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Research note

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Lipoprotein Lipase Hydrolysis Products Induce Pro-Inflammatory Cytokine Expression in Triple-Negative Breast Cancer Cells

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Abstract

Objectives: Lipoprotein lipase (LPL) is an extracellular lipase that hydrolyzes triacylglycerols and phospholipids from lipoproteins. LPL is highly expressed in adipose tissue and expressed in some breast cancer cell lines. Hydrolysis products generated by LPL can be used by cells as components of the cell membrane, as an energy supply, or as signaling molecules. Therefore, LPL on or around cancer cells may contribute to breast cancer growth and progression. We hypothesized that hydrolysis products generated by LPL from total lipoproteins can promote pro-inflammatory cytokine secretion from breast cancer cells and/or affect viability.

Results: Using cytokine arrays, we found that the secretion of seven cytokines was increased by MDA-MB-231 cells treated with lipoprotein hydrolysis products. An increased secretion of TNF-α and IL-6 was also seen by MDA-MB-468 cells, and an increase in IL-4 secretion was seen by MDA-MB-468 and SKBR3 cells. In contrast, MCF-7 cells showed a decreased secretion of only two cytokines. The changes to cytokine secretion profiles by the breast cancer cell types, including by non-cancerous MCF-10a breast cells, were independent of increased cell metabolic activity. Overall, these results provide information on how lipoprotein hydrolysis products within the tumor microenvironment might affect breast cancer cell viability and tumor progression.

Keywords: lipoprotein lipase, lipoproteins, breast cancer, cytokines, metabolic activity, antibody arrays

Introduction

Lipoprotein lipase (LPL) is an extracellular cell surface-associated *sn*-1 lipase that hydrolyzes ester bonds within triacylglycerols and phospholipids that are carried by lipoproteins, to yield free fatty acids (FFA), mono- and diacylglycerols from triacylglycerols, and lysophospholipids from phospholipids [1]. In addition, LPL has a non-catalytic function that captures lipoproteins to bridge them to cell surface receptors, thus promoting lipid uptake by cells [2].

LPL is highly expressed in the adipose tissue, skeletal muscle, and cardiac muscle, but is also present in other tissues, including mammary tissue [3]. Of interest, *LPL* gene expression has been detected in select human breast cancer cell lines, and LPL protein and activity were identified in the Du4475 cell line and primary breast tumor tissues [4]. The presence of LPL in the breast cancer microenvironment can result in increased delivery of FFA to tumor cells for energy production via β-oxidation [5]. However, metabolites generated by LPL could also carry out other functions. Of note, LPL hydrolysis products generated from very low-density lipoproteins were shown to increase tumor necrosis factor (TNF)-α secretion from endothelial cells [6], and to increase intercellular adhesion molecule-1 (ICAM-1) expression within endothelial cells [6]. In breast cancer, ICAM-1 is upregulated by TNF-α and it is thought that ICAM-1 is involved in tumor cell invasion and metastasis by promoting intravasation [7].

While LPL hydrolysis products can support the energy requirements of the highly proliferative tumor cells, the effects of hydrolysis products on signaling pathways, gene expression profiles, and cytokine secretion on breast cancer cells remain to be thoroughly investigated. For the current study, we hypothesize that hydrolysis products generated by LPL

from total lipoproteins will lead to increased pro-inflammatory cytokine secretion and cell metabolic activity in breast cancer cells. We examined our hypothesis by exposing breast cancer cells with differing receptor status to total lipoprotein hydrolysis products, generated by LPL. Cytokine expression profiles and metabolic activity were then determined.

Main text

Methodology

Lipoprotein hydrolysis products were generated by incubating recombinant LPL with total lipoproteins from normolipidemic human plasma; medium containing no LPL (mock) incubated with total lipoproteins was used as a negative control. Hydrolysis products generated by LPL were diluted to 0.68 mM in conditioned media and incubated for 24 h with human breast cancer cells (MDA-MB-231, MDA-MB-468, SKBR3, MCF-7, and T47D) or MCF-10a non-tumorigenic human breast cells. Media from cells were examined for cytokines using cytokine arrays and/or ELISAs. Cell metabolic activity was examined using an MTT assay. See also **Additional file 1: Detailed methodology**.

Results

Antibody array analyses of cytokines from MDA-MB-231 and MCF-7 cell lines in response to LPL hydrolysis products

We hypothesized that cytokine secretion from breast cancer cells would be affected by total lipoprotein hydrolysis products generated by LPL. To test this, we examined the presence and relative secretion levels of 36 different cytokines using antibody arrays (**Additional file 2: Table S1**) in the supernatants of triple-negative breast cancer (TNBC) MDA-MB-231 cells, and estrogen receptor (ER)+/progesterone receptor (PR)+/human epidermal growth factor 2 (HER2)-MCF-7 cells, treated for 24 h with either total lipoprotein hydrolysis products generated by LPL, or mock control media (without LPL). We observed differing cytokine secretion profiles between the two cell types. Across three independent experiments, seven cytokines were significantly increased in media from hydrolysis product-treated MDA-MB-231 cells; these include CXC motif chemokine ligand (CXCL) 1, CXCL11, ICAM-1, interleukin (IL)-4, IL-6, IL-8, and TNF-α (**Fig. 1A**). No detection or no changes were observed with other cytokines. On the other hand, these cytokines did not change in media from hydrolysis product-treated MCF-7 cells, and only two cytokines exhibited a significant change: decreased IL-1α and decreased IL-27 (**Fig. 1B**).

