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Research Article

Per- and Polyfluoroalkyl Substances Differentially Inhibit Placental Trophoblast Migration and Invasion *In Vitro*

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ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) are used as industrial surfactants and chemical coatings for household goods such as Teflon. Despite regulatory efforts to phase out legacy PFAS, they remain detectable in drinking water throughout the United States. This is due to the stability of legacy PFAS and the continued use of replacement compounds. In humans, PFAS have been detected in placenta and cord blood and are associated with low birth weight and preeclampsia risk. Preeclampsia is a leading cause of maternal mortality and is driven by insufficient endometrial trophoblast invasion, resulting in poor placental blood flow. PFAS alter invasion of other cell types, but their impact on trophoblasts is not understood. We therefore assessed the effects of PFAS on trophoblast migration, invasion, and gene expression *in vitro*. Trophoblast migration and invasion were assessed using a modified scratch assay in the absence or presence of Matrigel, respectively. Treatment with perfluorooctanoic sulfate (PFOS), perfluorooctanoic acid (PFOA), and GenX (1000 ng/ml) each decreased trophoblast migration over 24 h. However, only GenX (1000 ng/ml) significantly inhibited trophoblast invasion. Treatment with PFOS, PFOA, and GenX also decreased trophoblast expression of chemokines (eg, *CCL2*), chemokine receptors (eg, *CCR4*), and inflammatory enzymes (eg, *ALOX15*) involved in migration. Inhibition of chemokine receptors with pertussis toxin (10 ng/ml), a G-protein inhibitor, inhibited trophoblast migration similar to the PFAS. Taken together, PFAS decrease trophoblast migration, invasion, and inflammatory signaling. By understanding the mechanisms involved, it may be possible to identify the biological and exposure factors that contribute to preeclampsia.

Key words: PFAS; placenta; trophoblast; preeclampsia; inflammation.

Per- and polyfluoroalkyl substances (PFAS) are used as industrial surfactants and chemical coatings for common household goods such as Teflon and scotch guard (Renner, 2006; Sajid and Ilyas, 2017). In addition, PFAS are frequently employed in industrial manufacturing processes due to their hydrophobicity and stability, 2 key factors that contribute to their persistence in the environment (Kovarova and Svobodova, 2008). Despite regulatory efforts in the United States to phase out production of legacy PFAS perfluorooctanoic acid (PFOA) and perfluorooctanoic sulfate (PFOS), their stability and the introduction of replacement compounds, such as hexafluoropropylene oxide dimer acid (ammonium salt with trade name: GenX), result in detectable levels of PFAS throughout drinking water in the United States (Rayne and Forest, 2009). According to the National Health and Nutrition Examination Survey, most people in the United States and other industrialized countries have detectable levels (median 10 ng/ml total) of PFAS in their blood (NHANES, 2014). PFAS are detectable even after steady low-level

exposure because of their long biological half-life. PFAS have also been detected in both placenta (approximately 0.1–10 ng/g wet weight) and cord blood in a number of studies worldwide indicating that PFAS are prevalent, being retained in maternal serum, and crossing the placenta into the fetal circulation (Fisher *et al.*, 2016; Lee *et al.*, 2016; Mamsen *et al.*, 2017; Spratlen *et al.*, 2019; Wang *et al.*, 2016).

Legacy PFAS are well documented for their role as developmental and metabolic toxicants with impact on later life children's health (Braun, 2017). There is also evidence of concern to pregnant women, that PFAS are associated with detriments to the health of the pregnancy and to the developing fetus (Bach *et al.*, 2015; Darrow *et al.*, 2013; Huang *et al.*, 2019; Stein *et al.*, 2009; Wikström *et al.*, 2019). Specifically, placental PFOS content has been associated with the incidence of preeclampsia across multiple studies (Darrow *et al.*, 2013; Huang *et al.*, 2019; Stein *et al.*, 2009; Wikström *et al.*, 2019). Placental, maternal serum, and cord serum PFAS concentrations during pregnancy have also been correlated to low birth weight, which is often a consequence of placental insufficiency, in multiple studies (Ashley-Martin *et al.*, 2017; Rokoff *et al.*, 2018; Sagiv *et al.*, 2018; Valvi *et al.*, 2017; Verner *et al.*, 2015; Washino *et al.*, 2009; Woods *et al.*, 2017). Little is known, however, about the mechanistic interactions between PFAS and the placenta as it pertains to pregnancy complications such as preeclampsia.

