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Progestogens and Breast Cancer Risk – In Vitro Investigations with Human Benign and Malignant Epithelial Breast Cells

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1. Introduction

Two recent studies, the Women's Health Initiative (WHI) and the Million Women Study (MWS), have above all raised concerns over the relationship between progestogens and increased risk of breast cancer in the climacteric and postmenopause (Million Women Study collaborators, 2003; Writing Group, 2002). The Women's Health Initiative study was terminated early after five years, due to an increased incidence of breast cancer in the group treated with combined estrogen and progestogen therapy (EPT). The MWS concluded that breast cancer risk was increased two-fold in current users of combined HRT compared to a factor of 1.3 for estrogen-only therapy.

A crucial role of progestogens in increasing breast cancer risk was supported by the WHI estrogen mono-arm showing no increase but rather a reduction of breast cancer risk, which was significant for patients with more than 80% adherence to study medication (The Women's Health Initiative Steering Committee, 2004).

However, in the French E3N-EPIC trial of over 80 000 postmenopausal women it was reported that hormone therapy containing the progestin medroxyprogesterone acetate or norethisterone was associated with a significant increase in risk of breast cancer, whereas hormone therapy including progesterone and certain other progestins did not induce an increased risk (Fournier et al., 2008).

By stimulating the production of survival factors, estradiol (E2) and other steroid hormones may influence cell proliferation. These survival factors include growth factors and cytokines. Epithelial and stromal cell-derived growth factors are understood to be significant in the regulation of breast epithelial cells directly via autocrine, paracrine, juxtacrine or intracrine pathways. Further responses stimulated by growth factors may activate signalling pathways which support the growth of cancer cells (Dickson & Lippman, 1995).

Progestogens are conventionally thought to act via the activation of the intracellularly-located progesterone receptors (PR), PR-A and PR-B. Several *in vitro* studies indicate that progestogens may exert an antiproliferative effect by activation of these receptors in human breast cancer cells (Cappellatti et al. 1995; Krämer et al., 2006; Schoonen et al., 1995). These data are in contrast to the above mentioned clinical data. Other data suggested a proliferative effect of synthetic progestogens (Catherino et al., 1995; Franke & Vermes, 2003). Thus the mechanisms by which progestogens act on human breast cells remain unclear.

Recent experimental data revealed that in addition to the intracellular-located receptors, progesterone receptor membrane component-1 (PGRMC1) is associated with a membrane-associated progesterone receptor activity (Cahill, 2007). PGRMC1 was originally cloned from the endoplasmatic reticulum from porcine hepatocytes (Meyer et al., 1996). It contains several predicted motifs for protein interactions, and overlapping sites for phosphorylation, whose phosphorylation status might correlate with its localisation in the cell (Ahmed et al., 2010, Cahill, 2007; Munton et al. 2007). PGRMC1 has been detected in several cancers and cancer cell lines e.g. breast cancer (Neubauer et al., 2008, 2009). It is overexpressed in lung cancer and colon cancer (Cahill, 2007).

There is a long-standing link between PGRMC1 and progesterone signaling. However, because bacterially expressed PGRMC1 does not bind to progesterone (Min et al., 2005), and since the majority of PGRMC1 is not localized to the plasma membrane (Crudden et al., 2005; Nolte et al., 2000; Peluso et al., 2008) it is now tentatively assumed that PGRMC1 does not bind P4 by itself (Cahill, 2007), but requires an unknown protein that is associated only in partially purified PGRMC1 preparations (Peluso et al., 2008). PGRMC1-associated progesterone binding is functionally important in cancer cells because progesterone inhibits apoptosis in granulosa cells, and this anti-apoptotic activity requires PGRMC1 (Peluso et al., 2008a, 2008b). However, it is unclear how PGRMC1 transduces anti-apoptotic signaling by progesterone. Expression of PGRMC1 has been identified in several subcellular compartments including cell membrane, cytoplasm, endoplasmatic reticulum and nucleus (reviewed in Cahill, 2007). Swiatek-De Lange et al. (2007) reported that PGRMC1 localizes to the plasma membrane and microsomal fraction of retinal cells.

In the following our investigations on the effect of progesterone and various synthetic progestins on the proliferation of human benign and malignant breast epithelial cells with and without expressing PGRMC1 are summarized.

2. Normal breast epithelial cells

MCF10A, a human, non-tumorigenic, estrogen and progesterone receptor-negative breast epithelial cell line was used for these experiments (Catherino et al., 1995, Soule et la., 1990). Progesterone (P4), chlormadinone acetate (CMA), norethisterone (NET), medroxyprogesterone acetate (MPA), gestodene (GSD), 3-ketodesogestrel (KDG) and dienogest (DNG) were tested at the concentration range of 10^{-9} to 10^{-6} M. For stimulation of the MCF-10A cells a mixture of growth factors was used. As outcome proliferation and apoptosis were measured and the ratio of apoptosis to proliferation was compared. Proliferation is quantified by measuring light emitted during the bioluminescence reaction of luciferene in the presence of ATP and luciferase. Apoptosis was measured by the Cell Death Assay, which is based on the quantitative sandwich-enzyme-immunoassay principle using mouse monoclonal antibodies directed against DNA and histones. Photometric enzyme immunoassay quantitatively determines cytoplasmic histone-associated DNA fragments after induced cell death.

The combination of the stroma-derived growth factors epithelial growth factor (EGF),basic-fibroblastic growth factor (FGF) and insulin-like growth factor-I (IGF-I) alone confirmed a proliferative response compared to the assay medium-only control. These growth factors were chosen, since they have been shown to be most effective in terms of breast epithelial cell proliferation (Dickson & Lippman, 1995).