ELISA analyses of cytokines from various breast cancer cell lines in response to LPL hydrolysis products

Our array data suggested that breast cancer cell receptor status will result in differing cytokine secretion profiles in response to lipoprotein hydrolysis products. Thus, we examined conditioned media from MCF-7, T47D (ER+/PR+/HER2-), SKBR3 (ER-/PR-/HER2+), MDA-MB-231, MDA-MB-468 (TNBC), or MCF-10a (non-tumorigenic mammary epithelial – included as a

control) cells treated for 24 h with lipoprotein hydrolysis products (or mock control) for the presence of TNF-α, IL-4, and IL-6 by ELISA. Across all cell lines, TNF-α was only detected in conditioned media from MDA-MB-231 and MDA-MB-468 cells treated with hydrolysis products (**Fig. 2A**). These data are generally consistent with the expression pattern of the TNF-α data obtained for MDA-MB-231 and MCF-7 cells by the cytokine arrays. Compared to control, IL-4 was significantly increased in conditioned media from MDA-MB-231 cells treated with hydrolysis products (**Fig. 2B**). IL-4 was not detected in conditioned media from control-treated MDA-MB-468 cells, but it was present in the media of MDA-MB-468 cells treated with hydrolysis products (**Fig. 2B**). No other cell lines tested had detectable levels of IL-4 in their media, regardless of treatment (**Fig. 2B**). For both the MDA-MB-231 and MDA-MB-468 cells, IL-6 was significantly increased in conditioned media of cells treated with hydrolysis products compared to control (**Fig. 2C**). The levels of IL-6 were also significantly increased in conditioned media of SKBR3 cells treated with hydrolysis products compared to control (**Fig. 2C**). However, media from MCF-7, T47D, and MCF-10a cell lines did not have any detectable IL-6, regardless of treatment (**Fig. 2C**).

Metabolic activities of various breast cancer cell lines in response to LPL hydrolysis products

To determine the effects of total lipoprotein lipid hydrolysis products generated by LPL on metabolic activities of breast cancer cells of different subtypes, MCF-7, T47D, SKBR3, MDA-MB-231, MDA-MB-468 and MCF-10a cells were cultured and treated for 24 h with hydrolysis products, or with mock control media. The metabolic activity of all cell lines treated with LPL-generated hydrolysis products was significantly increased by approximately 20% compared to mock control (**Fig. 3**). Overall, the data suggest that cytokine expression profiles in response to LPL hydrolysis products from total lipoproteins are independent of alterations to metabolic activity.

Discussion

Breast cancer cells can secrete cytokines to induce changes in the surrounding cells, and similarly, cells of the microenvironment can secrete cytokines to support or reduce cancer survival [8]. Surprisingly, the cytokine profiles of conditioned media from MDA-MB-231 and MCF-7 cells in the presence of lipoprotein hydrolysis products were quite different. These cell lines were chosen as they represent the most and least aggressive breast cancer subtypes, respectively. This observation, combined with the observation that media from the MDA-MB-231 cells had increased levels of several pro-tumorigenic cytokines in the presence of lipoprotein hydrolysis products, led us to examine additional cell lines for levels of secreted TNF-α, IL-4, and IL-6.

The secretion profile of the TNBC cell lines we examined in the presence of lipoprotein hydrolysis products generated by LPL is pro-tumorigenic, with significant levels of secreted TNF-α, IL-4, and IL-6 compared to controls. Breast cancer cells can produce and utilize TNF-α to activate nuclear factor (NF)-κB signaling. NF-κB activation can induce tumor cell

proliferation, angiogenesis, immune evasion, and metastasis [9,10]. One result of NF-κB activation is increased expression of IL-8, which was also found to be significantly increased using the cytokine array. IL-8 promotes angiogenesis, tumor cell migration, and immune cell infiltration in breast cancer [11]. Similarly, NF-κB activation by TNF-α increases ICAM-1 expression in breast cancer, which is upregulated to promote metastasis and is associated with more aggressive subtypes [7]. Unsurprisingly, the cytokine array data revealed a significant increase in secreted ICAM-1 for the MDA-MB-231 cells, which suggests that lipoprotein hydrolysis products may promote development of a more aggressive tumor cell phenotype. CXCL1, which promotes immune cell invasion and angiogenesis, was also found to be upregulated. Interestingly, CXCL1 is also induced by TNF-α. It is thus possible that in our system, TNF-α induces the secretion of more pro-inflammatory cytokines, though this remains to be determined. On the other hand, IL-4 has been described as an anti-inflammatory cytokine that induces apoptosis in breast cancer cells [12]. However, IL-4 secretion activates M2-like tumor-associated macrophages, which are strongly pro-tumorigenic [13]. The pleiotropic cytokine IL-6 was also increased in response to lipoprotein hydrolysis products. IL-6 is involved in multiple aspects of tumor progression, including invasion, metastasis, angiogenesis, and resistance to therapy. The broad impact of IL-6 is due to its ability to activate many different signaling pathways that have pro-tumorigenic functions, such as the NF-κB [6] and JAK/STAT3 [14] pathways. Finally, increased secretion of CXCL11 in MDA-MB-231 cells was slightly unexpected. CXCL11 attracts mononuclear immune cells to the tumor microenvironment. Once in the tumor microenvironment, the immune cells may have a pro- or anti-tumorigenic effect depending on context. CXCL11 is primarily induced by interferon (IFN)-γ [15], yet IFN-γ secretion was not detected in this study. An explanation for this could be that the IFN-γ secretion was below detectable levels for the cytokine array. Taken together, these data suggest that hydrolysis products liberated by LPL from total lipoproteins promote tumorigenesis by upregulating pro-tumorigenic cytokine secretion in TNBC cells.