As a tool to study the biological mechanisms underlying preeclampsia, investigators can use placental cell lines to reproduce and examine trophoblast migration and invasion (Alvarez and Chakraborty, 2011; Brooks and Fry, 2017; Zhang *et al.*, 2019b). Efficient exchange of nutrients, waste, and dissolved gasses across the placenta requires sufficient maternal blood flow in the villous space. This is accomplished through the remodeling of the maternal endometrial spiral arteries, which is carried out by invasive extravillous trophoblasts (Anin *et al.*, 2004; Lyall *et al.*, 1999; Whitley and Cartwright, 2010). Defective spiral artery transformation and shallow trophoblast invasion are both known histological features of preeclampsia (Chaiworapongsa *et al.*, 2014; Falco *et al.*, 2017; Lyall *et al.*, 2013; Silva and Serakides, 2016). The precise regulation of extravillous trophoblast invasion is essential not only to placentation but also to fetal development. Environmental toxicants that interfere with the pathways regulating invasion can therefore potentially be detrimental to maternal and fetal health during pregnancy (Laine *et al.*, 2015). For instance, cadmium has been shown to decrease trophoblast migration *in vitro*, and placental cadmium content has been associated with preeclampsia (Brooks and Fry, 2017; Laine *et al.*, 2015). Environmental contaminants that are both associated with preeclampsia and affect *in vitro* trophoblast migration raise concerns about their risk particularly during pregnancy.

Although PFAS are established to alter migration and invasion in other cell types such as ovarian, colorectal, and breast cancer cells (Chou *et al.*, 2017; Li *et al.*, 2018; Pierozan *et al.*, 2018; Pierozan and Karlsson, 2018), no data exist on their effect on the same processes in trophoblasts. We therefore set out to investigate the impact of several PFAS on trophoblast migration and invasion and the molecular pathways involved in those effects. This investigation could provide mechanistic evidence for how PFAS contribute to placental dysfunction during pregnancy.

MATERIALS AND METHODS

Cell culture. The HTR-8/SVneo immortalized trophoblast cell line was purchased from the American Type Culture Collection (Manassas, Virginia). Cells were grown in Gibco RPMI 1640, supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, and 1 mM sodium pyruvate at 37°C in 5% CO_2.

Resazurin cytotoxicity assay. Cells were seeded at 10 000 cells/well, incubated overnight, and treated with 0.01–100 000 ng/ml PFOS, PFOA, GenX (Synquest Laboratories, Alachua, Florida) or 10–100 ng/ml pertussis toxin (PTX; Millipore Sigma, St. Louis, Missouri) for 24 h. Resazurin was then added to each well at a final concentration of 10 μg/ml, incubated for 4 h at 37°C in 5% CO_2, and fluorescence read on a Promega (Madison, Wisconsin) GloMax microplate spectrophotometer using 560 and 590 nm as the excitation and emission wavelength, respectively. Of all the chemicals tested, only 100 000 ng/ml PFOS was cytotoxic over 24 h.

Migration and invasion. To measure trophoblast migration and invasion, an ACEA S16 Real-Time Cell Analyzer (ACEA, San Diego, California) was utilized, which, using wells lined with gold microelectrodes, measures the electrical resistance associated with cell number and density. If done in conjunction with the traditional scratch assay, this setup allows for the collection of migration and invasion data in real time without relying on microscopy and image analysis (Al-Khayal *et al.*, 2017; Bucur *et al.*, 2018; Ragone *et al.*, 2017). For migration and invasion assays, cells were seeded at 25 000 cells/well in a 16-well E-Plate VIEW 16 (ACEA) and allowed to adhere overnight. For invasion assays in particular, wells were coated with 100 μg/ml Matrigel (Corning, Corning, New York) the day before seeding. Scratches were generated 48 h postseeding when growth had plateaued and the cells were 100% confluent. The day before scratching, cells were pretreated with PFAS (1000 ng/ml) for 24 h. This dose is biologically relevant as it is representative of median serum PFAS concentrations previously observed in occupationally exposed populations (Fu *et al.*, 2016; Zhou *et al.*, 2014). On the day of the experiment, a scratch was generated in each well with a 200 μl pipette tip, cells washed twice with culture medium. For migration assays, medium containing PFAS (1000 ng/ml) or PTX (10 ng/ml) was then added to each well (100 μl final volume). For invasion assays, scratched were filled in with 50 μl of treatment-containing Matrigel solution. The concentration of Matrigel used in this step was 800 μg/ml, which was determined in a separate experiment to reduce cell movement without completely preventing it (approximately 50% reduction in rate of movement compared with migration, data not shown). After 30 min at 37°C, the Matrigel solidified and an additional 100 μl of treatment-containing media was added on top (150 μl final volume). Data were collected and analyzed using Real-Time Cell Analysis software lite (ACEA, v2.2.2). To account for the nonlinear rate of cell movement, rate of migration and invasion was calculated in the software as the average of slopes for all 10 min intervals over 24 h postscratch. Each experiment and treatment condition was measured alongside a no-scratch control to account for differences in cell viability, attachment, or growth. No significant changes in no-scratch controls were seen in any treatment (data not shown).