In combination with growth factors, the ratio was reduced significantly compared to the growth factor alone by MPA and CMA (i.e., favouring an additional proliferative effect). MPA produced a four-fold reduction in the ratio in comparison to growth factors alone at 10^{-7} M and 10^{-6} M ($p<0.05$), CMA had a significant effect at 10^{-6} M only, reducing the ratio 3-fold. P4, NET, LNG, DNG, GSD and KDG had no significant effect on the growth factor-induced stimulation of MCF10A (Table 1).

	Normal cells
	Growth factors
Progesterone	Ø
Medroxyprogesterone acetate	--
Chlormadinone acetate	--
Norethisterone	Ø
Levonorgestrel	Ø
3-Keto-desogestrel	Ø
Gestodene	Ø
Dienogest	Ø

Table 1. Effect of various progestins on the ratio of apoptosis to proliferation in normal breast epithelial cells in the presence of stroma-derived growth factors as stimulans.
(+ = increase; - = decrease of the ratio; Ø = no effect as compared to the stimulans alone)

3. Cancerous breast epithelial cells

HCC1500, a human estrogen and progesterone receptor-positive primary breast cancer cell line was used (Gazdar et al., 1998). For stimulation of the cells estradiol alone, a growth factor mixture alone as well as a combination of both was used.
The combination of the growth factors EGF, FGF and IGF-I alone confirmed a proliferative response compared to the assay medium-only control. MPA in combination with growth factors caused a significant increase in the ratio of apoptosis to proliferation at both concentrations compared to growth factors alone ($p<0.05$), the greatest effect being at 10^{-7} M, with a doubling of this ratio, i.e., an inhibitory effect. CMA also caused a significant increase in this ratio, with the greatest effect seen at 10^{-6} M, yielding over a 2-fold ratio increase. Conversely, NET, LNG, and DNG at both concentrations and GSD and KDG at 10^{-6} M led to a significant reduction in the ratio of apoptosis to proliferation, enhancing the initial proliferative effect induced by the growth factors. P4 had no significant effect at either concentration.
The results of the combination of the steroids and E2 on the estrogen-receptor positive (ER+) HCC1500 cells showed that the progestins CMA, MPA, NET, LNG, DNG, GSD and P4 significantly increased the ratio of apoptosis to proliferation towards an anti-proliferative

effect to varying degrees compared to E2 alone, with MPA having the greatest effect, followed by NET. KDG had no significant effect at either concentration. No progestin used was able to further enhance the stimulatory effect of E2 on HCC1500 cells, and all but KDG actually inhibited this effect.
The results of combining the steroids with the combination of growth factors (EGF, FGF and IGF-I) and E2 on HCC1500 cells revealed that MPA, GSD, CMA and NET all increased the ratio favouring an anti-proliferative effect compared to the proliferative effect of growth factors and E2 alone. P4, LNG, DNG and KDG had no significant effect at either concentration.

Progestin	**Cancerous cells**		
	Growth factors	Estradiol	Growth factors + Estradiol
Progesterone	+	+	+
Medroxyprogesterone acetate	++	++	++
Chlormadinone acetate	++	++	++
Norethisterone	--	++	++
Levonorgestrel	--	++	++
3-Keto-desogestrel	--	Ø	++
Gestodene	-	++	++
Dienogest	--	+	Ø

Table 2. Effect of various progestins on the ratio of apoptosis to proliferation in cancerous breast epithelial cells in the presence of stroma-derived growth factors, estradiol or a combination of both as stimulans. (+ = increase; - = decrease of the ratio; Ø = no effect as compared to the stimulans alone)

In summary these results indicate that progestins are different in their ability to induce proliferation or inhibit the growth of benign or malignant human breast epithelial cells dependently or independently of the effects of stromal growth factors and E2. Thus on the basis of experimental data the choice of progestin for hormone therapy may be important in terms of influencing a possible breast cancer risk.
A further important result from our experimental research seems to be the fact that the influence of the progestins can differ largely between normal and cancerous breast epithelial cells. This would have clinical relevance for the use of HRT after breast cancer, which is of course contraindicated in routine therapy. But as even in the normal population women express malignant cells, shown by post mortem analyses (Black & Welch, 1993), different, may be contrary progestins effects in benign or malignant cells may have relevance for the primary breast cancer risk of postmenopausal women treated with HRT. Therefore this field should be further investigated.

4. Cancerous breast epithelial cells cells overexpressing PGRMC1

Since the results of the WHI mono arm were published, indicating a negative effect of progestins on breast cancer risk, the molecular pathway responsible for this effect and the many questions on the extrapolation of the WHI results to all synthetic progestins and to

natural progesterone remain unknown. We have published for the first time results suggesting that signaling of synthetic progestins via PGRMC1 could be one explanation (Neubauer et al., 2009).
For the experiments two synthetic progestins have been chosen that are widely used in hormone therapy, i.e. MPA and NET, as well as a new synthetic progestin, i.e. DRSP, which might differ in its behaviour to MPA and NET because of a different chemical structure. In addition progesterone and progesterone-3-(O-carboxymethyl) oxime: BSA-fluorescein-isothio cyanate conjugate (P4:BSA-FITC) was tested.