The cytokine array data for conditioned media from MCF-7 cells are more difficult to interpret. MCF-7 cells only showed a significant decrease in IL-1α and IL-27. IL-1α is a pro-inflammatory cytokine that can promote cancer progression by activating NF-κB and JAK/STAT3 signaling. Because of the pro-tumorigenic effects of IL-1α [16], it would be expected that IL-1α would be upregulated in breast cancer cells following lipoprotein hydrolysis products treatment. However, IL-1α has been shown to inhibit MCF-7 cell proliferation by causing cell cycle arrest [17]. This is an interesting and counterintuitive observation because it indicates that IL-1α downregulation by lipoprotein hydrolysis products may function to sustain tumorigenesis in MCF-7 cells. Similarly, IL-27 is known to have both pro- and anti-tumorigenic effects. IL-27 can induce T cell activation in the tumor microenvironment, resulting in different effects depending on the degree of progression. IL-27 can also activate STAT1, which has potent anti-proliferative effects, induces apoptosis, and enhances immune cell elimination of cancer cells [18]. Like IL-1α, IL-27 downregulation by lipoprotein hydrolysis products may be pro-tumorigenic in our model. Because of their multifunctional roles, IL-1α and IL-27 likely have

both pro- and anti-inflammatory effects *in vivo*. However, these data suggest that LPL hydrolysis products may also promote tumorigenesis in luminal A breast cancer.

The TNBC cell lines exhibited a clear pro-tumorigenic phenotype in response to hydrolysis products from total lipoproteins generated by LPL, and the changes to IL-1α and IL-27 in MCF-7 cells may lean toward a pro-tumorigenic phenotype. T47D cells, like MCF-7 cells, are ER+/PR+/HER2-. While we did not examine the T47D cell line in the cytokine array, like the MCF-7 cells, we did not detect TNF-α, IL-4, and IL-6 in the media using ELISA, regardless of treatment; however, while future work, we anticipate it may also show changes to changes to IL-1α and IL-27 expression. The ER-/PR-/HER2+ SKBR3 cell line did release excess IL-6 in the presence of lipoprotein hydrolysis products, but not TNF-α or IL-4.

Collectively, our data suggest that components within total lipoprotein lipid hydrolysis products generated by LPL change the cytokine secretion profile of breast cancer cells in a subtype-specific manner through one or more mechanisms. At least for the MDA-MB-468 cell line, it appears that one or more components within the FFA component may be responsible for affecting TNF-α expression, as our data show a 12.4-fold increase in *TNFA* mRNA expression compared to control treatments (**Additional file 3: Fig. S1**). This observation is similar to that observed within THP-1 macrophages [19].

Conclusions

Our study has shown that products of lipid hydrolysis from total lipoproteins by LPL may directly promote breast cancer growth and progression by inducing a pro-tumorigenic cytokine secretion profile from tumor cells, independent of cell metabolic activity. Of interest, the lipoprotein hydrolysis products induce different pro-tumorigenic cytokine expression profiles in different breast cancer subtypes. This suggests that lipoprotein hydrolysis products have distinct effects between breast cancer cell subtypes, that could result in promoting breast cancer progression.

Limitations

Our data reflect changes to cytokine expression using hydrolysis products from lipoproteins obtained from normolipidemic subjects. It is possible that lipoproteins from subjects who are obese, hyperlipidemic, or on long-term dietary interventions may yield differing outcomes. This remains to be examined.

Abbreviations

CXCL: CXC motif chemokine ligand; ER: estrogen receptor; FFA: free fatty acid; HER2: human epidermal growth factor 2; ICAM-1: intercellular adhesion molecule-1; IFN: interferon; IL: interleukin; LPL: lipoprotein lipase; NF: nuclear factor; PR: progesterone receptor; TBNC: triple-negative breast cancer; TNF: tumor necrosis factor.

Supplementary information

The online version contains supplementary material available at https://doi.org/10.1186/xxxxxx.

Additional file 1: Detailed methodology.

Additional file 2: Table S1. Cytokines examined using the Proteome Profiler™ Human Cytokine Array.

Additional file 3: Fig. S1. Expression of *TNFA* in MD-MBA-468 cells in response to the FFA component of total lipoprotein hydrolysis products generated by LPL. The FFA component that is generated from the hydrolysis of LPL was reconstituted as previously described – see Additional file 1: Detailed methodology. MDA-MB-468 cells were treated with either the FFA component or vehicle control for 18 h, as previously described – see Additional file 1: Detailed methodology. Following treatment, RNA was extracted from cells and examined for the expression of *TNFA* and normalized against the expression data for *ACTB*. Primer information and qPCR conditions were previously reported – see Additional file 1: Detailed methodology. Data are the average ± SD of two biological experiments.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated and analysed during this study are included in this article and its supplementary information files. All data may be requested from the corresponding authors upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

AJT, SLC, and RJB conceived the study. AJT and NPN acquired the data. All authors analyzed and interpreted the data. AJT, SLC, and RJB drafted the manuscript. All authors read and approved the final manuscript.

Acknowledgments

Not applicable.

Authors' information

AJT was a M.Sc. (Biochem.) student. NPN was a B.Sc. (Hons. Biochem.) student. Both SLC and RJB hold a Ph.D. and are Associate Professors.