mRNA expression assessment by quantitative real-time polymerase chain reaction. Cells were seeded at 100 000 cells/well in a 12-well plate and allowed to adhere overnight. The day before treatment, cell culture media was replaced with Gibco Opti-pro serum-free media supplemented with 0.1% penicillin/streptomycin. The following day, cells were treated with PFAS (10–10 000 ng/ml) in the same media for 24 h. According to 2013–

2014 National Health and Nutrition Examination Survey, 10 ng/ml is approximately representative of the median total serum PFAS concentration in the United States (NHANES, 2014). The concentrations of 100–10 000 ng/ml included in this study represent previously observed median serum PFAS concentrations in occupationally exposure populations (Fu *et al.*, 2016; Zhou *et al.*, 2014). Treated and untreated cells were harvested in 350 µl of buffer RLT plus and placed in a QIAcube (Qiagen, Valencia California) for RNA and DNA extraction using the AllPrep DNA/RNA/miRNA Universal Kit (Qiagen) according to the manufacturer's protocol. RNA was quantified using a Nanodrop 1000 spectrophotometer (Thermo Scientific, Waltham, Massachusetts). RNA integrity was analyzed with the use of the QIAxcel Screengel (Qiagen) according the manufacturer's protocol. To analyze gene expression, extracted RNA was converted to cDNA using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, California). Following this, mRNA expression was preamplified and analyzed by the UNC Advanced Analytics Core using the Fluidigm Biomark HD 96.96 array (Fluidigm, San Francisco, California) and validated Taqman Primers (Thermo Scientific) according to the manufacturer's protocol. Real-time RT-qPCR Ct values were then normalized against the geometric mean of housekeeping genes glyceraldehyde 3-phosphate dehydrogenase, succinate dehydrogenase complex flavoprotein subunit A, and TATA-Box Binding Protein, and fold changes in expression were calculated based on the ΔΔCT method (Livak and Schmittgen, 2001).

Chemokine and eicosanoid measurement in cell culture supernatants. Cells were seeded at 250 000 cells/well in a 6-well plate and allowed to adhere overnight. The day before treatment, cell culture media was replaced with Gibco Opti-pro serum-free media supplemented with 0.1% penicillin/streptomycin. The following day, cells were treated with PFAS (10–10 000 ng/ml) in the same media for 24 h. Supernatants were then collected and spun down at 2000 × g for 5 min to remove cellular debris and stored at −80°C until analysis. Cells were lysed and collected in 350 µl RIPA buffer, spun down at 10 000 × g, and analyzed for protein content using the Bicinchoninic Acid Protein Assay according to the manufacturer's protocol (Thermo Scientific). Chemokine ligand (CCL) 7, C-X-C motif chemokine ligands (CXCL) 2 and 8 and the lipoxygenase 15 (*ALOX15*) metabolite 15-Hydroxyeicosatetraenoic acid (15-HETE) were quantified in supernatants using enzyme-linked immunosorbent assays according to the manufacturer's protocol (ab193769, ab184862, ab214030, Abcam, Cambridge, Massachusetts; 534721, Cayman Chemical, Ann Arbor, Michigan). CCL2 was quantified using the LANCE Ultra Time Resolved Fluorescent Resonance Energy Transfer (TR-FRET) Detection Kit according to the manufacturer's protocol (TRF1244C, Perkin Elmer, Waltham, Massachusetts). All colorimetric, fluorimetric, and TR-FRET measurements were conducted using a SpectraMax iD5 (Molecular Devices, San Jose, California).