4.1 Transfection of MCF-7 cells

MCF-7 cells were stably transfected with expression vector pcDNA3.1 containing hemeagglutinin-tagged (3HA) PGRMC1 using lipofectamine™ 2000, in accordance with the manufacture's recommendation. A total of $5x10^5$ cells were transfected and plated with RPMI-medium for 24h. Then medium was changed to RPMI complete medium containing 100µg/ml hygromycin B. Cells were cultured for 2 weeks for selection of stable integration events. Transfection rates were measured by cotransfection of a GFP expressing plasmid and immune fluorescence analysis. After 2 weeks single colonies had formed and limiting dilutions were performed three times to select for colonies grown from a single cell.
Stable transfection was verified by PCR using chromosomal DNA and primers spanning intron 1 to distinguish integrated PGRMC1 cDNA from the chromosomal sequence. The sequences of the primers were 5'- CTGCTGCATGAGATTTTCACG-3' hybridizing to nucleotides 71 to 91 of PGRMC1 open reading frame and 5'-GCATAGTCCGGGACGTCATA-3' hybridizing to the sequence coding for the HA tag. PCR products were sequenced.

4.2 Effect of synthetic progestins alone

Dose-dependent effects on cell proliferation of P4, P4:BSA-FITC, MPA, NET or DRSP were determined using MTT assay (Fig. 3). Between 10^{-9}M to 10^{-5}M P4 did not increase proliferation of either MCF-7 or MCF-7/PGRMC1-3HA cells (WT-12). However, proliferation of WT-12 cells was significantly increased when treated with P4:BSA-FITC or the synthetic progestogens: for P4:BSA-FITC at concentrations from 10^{-7} M to 10^{-5}M with a maximal effect at 10^{-6}M, for NET reaching its maximal effect compared to untreated control at 10^{-7}M, for MPA at concentrations higher than 10^{-6}M, and for DRSP at concentrations higher than 10^{-7}M. The effect of NET was significantly different to that one of DRSP at the concentrations of 10^{-9} and 10^{-8} M and to the effect of MPA at the concentrations of 10^{-9}, 10^{-8} and 10^{-7} M. DRPS showed a significant stronger effect as compared to MPA at the concentration of 10^{-7} M. No effects were observed in MCF-7 cells within the investigated concentration ranges for all the progestogens used in this experiment.
For further kinetic experiments 10^{-6}M was chosen for all progestogens. In comparison to all other synthetic progestins tested NET significantly increased proliferation almost to maximum even at 10^{-9}M, the lowest concentration that we tested. Taken together, the results strongly suggested that some synthetic progestins elicit a PGRMC1-dependent proliferative response.
To determine time-dependent proliferative effects of progestogens a kinetic analysis over 6 days was performed (Fig. 4). MCF-7 and WT-12 cells were incubated with P4, P4-BSA-FITC, DRSP, MPA and NET at 10^{-6}M and proliferation was determined by MTT assay. The results indicate that P4:BSA-FITC, DRSP, MPA and NET increased proliferation in WT-12 cells by approximately 3.5 to 4 fold on day 6 which is highly significant compared to the

simultaneously cultured untreated control cells. No effects on proliferation were observed for P4, DRSP, MPA and NET in MCF-7 cells. Only the membrane-impermeable P4-BSA-FITC caused a marginal increase of proliferation in the parental MCF-7 cells by approximately 1.5 fold compared to the control cells.

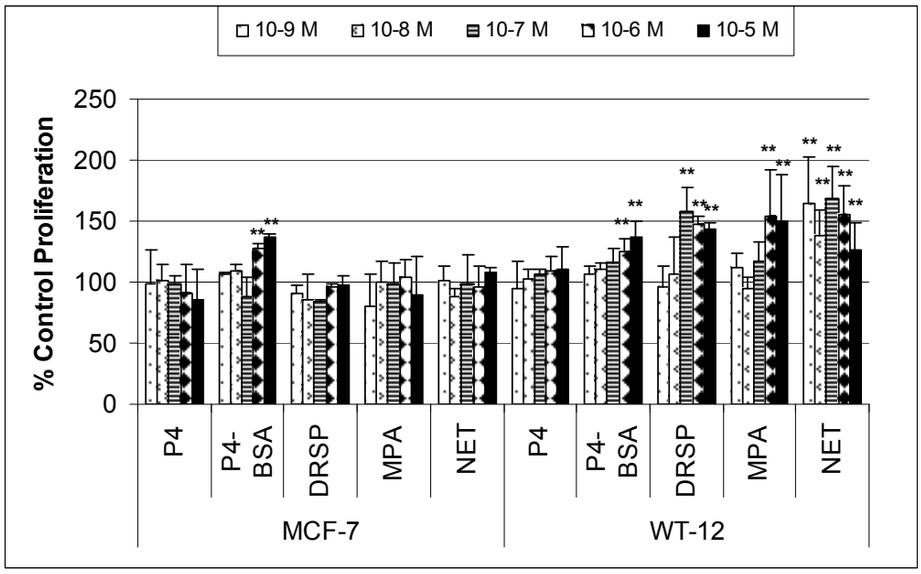


Fig. 3. Titration of progesterone and synthetic progestins. MCF-7 and MCF-7/PGRMC1-3HA (WT-12) cells were incubated with either progesterone (P4), P4:BSA-FITC, DRSP, MPA, and NET from 10^{-5}M to 10^{-9}M in tenfold dilution steps. Cell proliferation was measured after 4 days. Data were normalized to unstimulated controls. (means ± SD; ** $p < 0.01$ vs. controls)

