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Peroxisome proliferator-activated receptor-β/δ (PPARβ/δ) ligands inhibit growth of UACC903 and MCF7 human cancer cell lines

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Abstract

The development of peroxisome proliferator-activated receptor-β/δ (PPARβ/δ) ligands for the treatment of diseases including metabolic syndrome, diabetes and obesity has been hampered due to contradictory findings on their potential safety. For example, while some reports show that ligand activation of PPARβ/δ promotes the induction of terminal differentiation and inhibition of cell growth, other reports suggest that PPARβ/δ ligands potentiate tumorigenesis by increasing cell proliferation. Some of the contradictory findings could be due in part to differences in the ligand examined, the presence or absence of serum in cell cultures, differences in cell lines, or differences in the method used to quantify cell growth. For these reasons, this study examined the effect of ligand activation of PPARβ/δ on cell growth of two human cancer cell lines, MCF7 (breast cancer) and UACC903 (melanoma) in the presence or absence of serum using two highly specific PPARβ/δ ligands, GW0742 or GW501516. Culturing cells in the presence of either GW0742 or GW501516 caused upregulation of the known PPARβ/δ target gene angiopoetin-like protein 4 (ANGPTL4). Inhibition of cell growth was observed in both cell lines cultured in the presence of either GW0742 or GW501516, and the presence or absence of serum had little influence on this inhibition. Results from the present studies demonstrate that ligand activation of PPARβ/δ inhibits the growth of both MCF7 and UACC903 cell lines and provide further evidence that PPARβ/δ ligands are not mitogenic in human cancer cell lines.

Keywords

peroxisome proliferator-activated receptor; melanoma; breast cancer; nuclear receptor; cell proliferation

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Introduction

Ligands for peroxisome proliferator-activated receptor-β/δ (PPARβ/δ) have great potential for treating metabolic syndrome, diabetes and obesity. This is due to their ability to increase serum HDL cholesterol (Leibowitz et al. 2000; Oliver et al. 2001; Sprecher et al. 2006; van der Veen et al. 2005; Wallace et al. 2005), increase fatty acid catabolism in skeletal muscle, improve insulin resistance and inhibit inflammation (reviewed in (Barish et al. 2006)). However, there is considerable controversy regarding the safety of PPARβ/δ ligands due to contradictory reports in the literature, in particular those describing effects in cancer models. For example, ligand activation of PPARβ/δ is reported to increase proliferation of human liver, cholangiocarcinoma, breast and prostate cancer cell lines (Stephen et al. 2004; Xu et al. 2006a; Xu et al. 2006b). In contrast, colon cancer cell lines fail to exhibit an increase in cell growth by PPARβ/δ in either the presence or absence of serum (Shimada et al. 2002; Stephen et al. 2004). Additionally, more recently it was shown that two different PPARβ/δ ligands inhibit cell growth of human liver and colon cancer cell lines, independent of culture medium serum (Hollingshead et al. 2007a).

While some reports suggest that PPARβ/δ ligands promote the growth of cancer cell lines, there are many observations inconsistent with this hypothesis. Anti-inflammatory activity of PPARβ/δ and/or PPARβ/δ ligands has been shown in a number of different models including immune cells, colon epithelium, macrophages, cardiomyocytes, keratinocytes, myoblasts, endothelial cells, nerve tissue and hepatocytes (Ding et al. 2006; Graham et al. 2005; Hollingshead et al. 2007b; Jakobsen et al. 2006; Kim et al. 2006; Nagasawa et al. 2006; Peters et al. 2000; Polak et al. 2005; Rival et al. 2002; Schmuth et al. 2004; Welch et al. 2003; Woo et al. 2006). There is also strong evidence that ligand activation of PPARβ/δ promotes terminal differentiation in intestinal epithelium, breast and colon cancer cell lines, trophoblasts and primary keratinocytes (Aung et al. 2006; Burdick et al. 2007; Kim et al. 2006; Marin et al. 2006; Nadra et al. 2006; Schmuth et al. 2004; Tan et al. 2001; Varnat et al. 2006; Westergaard et al. 2001). Evidence from a large number of independent laboratories also shows that cell growth is inhibited by PPARβ/δ and its ligands in colonocytes, keratincytes, cardiomyocytes, fibroblasts, endothelial cells and a variety of cancer cell lines (Ali et al. 2005; Aung et al. 2006; Burdick et al. 2007; Fukumoto et al. 2005; Hollingshead et al. 2007a; Kim et al. 2004; Kim et al. 2006; Kim et al. 2005; Man et al. 2007; Marin et al. 2006; Martinasso et al. 2006; Matthiessen et al. 2005; Michalik et al. 2001; Müller-Brüsselbach et al. 2007; Nadra et al. 2006; Ou et al. 2007; Peters et al. 2000; Planavila et al. 2005; Schmuth et al. 2004; Tan et al. 2001; Teunissen et al. 2007; Varnat et al. 2006; Westergaard et al. 2001). Given the potential of PPARβ/δ ligands as therapeutic agents, it is of great importance to determine the effect of ligand activation of PPARβ/δ on cell growth in vitro and in vivo.