Statistical analysis. Gene expression, chemokine and eicosanoid secretion, migration, and invasion data were analyzed using 1-way ANOVA ($p < .05$) in Partek Genomics Suite v 7.18 (Partek, Inc, St. Louis, Missouri) and q values ($q < .2$) were calculated for gene expression data using the Benjamini-Hochberg FDR correction (Benjamini and Hochberg, 1995). Unless otherwise stated, Dunnett's post hoc test was performed for pairwise comparisons versus control ($p < .05$).

RESULTS

Migration and Invasion

Using an *in vitro* model to examine the effects of toxic substances on the development of preeclampsia, we examined the impact of PFAS treatment on trophoblast migration and invasion. This was accomplished using a modified scratch assay in the absence and presence of Matrigel, respectively (Hulkower and Herber, 2011). HTR-8/SVneo cells were treated for 24 h with PFAS at a concentration of 1000 ng/ml, representative of median serum PFAS concentrations in occupationally exposed populations (Fu *et al.*, 2016; Zhou *et al.*, 2014). PFOS, PFOA, and GenX decreased rates of HTR-8/SVneo migration to 72.1%, 80.7%, and 68.8% of the control (100%), respectively (Figure 1). This experiment was also conducted with 10 ng/ml of each compound, but no statistically significant decrease was observed (data not shown).

GenX decreased the rate of HTR-8/SVneo invasion to 63.7% that of the control (Figure 1). PFOS and PFOA decreased HTR-8/SVneo invasion to 85.6% and 90.2% of the control, although this was not statistically significant.

Inflammatory Gene Expression

We next set out to examine the cellular pathways that displayed altered expression in relation to PFAS inhibition of trophoblast migration and invasion. Trophoblast invasion is regulated by maternal and placental-immune cells (such as natural killer T cells and Hofbauer cells, respectively), and PFAS are known immunomodulators (Liew *et al.*, 2018; Sunderland *et al.*, 2019). We therefore set out to examine the effect of PFAS treatment on HTR-8/SVneo inflammatory mRNA expression of targeted genes.

Using qRT-PCR, we measured a panel of 98 inflammatory genes that are expressed in the human placenta and involved in preeclampsia, cellular movement, or both after 24 h treatment with a range of PFOS, PFOA, or GenX concentrations (10–10 000 ng/ml). Of the 98 transcripts measured, 77 were detected and 18 were significantly ($p < .05$, $q < .2$) altered by 1 or more PFAS treatment (Supplementary Table 1). The mean relative expression of all detected transcripts under each condition is displayed in Figure 2. All 3 PFAS tended to decrease the expression of those 77 transcripts that were detected with a few exceptions (Figure 2, Supplementary Table 1), and all statistically significant changes corresponded to a decrease in gene expression. Figure 3 highlights the representative genes belonging to 4 biological categories: chemokines, chemokine receptors, enzymes, and toll-like receptors.

Chemokines and chemokine receptors, which together direct cellular migration (Bhusal *et al.*, 2020), were most responsive to PFOS treatment relative to PFOS and GenX (Figure 3). Expression of CCL2, CCL7, CXCL2, and CXCL8 were all significantly reduced by PFOS treatment. PFOS also decreased secretion of CCL2 and CCL7 and expression of interleukin 1 receptor 1 (*IL1R1*), *CCR4*, and *CCR7*. In comparison, PFOA decreased CCL7 and CXCL2 secretion and *CCR7* expression and GenX had no significant impact on chemokines or their receptors. Toll-like receptors, which are activated by pathogen-associated molecular patterns and damage-associated molecular patterns, were also measured. *TLR3* and *TLR9* displayed decreased trends in expression in relation to all 3 PFAS, but only the highest dose (10 000 ng/ml) of PFOA had a statistically significant impact on *TLR3* expression.