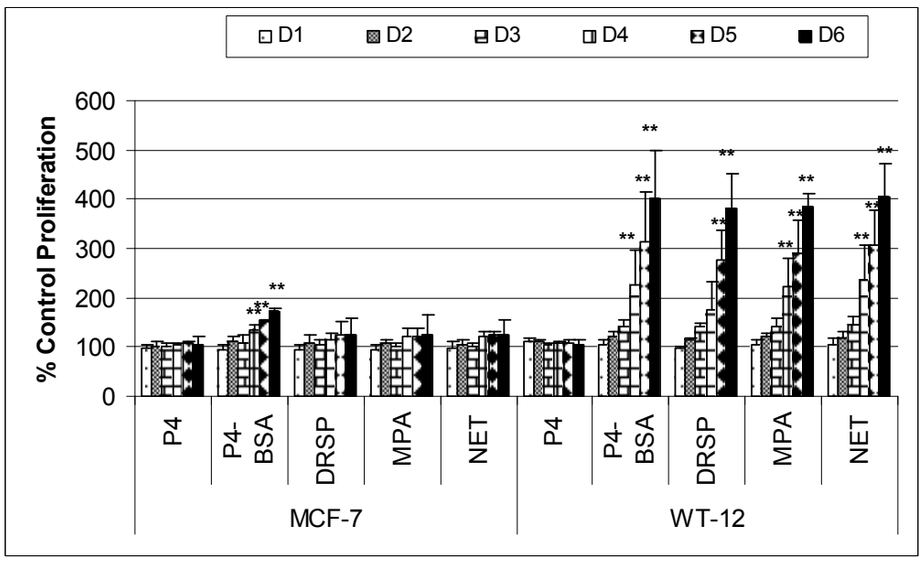


Fig. 4. Kinetic analysis of proliferation. MCF-7 and MCF-7/PGRMC1-3HA (WT-12) cells were incubated with either progesterone (P4), P4:BSA-FITC, DRSP, MPA, and NET at 10^{-6}M. Cell proliferation was measured daily for 6 days (D1–D6). Data were normalized to unstimulated controls. (means ± SD; * $p < 0.05$; ** $p < 0.01$ vs. controls)

4.3 Combination of progestogens with estradiol in PGRMC1 overexpressing cells

In our further investigations we showed that estradiol in a dosage that increased cell numbers of MCF-7 cells was able to induce an effect in WT-12 cells that doubed the effect in MCF-7 cells (Neubauer et al, 2010). The concentration of 10^{-10} M was chosen, because it is equally to in vivo serum concentrations achieved with transdermal or low orally estradiol application. The concentration of 10^{-12} M was chosen in order to imitate very low serum estradiol concentrations that were not able to induce a measurable breast cancer risk. The E2 effect could be blocked by the addition of the potent estrogen receptor antagonist fulvestrant indicating that the intracellular estrogen receptor-alpha is involved. However, since the proliferation was twice as high as in MCF-7 cells, in the presence of PGRMC1 a mechanistic interaction between the estrogen receptor-alpha and PGRMC1 signaling systems seems to be highly possible. The mechanism(s) of interaction is currently unknown. Of special significance are our findings in terms of adding progesterone or medroxyprogesterone acetate to estradiol. When PGRMC1 is overexpressed the E2-induced effect is more pronounced, but P4 still displayed a neutral effect. However, the addition of MPA triggered a strong proliferative signal in the presence of this E2 concentration (Fig. 5). The effect of other synthetic progestogens in combination with E2 on the proliferation of MCF-7 cells overexpressing PGRMC1 is currently under investigation.

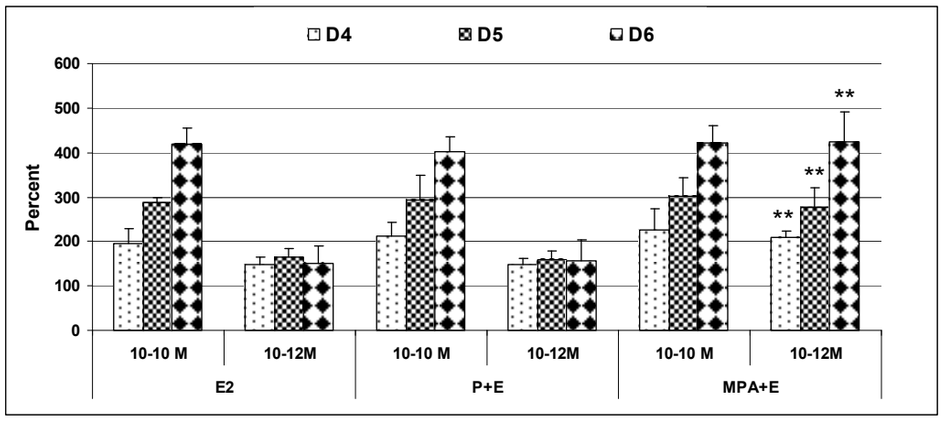


Fig. 5. MCF-7/PGRMC1-3HA (WT-12) cells were incubated with estradiol (E2, 10^{-10} M or 10^{-12} M) alone and in combination with either progesterone (P, 10^{-6} M) or medroxyprogesterone acetate (MPA, 10^{-6} M). Cell proliferation was measured after 4, 5 and 6 days. Data were normalized to unstimulated controls. (Means ±SD; ** $p < 0.01$ vs. E2)

5. Discussion

The proliferation of normal and malignant cells is under the control of both estrogen and growth factors. In normal epithelial cells, estrogen-receptor expressing cells represent only a minority of the total cells and do not proliferate (Ali & Coombes, 2002). Current opinion is that estrogens act proliferatively in a paracrine fashion by inducing the production of stromal-derived growth factors and cytokines or their receptors via the activation of epithelial or stromal estrogen receptors. Growth factors may play an important role in the promotion of receptor-positive breast cancer by cross-talk with the steroid-receptor and are mainly responsible for the progression of estrogen-receptor negative breast cancer. Among

the growth factors which are important for cell growth are the epidermal growth factor (EGF) family, insulin-like growth factors I and II (IGF-I and IGF-II), fibroblast growth factors (FGFs), transforming growth factor-α (TGF-α) and platelet-derived growth factors (PDGFs).