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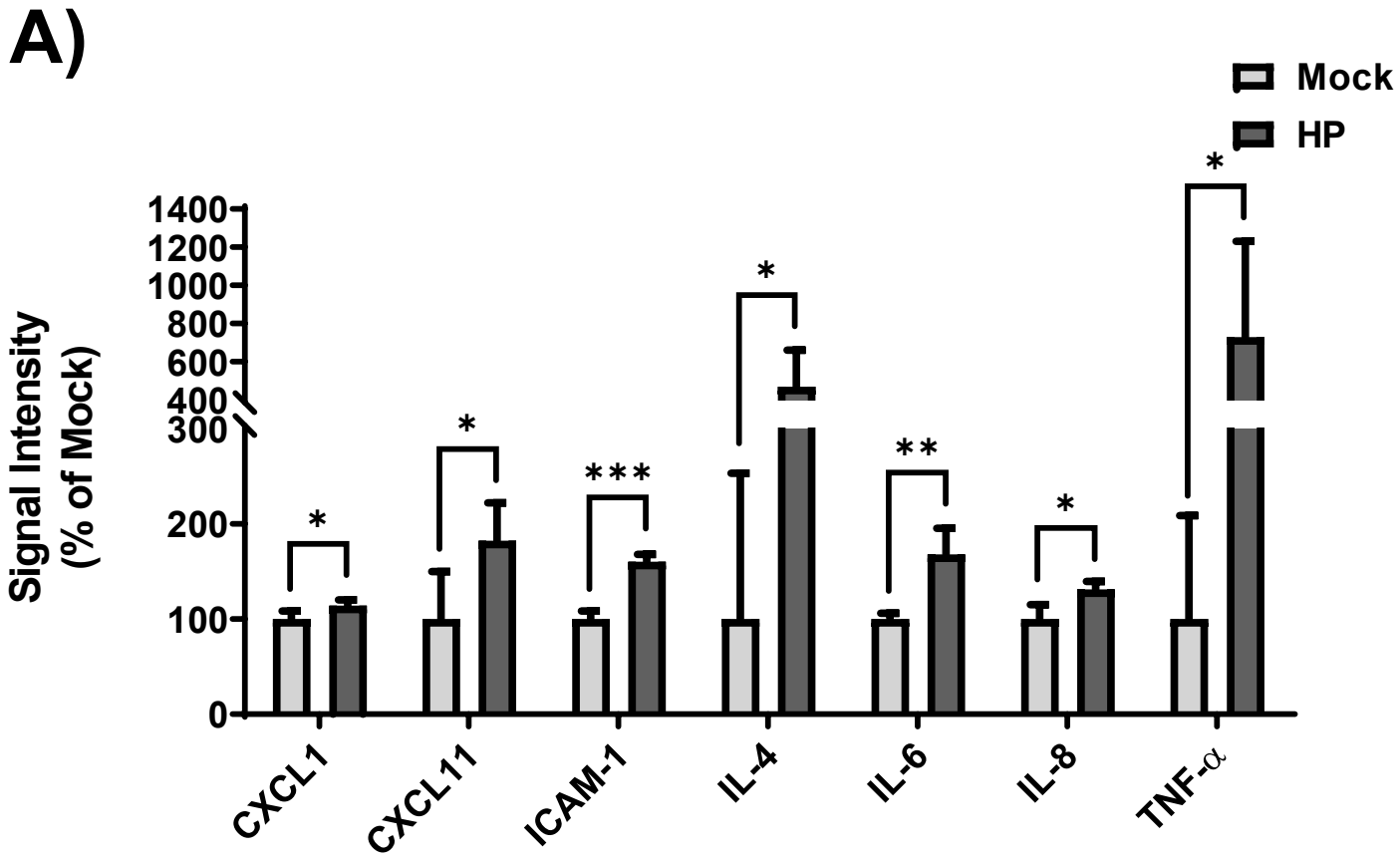
Figure legends

Fig. 1. Cytokine array analysis of MDA-MB-231 and MCF-7 cell supernatants following treatment of cells with total lipoprotein lipid hydrolysis products generated by LPL. Cells were treated with either total lipoprotein lipid hydrolysis products generated by LPL (HP) or mock heparinized media (Mock) for 24 h. Conditioned media from MDA-MB-231 cells (A) and MCF-7 cells (B) were examined for cytokines using an antibody array. Signal intensities were obtained by scanning densitometry, normalized to an internal control within the array, and presented as a percent of Mock. Data are presented as the mean of triplicate biological experiments, ± SD. *, $p<0.05$; **, $p<0.01$; *** $p<0.001$.

Fig. 2. TNF-α, IL-4, and IL-6 expression by ELISA of breast cancer and MCF-10a cell supernatants following treatment with total lipoprotein lipid hydrolysis products generated by LPL. Breast cancer cells and MCF-10a cells were treated with either total lipoprotein lipid hydrolysis products generated by LPL (HP) or mock heparinized media (Mock) for 24 h. Conditioned media were examined for (A) TNF-α, (B) IL-4, and (C) IL-6 by ELISA. Data are presented as the mean of triplicate biological experiments, ± SD. ND, not detected; *, $p<0.05$; **, $p<0.01$; *** $p<0.001$.

Fig. 3. MTT assay of breast cancer and MCF-10a cells following treatment with total lipoprotein lipid hydrolysis products generated by LPL. MCF-7, T47D, SKBR3, MDA-MB-231, MDA-MB-