There are a number of possible explanations for the reported differences described for the effect of PPARβ/δ ligands on cell growth including differences between high affinity PPARβ/δ ligands (Berger et al. 1999; Sznaidman et al. 2003) and differences due to the presence or absence of serum. The present studies evaluated the possible influence of these variables using two different PPARβ/δ ligands (GW0742 and GW501516), and comparing the effects of these ligands in two human cancer cell lines in the presence or absence of serum.

1. Experimental procedures

1.1 Chemicals

GW0742 was synthesized by GlaxoSmithKline. GW501516 was synthesized according to procedures previously described by others (Sznaidman et al. 2003; Wei and Kozikowski

2003). It was characterized using ^{1}H-NMR (DMSO-d_6) and MS, and determined to be 99% pure based on HPLC analysis.

1.2 Cell culture

MCF7 cells were obtained from ATCC in 2006. UACC903 cells were provided by Dr. Gavin Robertson. Cells were maintained in DMEM (Invitrogen, Carlsbad, CA) with 10% FBS at 37° C and 5% CO_2. Cells were plated on 6-well dishes at a density of 10,000 – 30,000 cells per well 24 hours prior to determining plating efficiency with a Z1 coulter particle counter® at time 0 (Beckman Counter, Inc., Hialeah, FL). Cells were then either serum starved for 24 hours, or not, prior to ligand treatment. After this 24 hour period, cells were maintained in respective culture medium with or without serum and treated with either GW0742 or GW501516 for 24, 48 and 72 hours at concentrations of 0 (DMSO control), 0.1 μM, 1 μM, or 10 μM. These concentrations of ligand were used because concentrations ranging from 0.1 μM to 1 μM are known to specifically activate PPARβ/δ (Kim et al. 2006). Cells were quantified every 24 hours with a Z1 coulter particle counter® (Beckman Counter, Inc., Hialeah, FL). Triplicate samples for each treatment were used for each timepoint for every treatment, and each replicate was counted three times.

1.3 RNA analysis

Total RNA was isolated from cells using Trizol reagent and the manufacturer's recommended procedures. The mRNA encoding angiogenin-related protein-like 4 (ANGPTL4), was quantified using real-time PCR analysis. The cDNA was generated using 2.5 μg total RNA with MultiScribe Reverse Transcriptase kit (Applied Biosystems, Foster City, CA). Primers were designed for real-time PCR using the Primer Express software (Applied Biosystems, Foster City, CA). Real-time PCR reactions were carried out using SYBR green PCR master mix (Finnzymes, Espoo, Finland) in the iCycler and detected using the MyiQ Realtime PCR Detection System (Bio-Rad Laboratories, Hercules, CA). The following conditions were used for PCR: 95 °C for 15 sec, 94 °C for 10 sec, 60 °C for 30 sec, and 72 °C for 30 sec, and repeated for 45 cycles. The PCR included a no template control reaction to control for contamination and/or genomic amplification. All reactions had >90% efficiency. Relative expression levels of mRNA were normalized to *GAPDH*.

2. Results

2.1 Expression of PPARβ/δ and activation by GW0742 and GW501516 in human breast cancer (MCF7) and melanoma (UACC903) cell lines

To verify that the MCF7 and UACC903 cells expressed a functional PPARβ/δ, mRNAs encoding PPARβ/δ and the known PPARβ/δ target gene (Ge et al. 2005), ANGPTL4, were quantified. As compared to mouse primary keratinocytes, expression of mRNA encoding PPARβ/δ was significantly less, but detectable in both human cancer cell lines (Fig. 1A). In response to either PPARβ/δ ligand, expression of ANGPTL4 was increased in both cancer cell lines (Fig. 1B, C). These results demonstrate that MCF7 and UACC903 express a functional PPARβ/δ and respond to both GW0742 and GW501516.