It is important to differentiate between normal and malignant estrogen-receptor positive breast cells. Therefore, for the first time, we have investigated the effect of eight different progestogens on the proliferation of benign and malignant breast epithelial cells in the presence of growth factors and/or estradiol.

Our results indicate that MPA may enhance the mitotic rate of normal epithelial breast cells in the presence of growth factors and thus may increase the probability of faults in DNA-replication when used in long-term. Indeed, the results of WHI indicate that patients who were not using hormones prior to the start of the study had no increased hazard ratio for breast cancer whereas subjects with prior hormone use for up to five, five to ten and more than 10 years showed an increasing risk (Writing Group, 2002). These data suggest that long-term use of MPA may increase breast cancer risk by enhancing the mitotic rate of normal epithelial cells.

We could further demonstrate that progesterone had a neutral effect on growth-factor stimulated healthy breast epithelial cells. In the case of cancerous breast cells, other groups have published supporting results, where E2-induced stimulation of MCF-7 cells has been shown to be inhibited by progesterone (Cappellatti et al., 1995; Mueck et al., 2004; Schoonen et al., 1995; Seeger et al., 2003). Up to now, there is a paucity of data available regarding the effects of CMA and LNG on the proliferation of normal and malignant epithelial breast cells. There are also conflicting epidemiological data concerning these progestogens (Ebeling et al., 1991; Nischan et al., 1984; Persson et al., 1996). DNG has been shown to elicit potent anti-tumour activity against hormone-dependent cancer types in an animal model and has exhibited slight concentration-dependent inhibitory effects in combination with E2, in agreement with our results (Katsuki et al., 1997). GSD and KDG have been shown to be able to inhibit cell proliferation of a specific sub-clone of MCF-7 in the presence of E2 (Schoonen et al., 1995). Our results support the inhibitory effects of both GSD and KDG in combination with E2, however, we found both exhibited a proliferative effect on HCC1500 cells with growth factors alone.

By comparing the cell death to proliferation ratio results of growth factors alone, E2 alone and combination of growth factor and E2 on HCC1500 cells, we also found that the single proliferative effects of growth factors or E2 alone are magnified when in combination with each other, which, however, was not always statistically significant. The mechanism of the stimulatory effect of MPA (and of CMA) on MCF10A cells is currently unknown, as this cell line is both estrogen and progesterone receptor negative. The effects of the steroids on HCC1500 cells appear to be receptor-dependent, since the time course clearly shows a long-term effect rather than a rapid non-genomic action.

For the first time we could present data suggesting that signaling of synthetic progestins via PGRMC1 could be one explanation for the clinically observed possible induction of breast cancer risk by progestins. We have chosen two synthetic progestins that are widely used in hormone therapy, i.e. MPA and NET, as well as a new synthetic progestin, i.e. DRSP, which might differ in its behaviour to MPA and NET, because of a different chemical structure.

The synthetic progestins MPA, NET and DRSP significantly induced a relatively large proliferative effect in MCF-7 cells that overexpress PGRMC1. For P4, however, no such effect was found. Since progesterone and the synthetic progestins used in HT are able to

activate PR-A/-B and PGRMC1 simultaneously, our data suggest that *in vivo* the balance of the expression levels of both receptors might influence whether epithelial cells proliferate or not in the presence of progestogens. Therefore, it may be instructive to determine the expression ratio of PGRMC1 and PR-A/-B before HT.

Interestingly, P4:BSA-FITC is able to induce a marginal proliferative signal in MCF-7 cells (Fig. 3). P4:BSA-FITC is thought to be unable to cross the plasma membrane and can therefore only bind to membrane associated progesterone receptors. MCF-7 cells express endogenous PGRMC1 at very low amounts (data not shown), which may transduce the weak proliferative signal since the classical PR-A/-B response is not triggered. The synthetic progestins and P4 bind to all progesterone receptors expressed by MCF-7 cells. Binding to PR-A/-B might transduce an antiproliferative signal, countermanding the proliferative signal induced by low levels of PGRMC1. In contrast, in WT-12 cells the exogenously expressed PGRMC1 might overrule the antiproliferative effect of PR-A/-B. In several human ovarian surface epithelial cell lines, P4 inhibits their proliferation (Syed et al., 2001). Because these cells express the PR-A/-B it has been assumed that P4's actions are mediated via these receptors. However, P4 exhibits antimitotic action only at micromolar doses, which have been used in these experiments (Syed et al., 2001). Given that the dissociation constant for the PR-A/-B is 1–5 nm (Stouffer, 2003) and for PGRMC1 is in the 0.20–0.3 μm range (Meyer et al., 1996), which is well within the levels of P4 in serum and in follicular fluid (Stouffer, 2003), in MCF-7 cells the classical PR-A/-B receptors are perhaps activated preferentially by gestagens inducing an anti proliferative signal. This concept is supported by a previous observation that at micromolar doses P4 inhibits granulosa cell and spontaneously immortalized granulosa cell (SIGC) mitosis (Fujii et al., 1983).