468 breast cancer cells and MCF-10a cells were treated with either total lipoprotein lipid hydrolysis products generated by LPL (HP) or mock heparinized media (Mock) for 24 h. At 4 h after the addition of MTT, the absorbance of the samples was read at 570 nm and 630 nm. Cellular metabolic activity was calculated by subtracting the 630 nm data from the 570 nm data. Data are presented as the mean of triplicate biological experiments with triplicate wells assessed per experiment, ± SD. *, $p<0.05$; **, $p<0.01$.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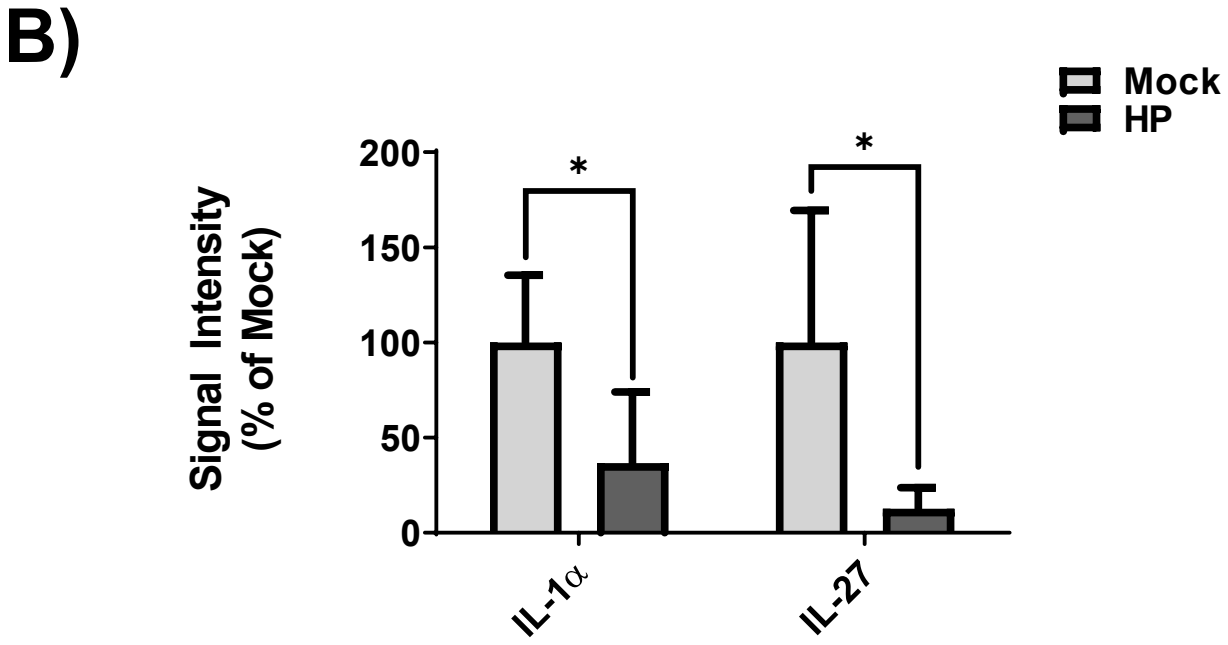
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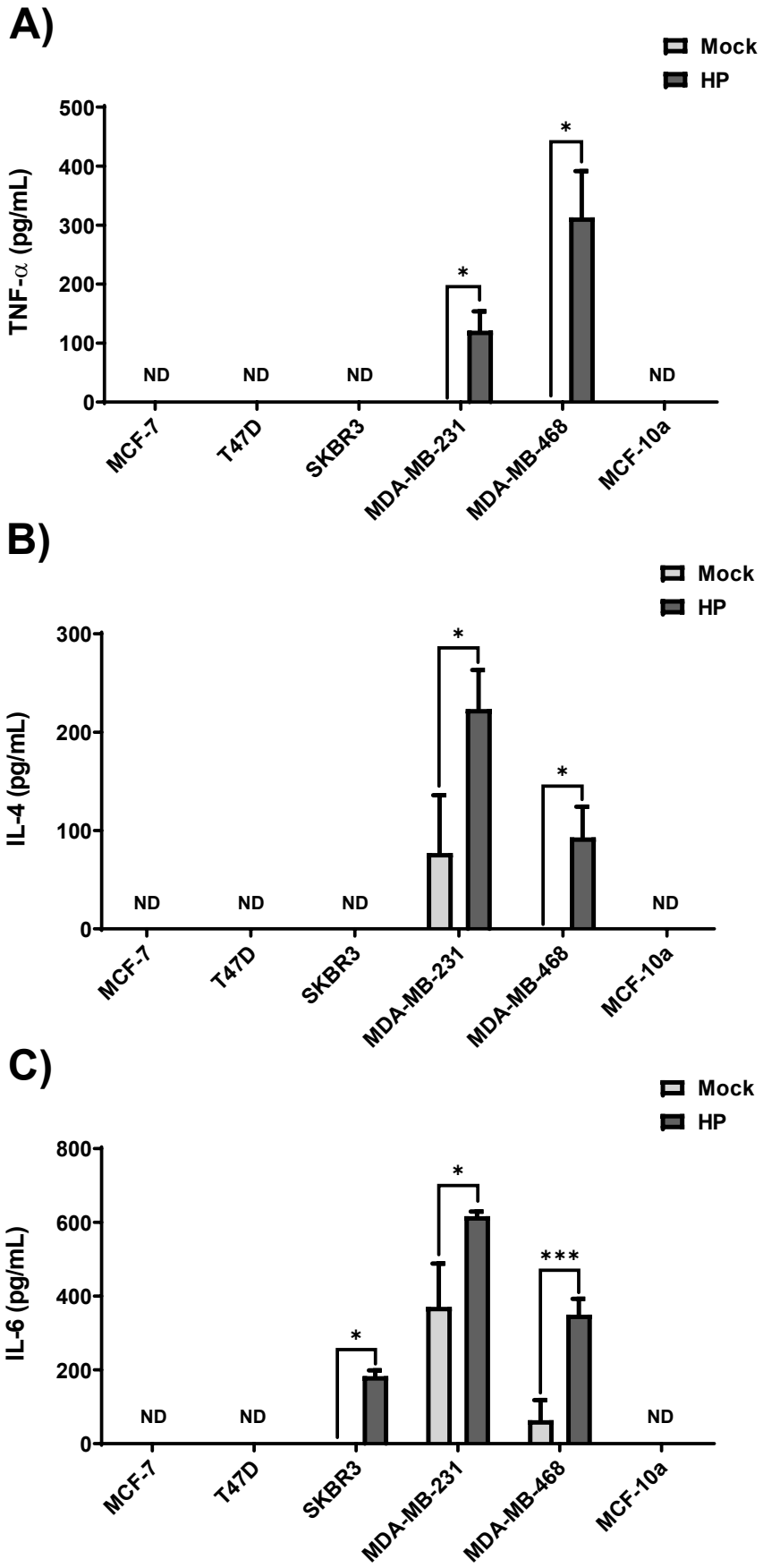
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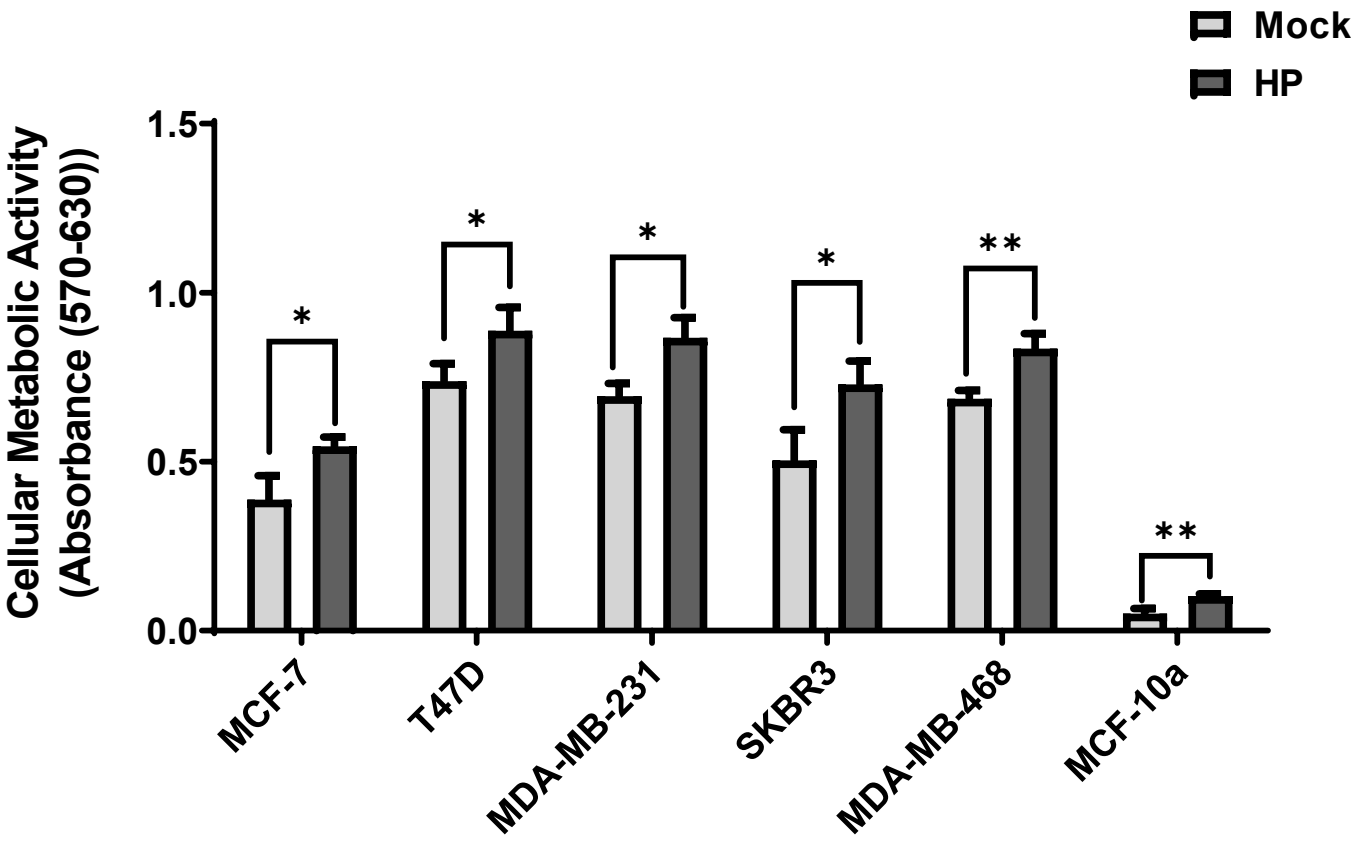
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MCF-10a