2.2 GW501516 and GW0742 inhibit growth of human breast cancer (MCF7) and melanoma (UACC903) cell lines

To examine the effect of PPARβ/δ ligands on cell growth of human breast cancer and melanoma cell lines, cell proliferation was quantified in the presence of either GW0742 or GW501516, in the presence or absence of serum. Inhibition of cell growth was observed by both GW0742 and GW501516, and this effect was typically observed only in cells cultured in the presence of 1–10 μM ligand (Figs. 2, 3). No significant increase in cell proliferation was observed in

either of the human cancer cell lines with either potent PPARβ/δ ligand (Figs. 2, 3). In the presence of serum, cell growth was faster in both MCF7 and UACC903 cells as compared to that found in the absence of serum (Figs. 2, 3).

3. Discussion

Results from the present study demonstrate with the use of two highly specific PPARβ/δ ligands and the most reliable method for quantifying cell number, that ligand activation of PPARβ/δ modestly inhibits cell growth in either MCF7 or UACC903 cells. This work is consistent with past studies showing that ligand activation of PPARβ/δ inhibits cell growth in human liver and colon cancer cell lines (Hollingshead et al. 2007a). Because of different culture conditions used by various laboratories, it is very difficult to draw firm conclusions on the role of PPARβ/δ in cell growth. For example, in some cases, serum is withheld from cells to synchronize the cell cycle while in other cases the cells are cultured in the presence of serum. Additionally, quantification of cell numbers using assays linked to enzyme activity has been used, which is flawed since PPAR agonists are known to increase activity of these enzymes (Conway et al. 1989; Tanaka et al. 2003). For this reason, in the present study, the effect of the presence or absence of serum was evaluated using Coulter counting, which accurately determines the number of cells over time. Using this approach, it is important to note that the only differences observed between treatment was that cells cultured in the presence of serum proliferated faster, consistent with past findings, and that the PPARβ/δ ligands only inhibited, rather than increased, cell growth of both MCF7 and UACC903 cells. It is important to note that measures of apoptosis were not evaluated in these studies. However, it was recently shown that human liver and colon cancer lines do not exhibit changes in poly ADP ribose polymerase cleavage (Hollingshead et al. 2007a) using similar culture conditions used for the present studies (e.g. with and without serum ± GW0742 or GW501516). Coupled with the observed lack of increase in the actual number of cells over time, these observations suggest that apoptosis is likely unaffected by ligand activation of PPARβ/δ in these models, but should be examined further to confirm this idea.

To date, only a few papers have examined the association between PPARβ/δ and cell growth in human breast cancer cell lines. Previous work by others suggests that ligand activation of PPARβ/δ increases the growth of MCF7 cells (Stephen et al. 2004). This increase in cell growth occurred in cells that were cultured only at low confluency and the serum used in these studies was stripped with charcoal. Additionally, cell growth was only assessed after seven or twelve days of culture using one concentration of ligand, rather than monitoring over time with increasing does of ligand as performed for the present studies. It remains possible that the previously reported increase in cell growth was influenced in part by charcoal stripping the medium. Additionally, since no dose response was examined, a mitogenic effect was not conclusively demonstrated (Stephen et al. 2004). In contrast to these findings, others have demonstrated an association between PPARβ/δ expression and differentiation in MCF7 cells (Aung et al. 2006). These studies suggest that expression of PPARβ/δ is likely involved in the induction of differentiation, which is consistent with the present findings indicating that ligand activation of PPARβ/δ inhibits cell growth. Given the relatively small number of studies that have examined the effect of ligand activation of PPARβ/δ on breast cancer cell growth, further studies are necessary before firm conclusions can be drawn.

The effect of ligand activation of PPARβ/δ on melanoma cell growth has not been examined to date. Thus, this is the first report to demonstrate that ligand activation of PPARβ/δ inhibits the growth of UACC903 cells. Since the removal of serum did not further influence this interpretation, serum deprivation does not appear to modulate the effect of ligand activation of PPARβ/δ on cell growth of UACC903 cells. It will be of interest to determine the mechanisms underlying the observed inhibition of cell proliferation in UACC903 cells, and

could include the induction of terminal differentiation that may or may not be dependent on concomitant cell growth inhibition and/or anti-inflammatory activity of PPARβ/δ.