The expression levels of genes that encode the eicosanoid synthesis enzymes, which produce metabolites that can directly

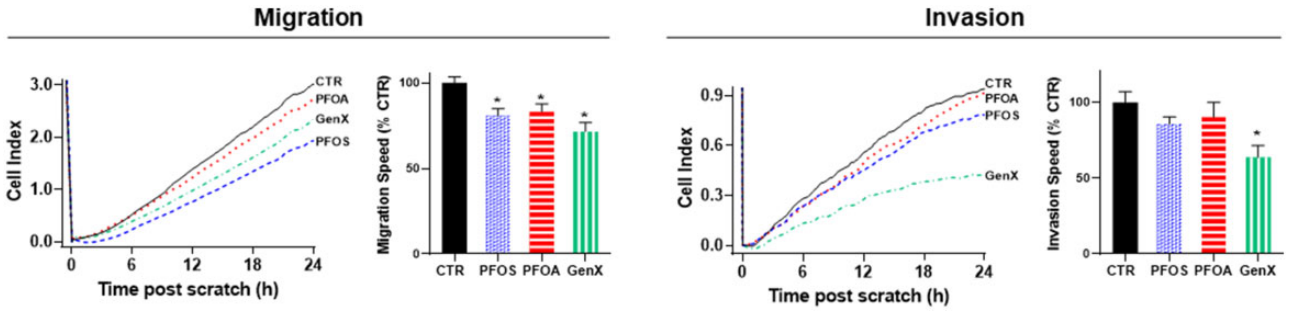

Figure 1. Per- and polyfluoroalkyl substances inhibit migration and invasion in a modified scratch assay in HTR-8/SVneo cells. Migration (left) and invasion (right) performed in the absence and presence of Matrigel, respectively. Cells were treated with 1000 ng/ml PFOS, PFOA, or GenX 24 h prior to and immediately following scratch. Line plots depict real-time data each from 1 representative experiment; bar plots represent the average rate of migration ± SEM from $N = 4$ experiments, $^{*}p < .05$.

or indirectly influence cellular movement (Qiu *et al.*, 2014; Silva *et al.*, 2017; Wu *et al.*, 2014), were significantly reduced by PFOS, PFOA, and GenX (Figure 3). *ALOX15* was reduced by all 3 PFAS tested, but only the changes by PFOS (100 ng/ml) and PFOA (10–10 000 ng/ml) were statistically significant. The metabolite of *ALOX15* was also measured in cell culture supernatants and was reduced by all treatments and doses but only statistically significant with 100 and 10 000 ng/ml PFOS.

Invasion-related Gene Expression

We next set out to determine the effect of PFAS on the expression of genes that regulate invasion in trophoblasts. We first measured transcripts of matrix-metalloproteinases (MMPs) 2 and 9, which, when secreted, serves to break down extracellular matrix proteins to aid in trophoblast movement through endometrial tissue. We found that both PFOS and PFOA significantly inhibited transcription of *MMP2*, and PFOS alone inhibited that of *MMP9* (Figure 4).

Trophoblast invasion also involves differentiation from an epithelial phenotype to a mesenchymal phenotype in a process termed epithelial-mesenchymal transition (EMT). We measured the effects of PFAS on the transcription of EMT-related genes and found only *SNAIL1* was significantly reduced with treatment of 1000 ng/ml PFOS (Figure 4).

G-protein Dependence of Trophoblast Migration

Considering that PFAS significantly decreased trophoblast migration and mRNA expression of *CCRs* and *CCLs*, we tested if inhibiting trophoblast CCR signal transduction would similarly decrease trophoblast migration. As CCRs are G-protein-coupled receptors, we measured trophoblast migration in the presence of the commonly employed G-protein inhibitor, PTX (Martinez-Olmedo and Garcia-Sainz, 1984). We observed in this experiment that 10 ng/ml PTX decreased trophoblast migration by 50.7% (Figure 5).

DISCUSSION

PFAS are prevalent and pervasive environmental contaminants that pose a global health risk as they have been shown to impact the heath of pregnancies (Darrow *et al.*, 2013; Huang *et al.*, 2019; Stein *et al.*, 2009; Wikström *et al.*, 2019). Despite their occurrence, little is known about their effect on the human placenta. In relation to disorders of pregnancy, placenta, or serum PFAS content has been associated with the incidence of preeclampsia (Darrow *et al.*, 2013; Huang *et al.*, 2019; Stein *et al.*, 2009; Wikström *et al.*, 2019). Preeclampsia is driven in part by shallow trophoblast invasion, and environmental contaminants that interfere with the pathways regulating invasion may contribute to preeclampsia (Chaiworapongsa *et al.*, 2014; Falco *et al.*, 2017; Lyall *et al.*, 2013; Rosen *et al.*, 2018; Silva and Serakides, 2016; Starling *et al.*, 2014; Wikström *et al.*, 2019). By recapitulating the process of trophoblast migration and invasion *in vitro*, it is possible to measure the specific impact of those environmental chemicals on placental functions dysregulated in preeclampsia and mechanisms therein. PFAS alter *in vitro* migration and invasion in other cell types (Chou *et al.*, 2017; Li *et al.*, 2018; Pierozan *et al.*, 2018; Pierozan and Karlsson, 2018), but the same has not been examined in trophoblasts. In this study, we examined the effect of PFAS on trophoblast migration and invasion and the molecular pathways involved in those effects. Three key findings were observed. First, PFOS, PFOA, and GenX all inhibit trophoblast migration. GenX was also associated with inhibited invasion. Second, PFAS decreased the mRNA expression and secretion of inflammatory genes and expression of MMPs involved in migration and invasion. Third, inhibiting G-protein activation independent of PFAS treatment decreased trophoblast invasion. These data provide novel information about the effects of PFAS on placental function.