Figures

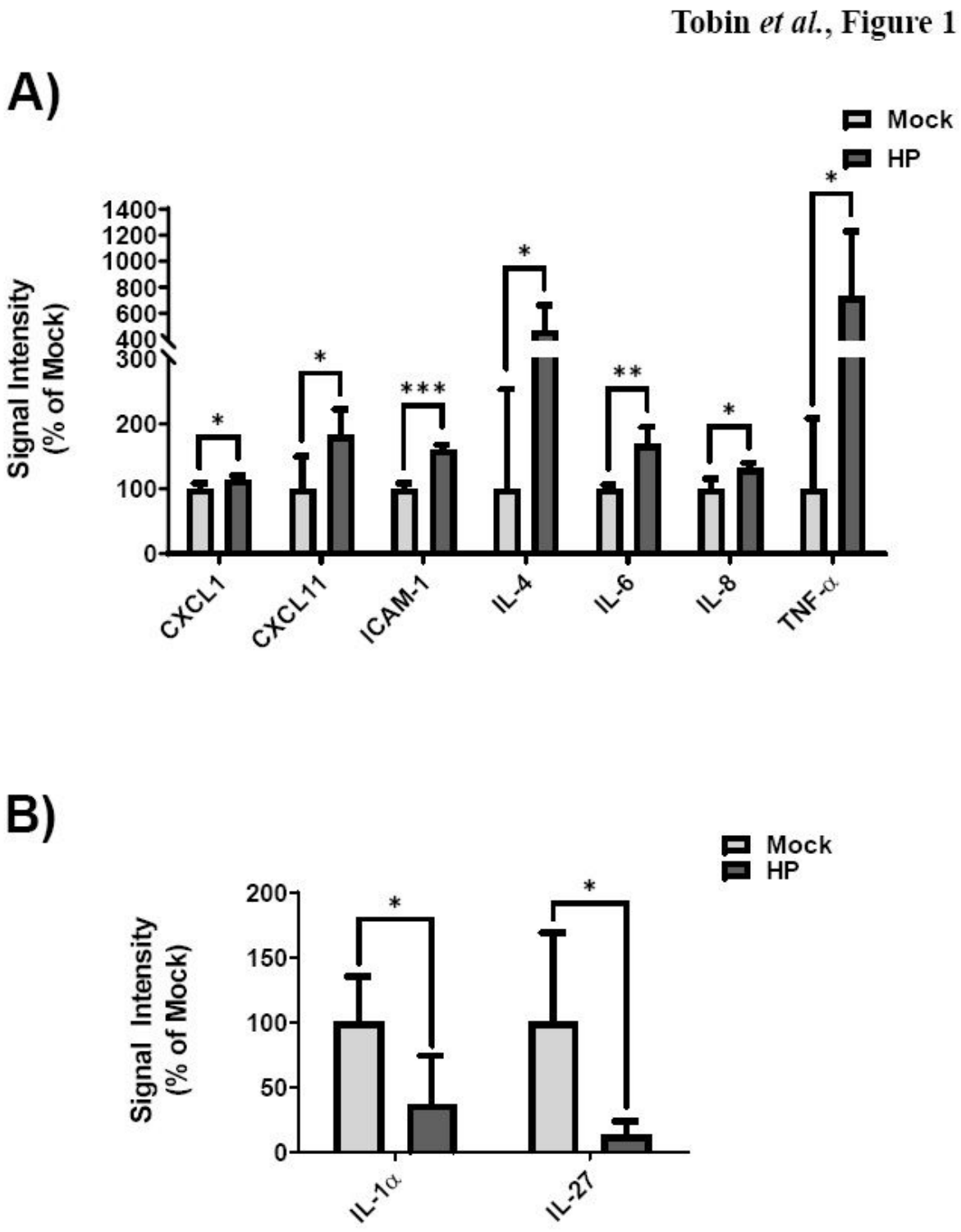

Figure 1

Cytokine array analysis of MDA-MB-231 and MCF-7 cell supernatants following treatment of cells with total lipoprotein lipid hydrolysis products generated by LPL. Cells were treated with either total lipoprotein lipid hydrolysis products generated by LPL (HP) or mock heparinized media (Mock) for 24 h. Conditioned

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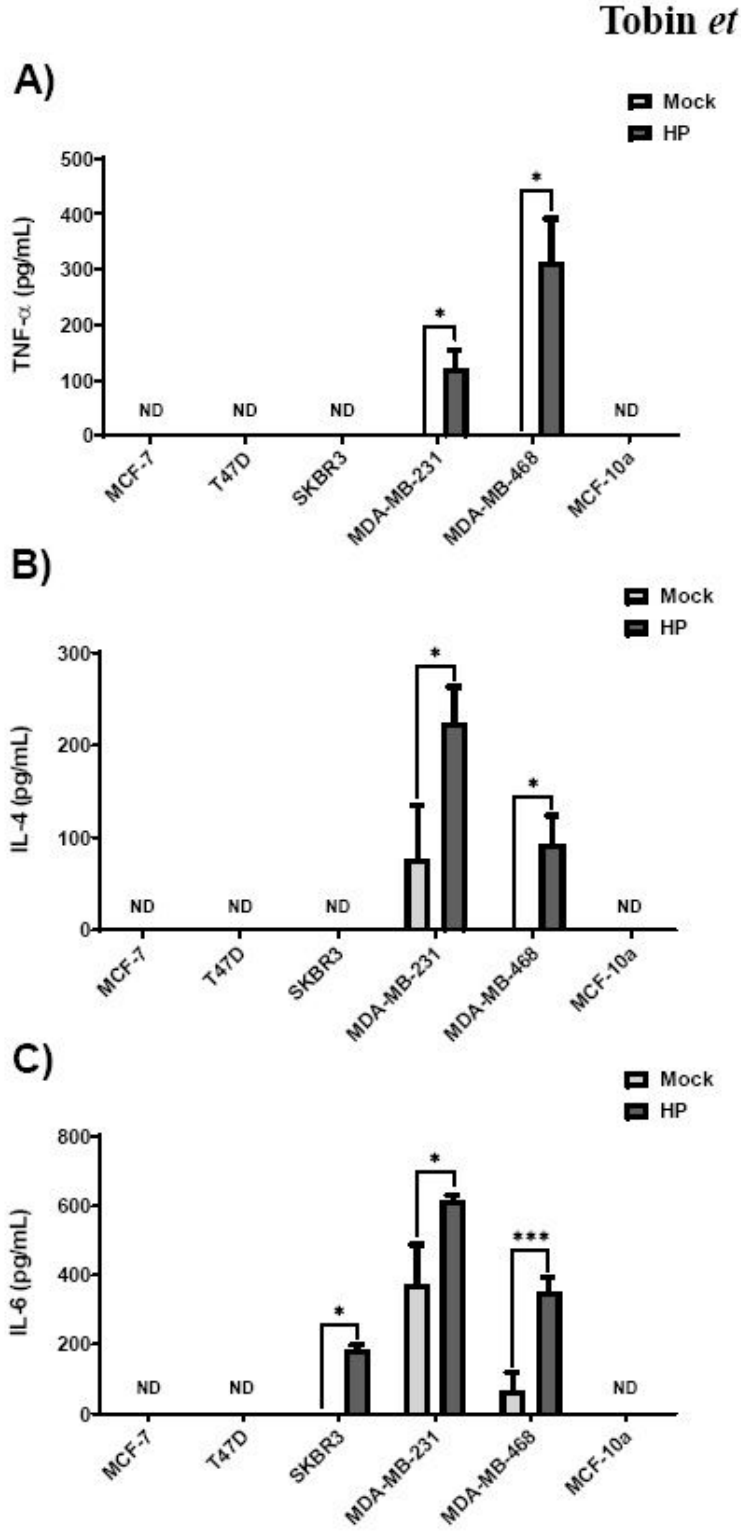