Interestingly, NET exerts its activity on proliferation already at the lowest concentration tested (10^{-9} M, Fig. 4) whereas DRSP and MPA increase proliferation only at higher concentrations (10^{-7} M and 10^{-6} M). This suggests that NET binds PGRMC1 with the highest affinity, followed by DRSP and MPA. Compared to PR-A/-B this is different since the latter binds MPA better than NET (Kuhl, 1998). These results indicate that HT including NET might result in an increased risk for breast cancer development. Indeed, some studies in which norethisterone- or levonorgestrel-derived progestogens were continuously administered a significantly higher risk for breast cancer was observed than for continuously administered progesterone-derived progestogens (Lyytinen et al., 2009; Magnusson et al., 1999). In one study the use of norethisterone acetate was accompanied with a higher risk after 5 years of use (2.03, 1.88-2.18) than that of medroxyprogesterone acetate (1.64, 1.49-1.79) (Lyytinen et al., 2009). It is known that NET can be converted in vivo into ethinylestradiol (Kuhnz et al., 1997). In as far this conversion may influence the observed NET effect is currently unknown and is under investigation.

Despite their widespread use, *in vitro* models have certain limitations: the choice of culture conditions can unintentionally affect the experimental outcome, and cultured cells are adapted to grow *in vitro*; the changes which have allowed this ability may not occur *in vivo*. Limitations of this *in vitro* study might be the high concentrations needed for an effective antiproliferative effect. The clinically relevant blood concentrations for the progestogens most commonly used for HRT, MPA and NET, are in the range of $4x10^{-9}$M to 10^{-8}M for MPA (Svensson et al., 1994) and around 10^{-8}M for NET (Stanczyk et al., 1978). However, higher concentrations may be required *in vitro* in short-time tests in which the reaction threshold can only be achieved with supraphysiological dosages. Higher concentrations may also be

reached *in vivo* in the vessel wall or organs compared to the concentrations usually measured in the blood.

A further limitation of our work is the short incubation period of the cells with the substrates under investigation, in comparison to the longer time period for which hormone therapy is usually prescribed. That duration of therapy may indeed be an important factor for breast cancer risk is emphasized by the results of WHI, where breast cancer risk was significantly higher compared to placebo only in women given combined HRT for 10 years or more, but not in those treated only for the duration of the study period, i.e. 5.2 years (Writing Group, 2002). In vitro experiments can support, but not replace clinical trials, and therefore, further clinical studies are needed to determine which progestogens, if any, have the lowest breast cancer risk.

6. Conclusion

Experimental data with the comparison of various synthetic progestins in the same in vitro model present rather high evidence that there may be differences between the various progestins regarding breast cancer risk. Especially of concern may be to differentiate between primary and secondary risk i.e. between benign and malignant breast epithelial cells. This differentiation seems to be especially important for the progestin MPA. Since even in 'clinically healthy' women malignant cells can be detected (Nielsen et al., 1987), this experimental finding may have relevance and should be further investigated.

The effect of progestins on breast cancer tumorigenesis may depend on the specific progestin used for hormone therapy and the expression of PGRMC1, PR-A and PR-B in the target tissue. However, in terms of the clinical situation it remains unknown how uniformly PGRMC1 is expressed in the normal breast epithelial cells between patients. Thus screening, which might be based on determining the expression ratio of PGRMC1 and PR in cells from nipple aspirate fluid (NAF), might be of interest to identify women who show an increased expression of PGRMC1 and who might thus be susceptible for breast cancer development under HT (Sauter et al., 1997). The data presented here are of dramatic importance in terms of progesterone and breast cancer risk in HT clinical studies so far (Writing Group for the Women's Health Initiative Investigators, 2002; The Women's Health Initiative Steering Committee, 2004). The epidemiological studies and especially the WHI trial, so far the only prospective placebo-controlled interventional study, demonstrate an increased risk under combined estrogen/progestin therapy, but they have the limitations that they up to now can not discriminate between the various progestins mostly due to too small or not comparable patient numbers in the subgroups with the various progestins. However, there is evidence that the natural progesterone, possibly also the transdermal usage of synthetic progestins, may avoid an increased risk, but this must be proven in further clinical trials.

7. References

Ahmed IS, Rohe HJ, Twist KE & Craven RJ. (2010) Pgrmc1 (progesterone receptor membrane component 1) associates with EGFR and regulates erlotinib sensitivity. *Biological Chemistry*, 285, pp. 24775-82

Ali S & Coombes RC. (2002). Endocrine-responsive breast cancer and strategies for combating resistance. *Nature Reviews Cancer*, 2, pp. 101-15

Black WC & Welch HG. (1993). Advances in diagnostic imaging and overestimations of disease prevalence and the benefits of therapy. *New England Journal of Medicine*, 328, pp. 1237-1243

Cahill MA. (2007). Progesterone receptor membrane component 1: an integrative review. *Journal of Steroid Biochemistry and Molecular Biology,* 105, pp. 16-36

Cappelletti V, Miodini P Fioravanti L & DiFronzo G. (1995). Effect of progestin treatment on estradiol- and growth factor-stimulated breast cancer cell lines. *Anticancer Research*, 15, pp. 2551-2556

Catherino WH, Jeng MH & Jordan VC. (1995) Norgestrel and gestodene stimulate breast cancer cell growth through an oestrogen receptor mediated mechanism. *British Journal of Cancer*, 67, pp. 945-952

Crudden G, Loesel R & Craven RJ. (2005). Overexpression of the cytochrome p450 activator hpr6 (heme-1 domain protein/human progesterone receptor) in tumors. *Tumour Biology*, 26, pp. 142–146

Dickson RB & Lippman ME. (1995). Growth factors in breast cancer. *Endocrine Reviews*, 16, pp. 559-89

Ebeling K, Ray R, Nischan P, Thomas DB. Kunde D & Stalsberg H. (1991). Combined oral contraceptives containing chlormadinone acetate and breast cancer: results of a case-control study. *British Journal of Cancer*, 63, pp. 804-08

Fournier A, Berrino F & Clavel-Chapelon F. (2008). Unequal risks for breast cancer associated with different hormone replacement therapies: results from the E3N cohort study. *Breast Cancer Research Treatment*, 107, pp. 103-111

Franke HR & Vermes I. (2003). Differential effects of progestogens on breast cancer cell lines. *Maturitas*, 46, Suppl1, pp. S55-S58

Fujii T, Hoover DJ & Channing CP. (1983). Changes in inhibin activity, and progesterone, oestrogen and androstenedione concentrations, in rat follicular fluid throughout the oestrous cycle. *Journal of Reproduction and Fertility*, 69, pp. 307-314.