Collectively, results from the present study demonstrate that ligand activation of PPARβ/δ modestly inhibits cell growth of a human breast cancer cell line (MCF7) and a human melanoma cell line (UACC903). These observations are consistent with recent findings made in other human cancer cell lines (Hollingshead et al. 2007a), as well as numerous reports linking PPARβ/δ with inducing terminal differentiation and/or inhibiting cell growth (Ali et al. 2005; Aung et al. 2006; Burdick et al. 2007; Fukumoto et al. 2005; Hollingshead et al. 2007a; Kim et al. 2004; Kim et al. 2006; Kim et al. 2005; Man et al. 2007; Marin et al. 2006; Martinasso et al. 2006; Matthiessen et al. 2005; Michalik et al. 2001; Müller-Brüsselbach et al. 2007; Nadra et al. 2006; Ou et al. 2007; Peters et al. 2000; Planavila et al. 2005; Schmuth et al. 2004; Tan et al. 2001; Teunissen et al. 2007; Varnat et al. 2006; Westergaard et al. 2001). The relatively modest activation of PPARβ/δ in both UACC903 and MCF7 cells as shown by increased mRNA encoding ANGPTL4 shows that these cells are not highly responsive to PPARβ/δ ligands as compared to other cells such as keratinocytes. However, this could also be due to the fact that these studies were performed in cell culture, which are not influenced by variables resulting from other cell types not found in an in vitro model. While the concentration of the PPARβ/δ ligands used for these studies are within the range that will specifically activate PPARβ/δ, the findings were not evaluated using gene knockdown. Thus, it also remains possible that the inhibition of cell growth observed could be due to off target events. Further studies are required to examine these possibilities.

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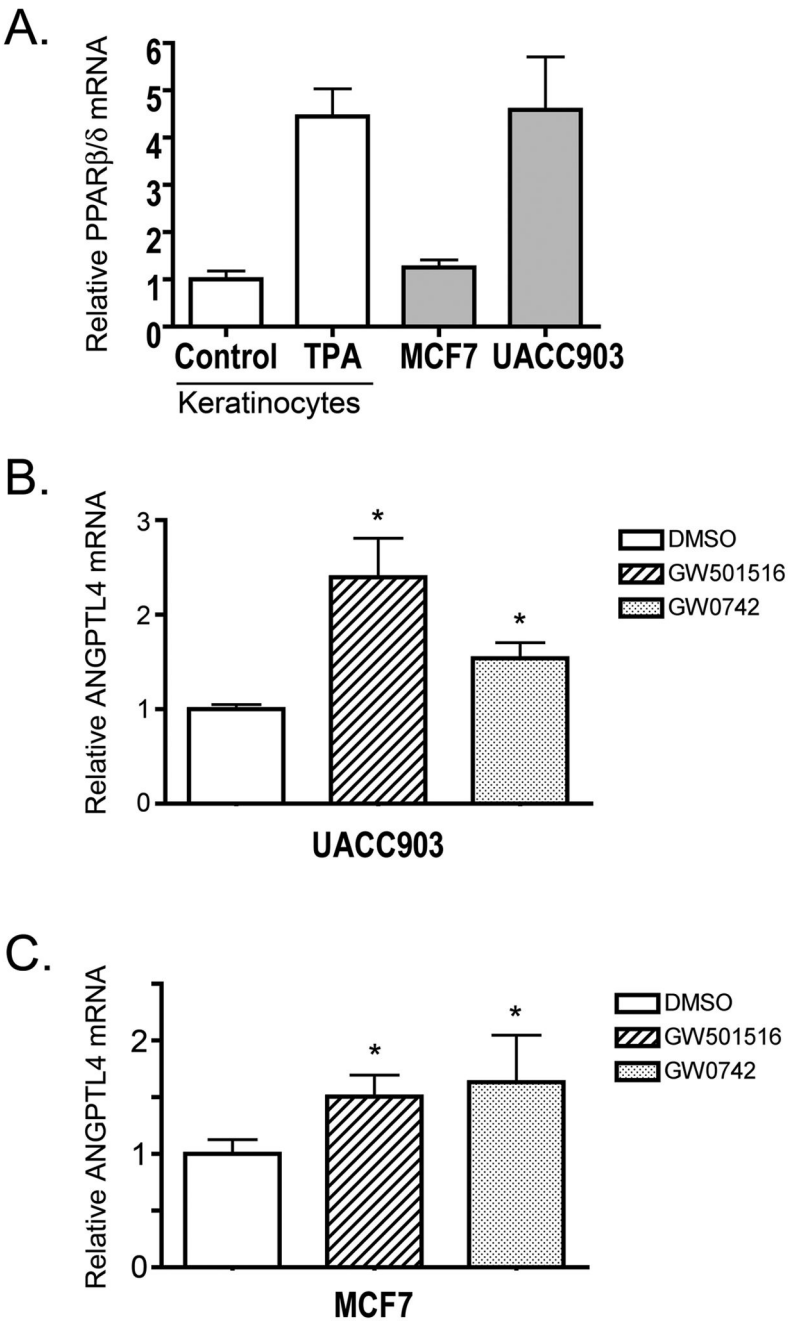

