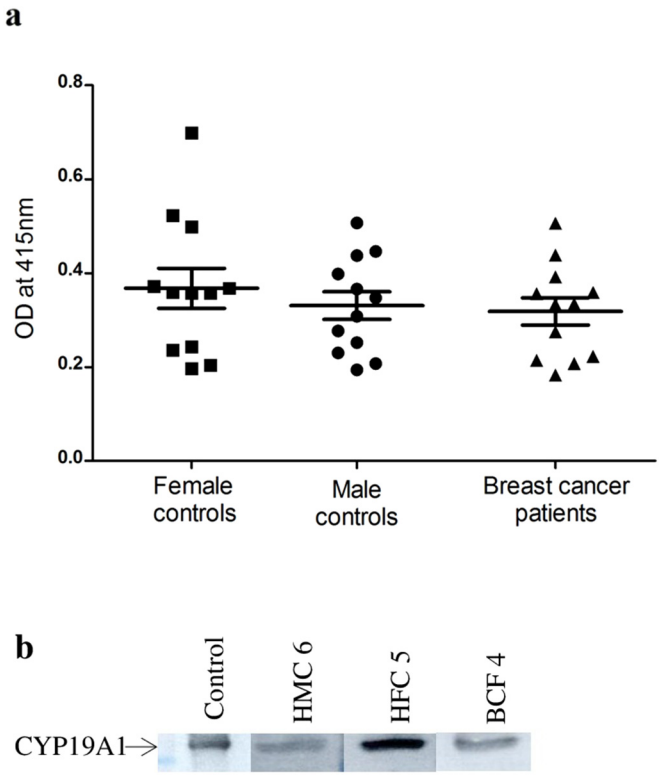

Fig. 4. ELISA assay and Western blot analysis for the detection of anti-CYP19A1 aAbs in healthy female controls, male controls and breast cancer patients' serum samples. (a) Detection of anti-CYP19A1 aAbs in the respective healthy controls and breast cancer patients' sera by ELISA. Recombinant CYP19A1 full-length protein was used to coat a ELISA plates. Wells were incubated with sera from healthy controls or female breast cancer patients (n = 12 in all cases) and probed with goat anti-human IgG/HRP labeled secondary antibody. The average of all samples in each group is displayed by the dark lines, with error bars representing the standard deviation for each group showed no significant difference between the groups' aromatase expression profiles. (b) Western blot analysis. Serum samples from in healthy male (HMC6) and female controls (HFC5) or from a female breast cancer patient (BCF4) that had high titers of anti-CYP19A1 aAbs in ELISA were used as primary antibodies in immunoblotting and probed against recombinant CYP19A1 protein; mouse anti-CYP19A1 IgG was used as positive control.

treatments [42,43]. According to our data, such treatment should raise CYP4Z1 expression levels more strongly in breast cancer cells than in normal breast cells, which would be an asset in a CYP4Z1-based prodrug strategy.

CYP19A1 (aromatase) catalyzes the last three steps in estrogen biosynthesis and aromatase inhibitors are an important class of drugs used in the treatment of breast cancer in postmenopausal women [23]. Elevated expression of CYP19A1 in breast tissue increases local estradiol concentrations and is associated with breast cancer development, but the precise mechanism by which estrogen causes breast cancer is not fully understood [44]. In this study, we observed a 21-fold higher CYP19A1 expression level in MCF-7 cells than in MCF-10A; treatment with either dexamethasone or progesterone did not have a significant effect on CYP19A1 expression in MCF-7 cells, while in MCF-10A cells progesterone treatment lead to doubling in CYP19A1 expression and dexamethasone had no effect (Fig. 1b). Thus, the effect of either progesterone or dexamethasone on MCF-10A and MCF-7 was basically identical, leading in both cases to a 13-fold higher expression level in the breast cancer cell line. When comparing the relative expression levels of the two CYPs under study with each other, CYP19A1 signals are much stronger than those of CYP4Z1 in MCF-7 cells, being 124-fold higher in untreated cells, 100-fold higher after progesterone treatment and 28-fold higher after dexamethasone treatment, respectively. By contrast, in MCF-10A cells the difference is much smaller, being 5.8-fold higher in untreated cells, 6.9-fold higher after progesterone treatment and 5.6-fold higher after dexamethasone treatment, respectively.