Gazdar AF, Kurvari V, Virmani A, Gollahon L, Sakaguchi M, Westerfield M, Kodagoda D, Stasny V, Cunningham HT, Wistuba II, Tomlinson G, Tonk V, Ashfaq R, Leitch AM, Minna JD & Shay JW. (1998). Characterization of paired tumor and non-tumor cell lines established from patients with breast cancer. *International Journal of Cancer*, 78, pp. 766-774

Katsuki Y, Shibutani Y, Aoki D & Nozawa S. (1997). Dienogest, a novel synthetic steroid, overcomes hormone-dependent cancer in a different manner than progestins. *Cancer*, 79, pp. 169-76

Krämer EA, Seeger H, Krämer B, Wallwiener D & Mueck AO. (2006). The effect of progesterone, testosterone and synthetic progestogens on growth factor- and estradiol-treated human cancerous and benign breast cells. *European Journal of Obstetrics, Gynecology and Reproduction*, 27, pp. 139-141

Kuhl H. (1998). Pharmakologie von Sexualsteroiden. *Gynäkologe*, 31, pp. 832-847

Kuhnz W, Heuner A, Hümpel M, Seifert W & Michaelis K. (1997). In vivo conversion of norethisterone and norethisterone acetate to ethinyl etradiol in postmenopausal women. *Contraception*, 56, pp. 379-85

Lyytinen H, Pukkala E & Ylikorkala O. (2009). Breast cancer risk in postmenopausal women using estradiol-progestogen therapy. *Obstetrics and Gynecology*, 113, pp. 65-73

Magnusson C, Baron JA, Correia N, Bergstrom R, Adami H-O & Persson I. (1999). Breast-cancer risk following long-term oestrogen- and oestrogen-progestin-replacement therapy. *International Journal of Cancer*, 81, pp. 339-344

Meyer C, Schmid R, Scriba PC & Wehling M. (1996) Purification and partial sequencing of high-affinity progesterone-binding site(s) from porcine liver membranes. *European Journal of Biochemistry*, 239, pp. 726-733

Million Women Study Collaborators. (2003). Breast Cancer and hormone replacement therapy in the Million Women Study. *Lancet*, 362, pp. 419-427

Min L, Strushkevich NV, Harnastai IN, Iwamoto H, Gilep AA, Takemori H, Usanov SA, Nonaka Y, Hori H, Vinson GP & Okamoto M (2005). Molecular identification of adrenal inner zone antigen as a hemebinding protein. *Febs J*, 272, pp. 5832–5843

Mueck AO, Seeger H & Wallwiener D. (2004). Comparison of the proliferative effects of estradiol and conjugated equine estrogens on human breast cancer cells and impact of continuous combined progestogen addition. *Climacteric*, 6, pp. 221-27

Munton RP, Tweedie-Cullen R, Livingstone-Zatchej M, Weinandy F, Waidelich M, Longo D, Gehrig P, Potthast F, Rutishauser D, Gerrits B, Panse C, Schlapbach R & Mansuy IM. (2007). Qualitative and quantitative analyses of protein phosphorylation in naive and stimulated mouse synaptosomal preparations. *Molecular and Cellular Proteomics*, 6, pp. 283-293

Neubauer H, Adam G, Seeger H, Mueck AO, Solomayer E, Wallwiener D, Cahill MA & Fehm T. (2009). Membrane-initiated effects of progesterone on proliferation and activation of VEGF in breast cancer cells. *Climacteric*, 3, pp. 230-239

Neubauer H, Clare SE, Wozny W, Schwall GP, Poznanović S, Stegmann W, Vogel U, Sotlar K, Wallwiener D, Kurek R, Fehm T & Cahill MA. (2008). Breast cancer proteomics reveals correlation between estrogen receptor status and differential phosphorylation of PGRMC1. *Breast Cancer Research*, 10, R85

Neubauer H, Yang Y, Seeger H, Fehm T, Cahill MA, Tong X, Ruan X & Mueck AO. (2010). The presence of a membrane-bound progesterone receptor sensitizes the estradiol-induced effect on the proliferation of human breast cancer cells. *Menopause,8,845-50*

Nielsen M, Thomsen JL, Primdahl S, Dyreborg U & Andersen JA. (1987). Breast cancer and atypia among young and middle-aged women: a study of 110 medico-legal autopsies. *Br J Cancer,*56, pp.814–819

Nischan P & Ebeling K. (1984). Oral contraceptives containing chlormadinone acetate and cancer incidence at selected sites in the German Democratic Republic—a correlation analysis. *International Journal of Cancer*, 34, pp. 671-74

Nolte I, Jeckel D, Wieland FT & Sohn K. (2000). Localization and topology of ratp28, a member of a novel family of putative steroid-binding proteins. *Biochimica and Biophysica Acta*, 1543, pp. 123–130