Treatment with all 3 PFAS tested decreased the rate of trophoblast migration over 24 h. PFAS also decreased the expression of chemokines and chemokine receptors in HTR-8/SVneo cells, which could, in part, explain their effects on migration. In addition, inhibition of G-protein signaling with PTX also decreased trophoblast migration. As CCRs are all G-protein-coupled receptors (Bhusal *et al.*, 2020), this serves to strengthen the association between PFAS, migration, and CCR expression. PFAS have been previously shown to alter cellular migration in ovarian, colorectal, and breast cancer cell lines (Chou *et al.*, 2017; Miao *et al.*, 2015; Zhang *et al.*, 2014) and decrease chemokine release in bronchial epithelial cells, colon myofibroblasts and astrocytes, among others (Chen *et al.*, 2018; Giménez-Bastida *et al.*, 2015; Sorli *et al.*, 2019). Trophoblast migration is regulated in part by chemokines and their receptors (Du *et al.*, 2014). Specifically, *CCL2*, *CCL7*, *CXCL2*, *CXCL8CCR4*, *CCR7*, and *IL1R1*, which were downregulated in response to PFAS treatment, regulate trophoblast migration and differentiation at the maternal-fetal interface (Du *et al.*, 2014). Moreover, each of those genes has been previously associated with preeclampsia (Boij *et al.*, 2015; Kieffer *et al.*, 2019; Liong *et al.*, 2018; Tersigni *et al.*, 2016; Zhang *et al.*, 2019a,b).

In this model of trophoblast invasion, only GenX significantly hindered cellular movement. The presence of extracellular matrices changes the processes involved in cellular movement. Trophoblast invasion requires EMT (DaSilva-Arnold

Figure 2. Per- and polyfluoroalkyl substances alter inflammatory-related gene expression in HTR-8/SVneo cells. Quantitative PCR results of inflammatory-related genes in HTR-8/SVneo cells following treatment with PFOS, PFOA, and GenX (10–10 000 ng/ml). Displayed are all 77 detected transcripts from a 98-target inflammation panel (see Supplementary Table 1). Each cell represents the mean relative expression of $N = 4$ experiments compared with control.