Figure 2

TNF-α, IL-4, and IL-6 expression by ELISA of breast cancer and MCF-10a cell supernatants following treatment with total lipoprotein lipid hydrolysis products generated by LPL. Breast cancer cells and MCF-10a cells were treated with either total lipoprotein lipid hydrolysis products generated by LPL (HP) or mock heparinized media (Mock) for 24 h. Conditioned media were examined for (A) TNF-α, (B) IL-4, and (C) IL-6 by ELISA. Data are presented as the mean of triplicate biological experiments, ± SD. ND, not detected; *, p<0.05; **, p<0.01; *** p<0.001.

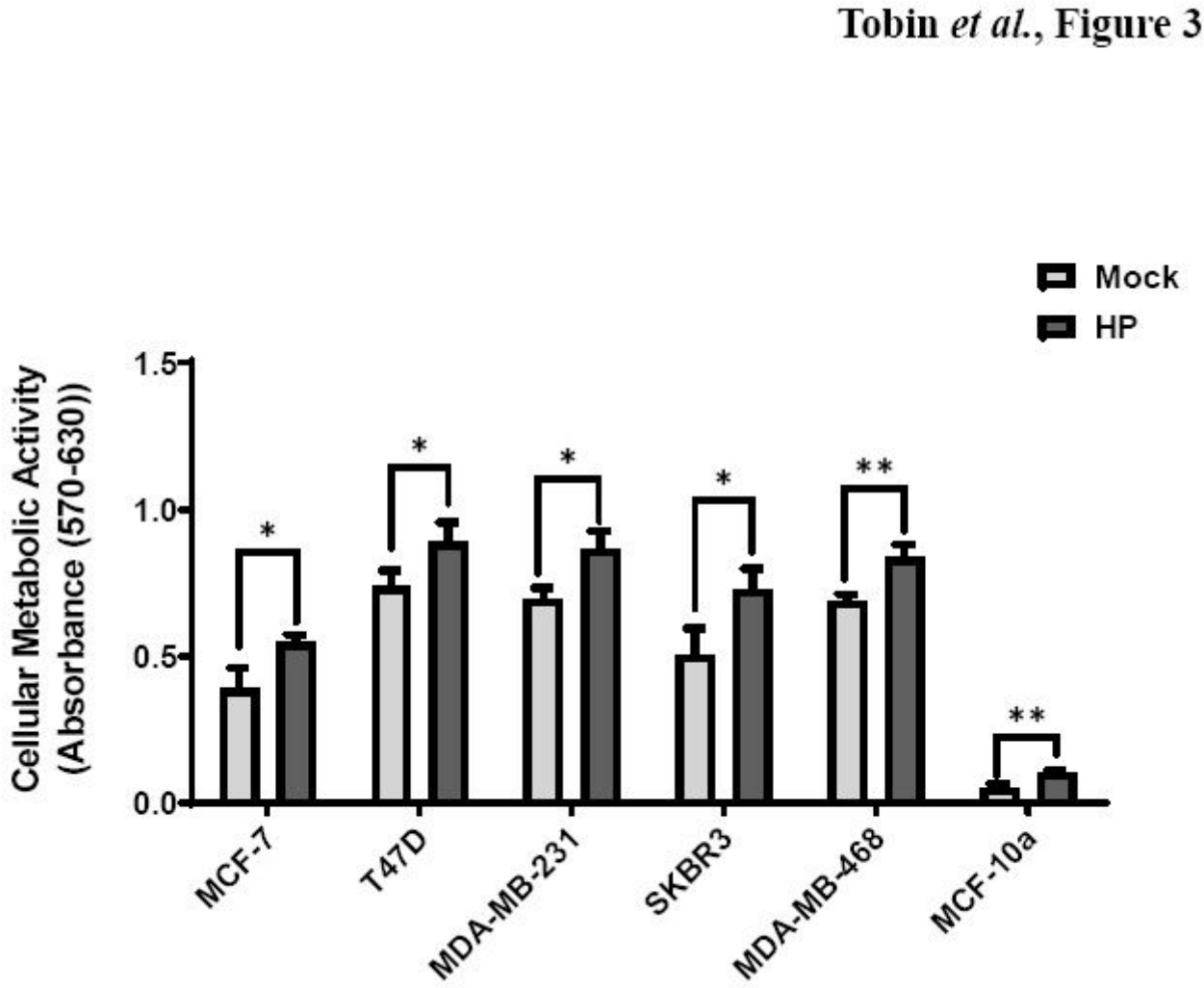

Figure 3

MTT assay of breast cancer and MCF-10a cells following treatment with total lipoprotein lipid hydrolysis products generated by LPL. MCF-7, T47D, SKBR3, MDA-MB-231, MDA-MB-468 breast cancer cells and MCF-10a cells were treated with either total lipoprotein lipid hydrolysis products generated by LPL (HP) or mock heparinized media (Mock) for 24 h. At 4 h after the addition of MTT, the absorbance of the samples was read at 570 nm and 630 nm. Cellular metabolic activity was calculated by subtracting the 630 nm data from the 570 nm data. Data are presented as the mean of triplicate biological experiments with triplicate wells assessed per experiment, ± SD. *, p<0.05; **, p<0.01.

Supplementary Files

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