Fig. 1.

Expression of PPARβ/δ and ligand activation of target genes in MCF7 and UACC903 cells. A. Expression of mRNA encoding PPARβ/δ was quantified in MCF7 and UACC903 cells by quantitative realtime PCR. For comparison, expression of mRNA encoding PPARβ/δ was quantified in control and phorbol ester (TPA)-treated mouse primary keratinocytes. Expression of mRNA encoding the known PPARβ/δ target gene ANGPTL4 was quantified in (B) UACC903 and (C) MCF7 cells by quantitative realtime PCR. *Significantly different from control, $P \leq 0.05$.

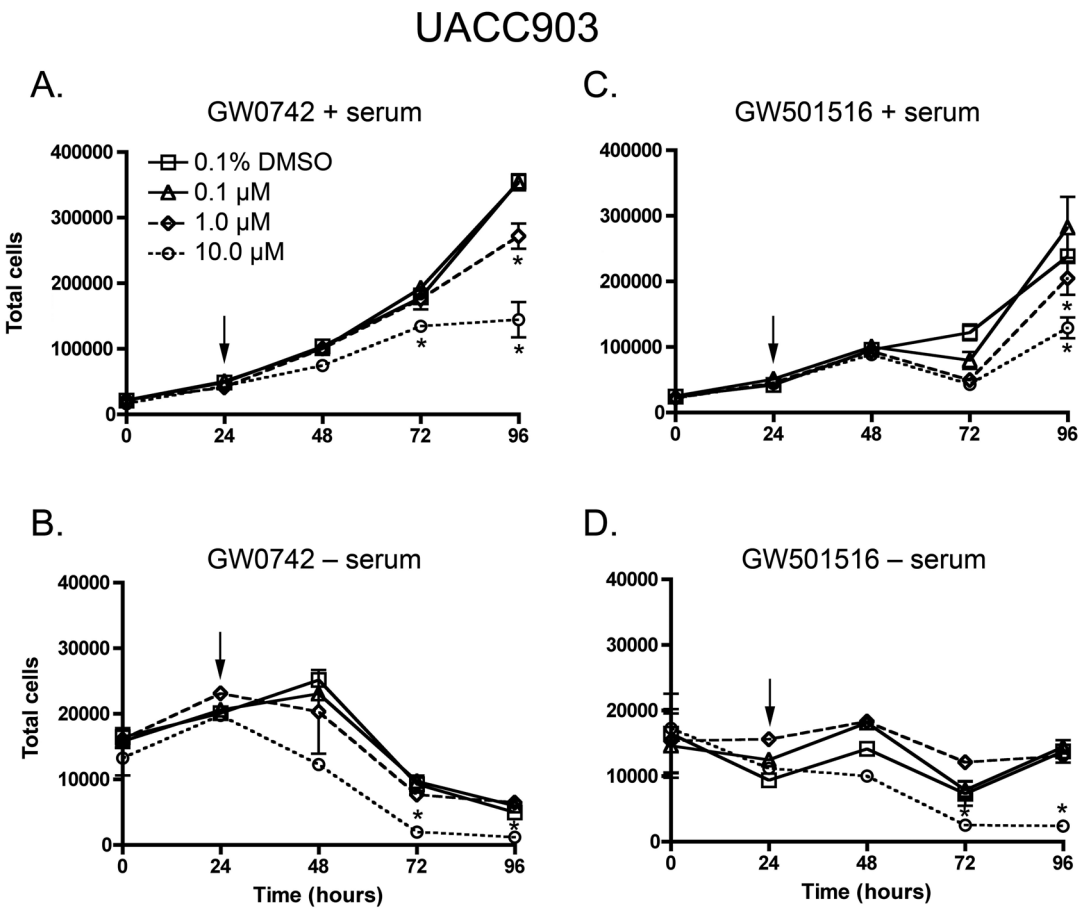

Fig. 2.