Peluso JJ, Liu X, Saunders MM, Claffey KP & Phoenix K. (2008a). Regulation of ovarian cancer cell viability and sensitivity to cisplatin by progesterone receptor membrane component-1. *Journal of Clinical and Endocrinololgical Metabolism*, 93, pp. 1592–1599

Peluso JJ, Pappalardo A, Losel R & Wehling M. (2006). Progesterone membrane receptor component 1 expression in the immature rat ovary and its role in mediating progesterone's antiapoptotic action. *Endocrinology*, 147, pp. 3133–3140

Peluso JJ, Romak J & Liu X. (2008b). Progesterone receptor membrane component-1 (PGRMC1) is the mediator of progesterone's antiapoptotic action in spontaneously immortalized granulosa cells as revealed by PGRMC1 small interfering ribonucleic acid treatment and functional analysis of PGRMC1 mutations. *Endocrinology*, 149, pp. 534–543

Persson I, Yuan J, Bergkvist L & Schairer C. (1996). Cancer incidence and mortality in women receiving estrogen and estrogen-progestin replacement therapy – long-term follow-up of a Swedish cohort. *International Journal of Cancer,* 67, pp. 327-32

Pirnia F, Pawlak M, Thallinger GG, Gierke B, Templin MF, Kappeler A, Betticher DC, Gloor B & Borner MM. (2009) Novel functional profiling approach combining reverse phase protein microarrays and human 3-D ex vivo tissue cultures: expression of apoptosis-related proteins in human colon cancer. *Proteomics*, 9, pp. 3535-3548

Sauter ER, Ross E, Daly M, Klein-Szanto A, Engstrom PF, Sorling A, Malick J & Ehya H. (1997). Nipple aspirate fluid: a promising non-invasive method to identify cellular markers of breast cancer risk. *British Journal of Cancer*, 76, pp. 494-501

Schoonen WGEJ, Joosten JWH & Kloosterboer HJ. (1995). Effects of two classes of progestins, Pregnane and 19-nortestosterone derivatives, on cell growth of human breast tumor cells: 1. MCF-7 cell lines. *Journal of Steroid Biochemistry and Molecular Biology*, 55, pp. 423-437

Seeger H, Wallwiener D & Mueck AO. (2003). The effect of progesterone and synthetic progestins on serum- and estradiol-stimulated proliferation of human breast cancer cells. *Hormone and Metabolic Research*, 35, pp. 76-80

Soule HD, Maloney TM, Wolman SR, Peterson WD, Brenz R, McGrath CM, Russo J, Pauley RJ, Jones RF & Brooks, SC. (1990) Isolation and characterization of a spontaneously immortalized human breast epithelial cell line, MCF-10. *Cancer Research*, 50, pp. 6075-6086.

Stanczyk FZ, Brenner PF, Mishell DR, Ortiz A, Gentzschein EKE & GoebelsmannU. (1978). A radioimmunoassay for norethindrone (NET): measurement of serum net concentrations following ingestion of NET-containing oral contraceptive steroids. *Contraception*, 18, pp. 615-33

Stouffer RL. (2003). Progesterone as a mediator of gonadotrophin action in the corpus luteum: beyond steroidogenesis. *Human Reproduction Update*, 9, pp. 99–117

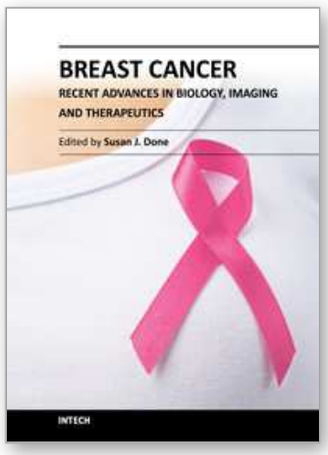
Svensson LO, Johnson SH & Olsson SE. (1994) Plasma concentrations of medroxyprogesterone acetate, estradiol and estrone following oral administration of Klimaxil, Trisequence/Provera, and Divina, A randomised, single-blind, triple cross-over bioavailability study in menopausal women. *Maturitas*, 18, pp. 229-38

Swiatek-De Lange M, Stampfl A, Hauck SM, Zischka H, Gloeckner CJ, Deeg CA & Ueffing M. (2007). Membrane-Initiated Effects of Progesterone on Calcium Dependent Signaling and Activation of VEGF Gene Expression in Retinal Glial Cells. *Glia*, 55, pp. 1061–1073

Syed V, Ulinski G, Mok SC, Yiu GK & Ho SM. (2001) Expression of gonadotropin receptor and growth responses to key reproductive hormones in normal and malignant human ovarian surface epithelial cells. *Cancer Research*, 61, pp. 6768–6776

The Women's Health Initiative Steering Committee. (2004). Effects of conjugated equine estrogen in postmenopausal women with hysterectomy. *JAMA*, 291, pp. 1701-1712

Writing Group for the Women's Health Initiative Investigators. (2002). Risks and benefits of estrogen plus progestin in healthy postmenopausal women. *JAMA*, 288, pp.321-333



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In recent years it has become clear that breast cancer is not a single disease but rather that the term encompasses a number of molecularly distinct tumors arising from the epithelial cells of the breast. There is an urgent need to better understand these distinct subtypes and develop tailored diagnostic approaches and treatments appropriate to each. This book considers breast cancer from many novel and exciting perspectives. New insights into the basic biology of breast cancer are discussed together with high throughput approaches to molecular profiling. Innovative strategies for diagnosis and imaging are presented as well as emerging perspectives on breast cancer treatment. Each of the topics in this volume is addressed by respected experts in their fields and it is hoped that readers will be stimulated and challenged by the contents.

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