Remarkably, plasma membrane localization of CYP4Z1 was clearly demonstrated in MCF-7 cells regardless of treatment, while no such signal could be observed in MCF-10A cells (Fig. 2). Our data suggest that CYP4Z1 translocation to the plasma membrane is not simply a function of the intracellular expression level, as otherwise untreated MCF-10A and MCF-7 cells (with qPCR signals), should display similar signals; rather, the breast cancer cells show this translocation while the normal breast cells do not. This notion is corroborated by the study of the plasma membrane expression of CYP19A1, as here the opposite effect is to be seen: despite the huge difference in mRNA expression levels, both cell lines show clear plasma membrane signals for CYP19A1 regardless of treatment (Fig. 3). In addition, this is the first demonstration of either human or animal CYP19A1 protein being present at the plasma membrane. The molecular basis of the targeting of CYPs the plasma membrane is much less well understood than the bimodally targeting of CYPs to both ER and mitochondria [45]. However, CYPs were found recently to be cargo of the adaptor protein complex 4, which is involved in the sorting of integral membrane proteins at various stages of the endocytic and secretory pathways [46]. If such complexes have varying affinities to different CYPs and, moreover, are differentially regulated in normal or cancer cells, they might contribute to the effects observed in this study. But clearly more research in this direction is needed to understand the differences in CYP plasma membrane translocation displayed by different cell types.

We recently reported the detection of anti-CYP4Z1 aAbs in sera from breast cancer patients, but not in those of healthy controls [21]. It was therefore of interest to also look for anti-CYP19A1 aAbs in breast cancer sera and controls by indirect ELISA and Western blot analysis. These experiments showed that the presence of anti-CYP19A1 aAbs in female breast cancer patient sera as well as in male and female controls, respectively (Fig. 4a). In all three groups tested there were samples with high aAb titers as well as those with low signals. In total, the results obtained for each group were not significantly different from those of either of the other two groups. Additional Western blot analysis confirmed the specificity of the ELISA signals and the recognition of at least one linear epitope within the CYP19A1 amino acid sequence by these aAbs (Fig. 4b). Taken together, these data show for the first time that there are anti-CYP19A1 aAbs in human blood.

In an attempt to integrate all the data presented above into a single model, we developed a hypothesis for the generation of anti-CYP aAbs (Fig. 5). In this model, a certain amount of some CYPs is translocated to the plasma membrane in both normal and cancerous cells, even if the CYP protein level in normal cells is much lower. Whether this unusual location of CYPs is purely aberrant or whether it serves some physiological purpose is currently unknown. The presentation of the CYP protein on the plasma membrane elicits the formation of aAbs directed against it. As this presentation occurs irrespective of neoplasia, such anti-CYP aAbs should, therefore, be found in both healthy persons and cancer patients. CYP19A1 would be an example of a member of this group of CYPs. However, there is also a second group, which encompasses those family members that are not translocated to the plasma membrane in normal cells, but only in cancerous cells. Importantly, the relative CYP expression level in the normal or cancer cells need not to be different. For these CYPs, presentation on the plasma membrane also leads to the formation of aAbs; but since this presentation only occurs on cancer cells, the respective aAbs are only found in cancer patients, but not in healthy controls. Obviously, CYP4Z1 would be a member of this second group. It will be intriguing to see the results of more studies in the future that either corroborate or disprove this hypothesis.