Effect of GW0742 and GW501516 on cell proliferation in the UACC903 melanoma cell line, in the presence (A, C) or absence (B, D) of culture medium serum. Cells were treated with the indicated concentration of ligand (arrow) and cell number quantified using Coulter counting as described in Materials and Methods. Values represent the mean ± S.E.M. *Significantly different than DMSO control, $P \leq 0.05$.

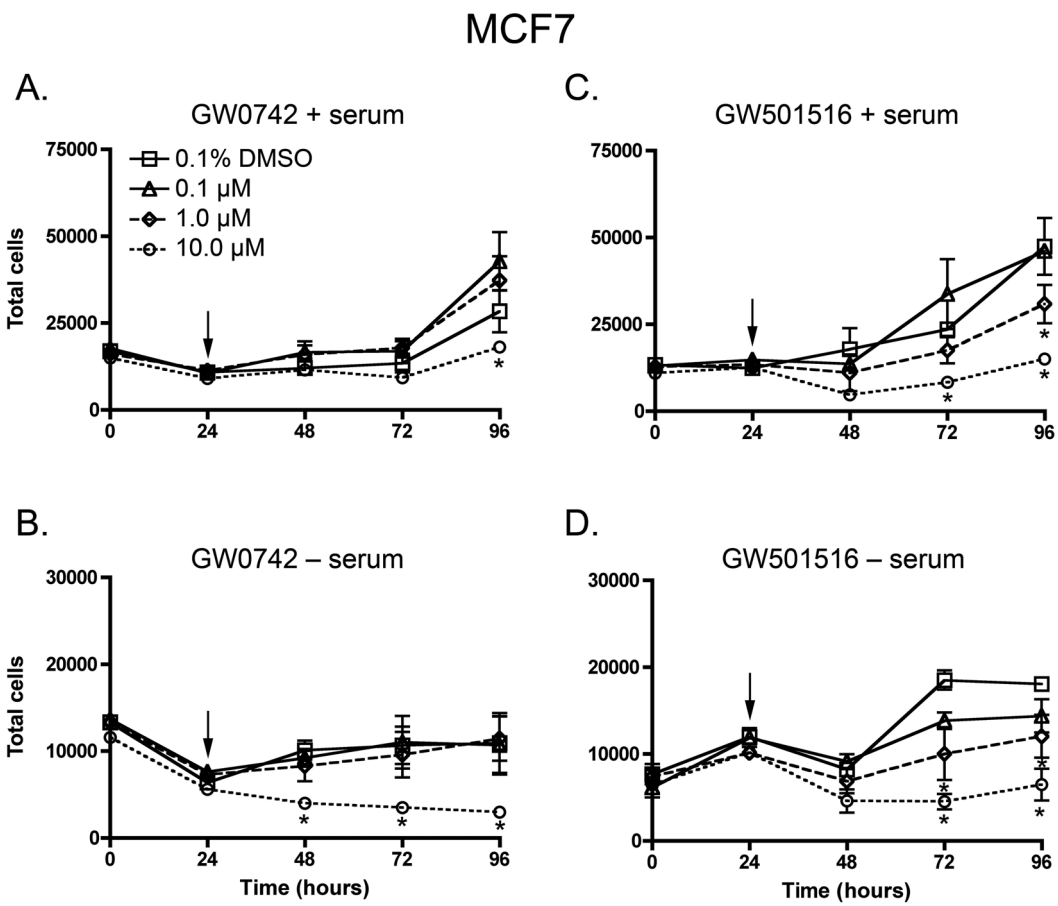

Fig. 3.

Effect of GW0742 and GW501516 on cell proliferation in the MCF7 breast cancer cell line, in the presence or absence of culture medium serum. Cells were treated with the indicated concentration of ligand (arrow) and cell number quantified using Coulter counting as described in Materials and Methods. Values represent the mean ± S.E.M. *Significantly different than DMSO control, $P \leq 0.05$.