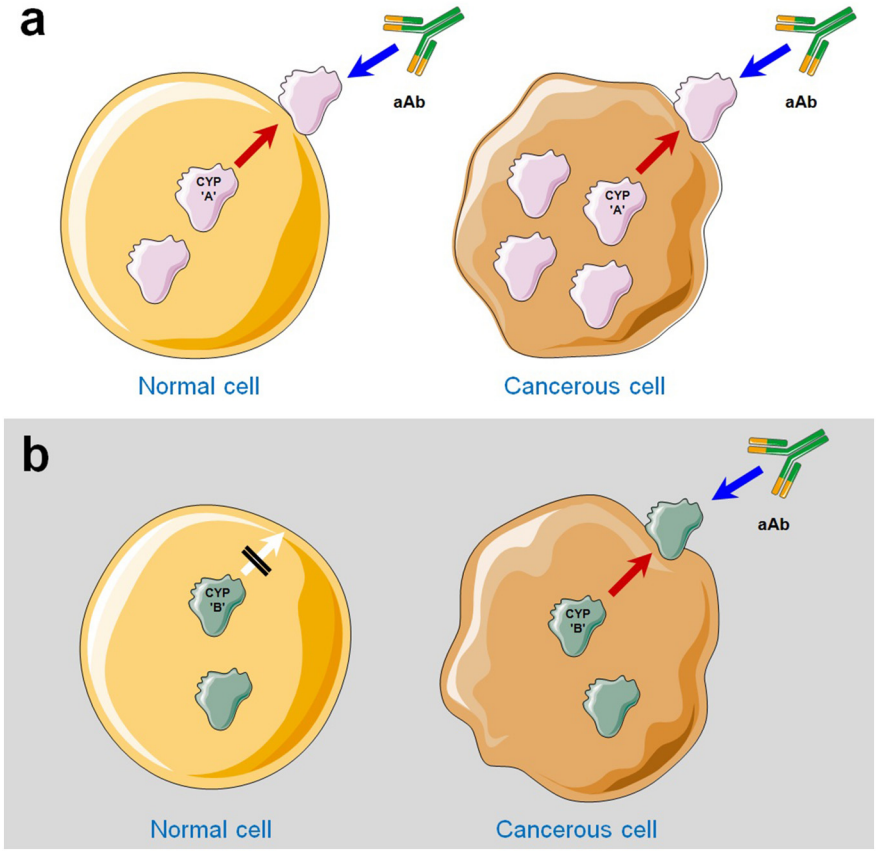

Fig. 5. Model for the generation of anti-CYP aAbs in normal and cancer cells. (a) For some CYPs (CYP 'A', shown in pink) a certain amount of CYP protein is translocated to the plasma membrane (red arrows) in both normal and cancerous cells, even if the CYP protein level in normal cells is much lower. The CYP on the plasma membrane elicits the formation of aAbs against itself, which thus leads to the presence of anti-CYP aAbs in both healthy persons and cancer patients. An example is CYP19A1. (b) In the case of other CYPs (CYP 'B', shown in green) no translocation to the plasma membrane occurs in normal cells (white arrow), but it does happen in cancerous cells, even if the CYP protein level is comparable. The CYP on the plasma membrane then causes the formation of aAbs, which leads to the presence of anti-CYP aAbs only in cancer patients. This is the case for CYP4Z1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Conflict of interest

The authors declare no conflict of interest.

Acknowledgement

We acknowledge the invaluable contributions of the study participants and we wish to thank Birgit Heider-Lips for preparation of CYP19. The Genotype-Tissue Expression (GTEx) Project was supported by the Common Fund of the Office of the Director of the National Institutes of Health, and by NCI, NHGRI, NHLBI, NIDA, NIMH, and NINDS. The data used for the analyses described in this manuscript were obtained from GTEx Analysis Release V7 (dbGaP Accession phs000424.v7.p2), accessed on 02/13/2018. Fig. 5 was made using material from the Servier Medical Art website (https://smart.servier.com).

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