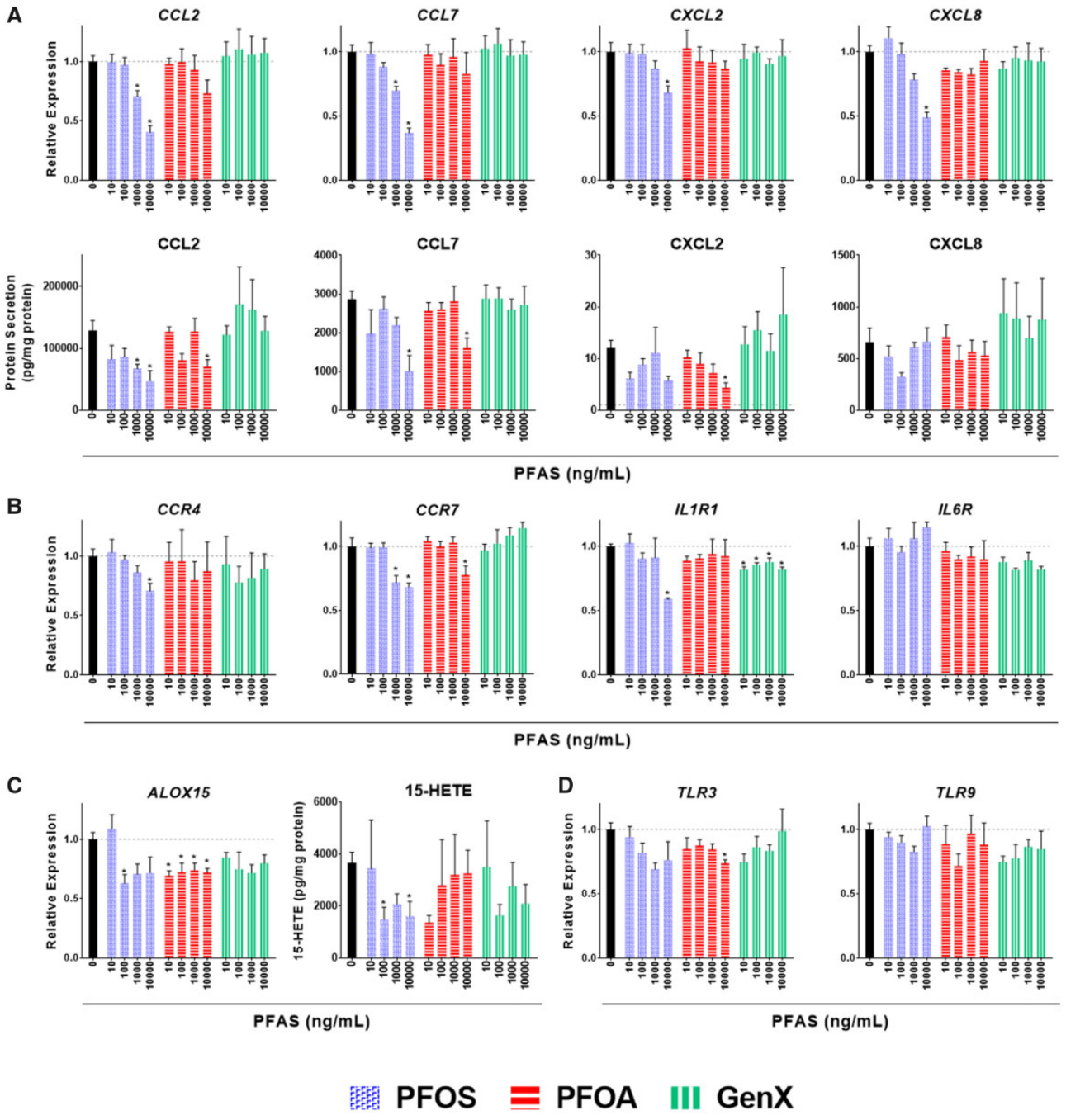

Figure 3. Expression and secretion of selected inflammatory genes in HTR-8/SVneo cells responsive to per- and polyfluoroalkyl substances treatment. Quantitative PCR and secretion of inflammatory-related genes in HTR-8/SVneo cells following treatment with PFOS, PFOA, and GenX (10–10 000 ng/ml). Genes displayed selected from a 98-target inflammation panel (see Supplementary Table 1), and include chemokines with their secreted proteins (A), chemokine receptors (B), *ALOX15* and secretion of its metabolite, 15-HETE (C), and toll-like receptors (D). Bars and lines represent the mean ± SEM relative to control. N = 4 experiments, $^{*}p < .05$.

et al., 2015, 2018, 2019), although none of the EMT markers measured here were significantly altered by PFAS. This may be due to the fact that the HTR-8/SVneo cell line is already differentiated to the mesenchymal phenotype (Abou-Kheir *et al.*, 2017; Graham *et al.*, 1993). In addition, trophoblasts secrete MMPs to digest extracellular proteins (Chen and Khalil, 2017; Espino *et al.*, 2017). When we measured MMP expression, however, only exposure to PFOS resulted in an effect. There may, however, be a difference in trophoblast MMP secretion as a result of GenX exposure. Future studies can comprehensively assess the differentiation and secretome of primary trophoblasts exposed to PFAS to establish the mechanisms underlying the observations in this study.

Several factors should be considered in the interpretation of these data. First, the effects of PTX are not specific to CCRs but rather all cellular G-protein-dependent process (Martinez-Olmedo and Garcia-Sainz, 1984). Moreover, because the changes in CCL and CCR expression and CCL secretion are modest and limited to PFOS and PFOA, it is difficult to conclude that this is the primary mechanisms driving the observed decrease in

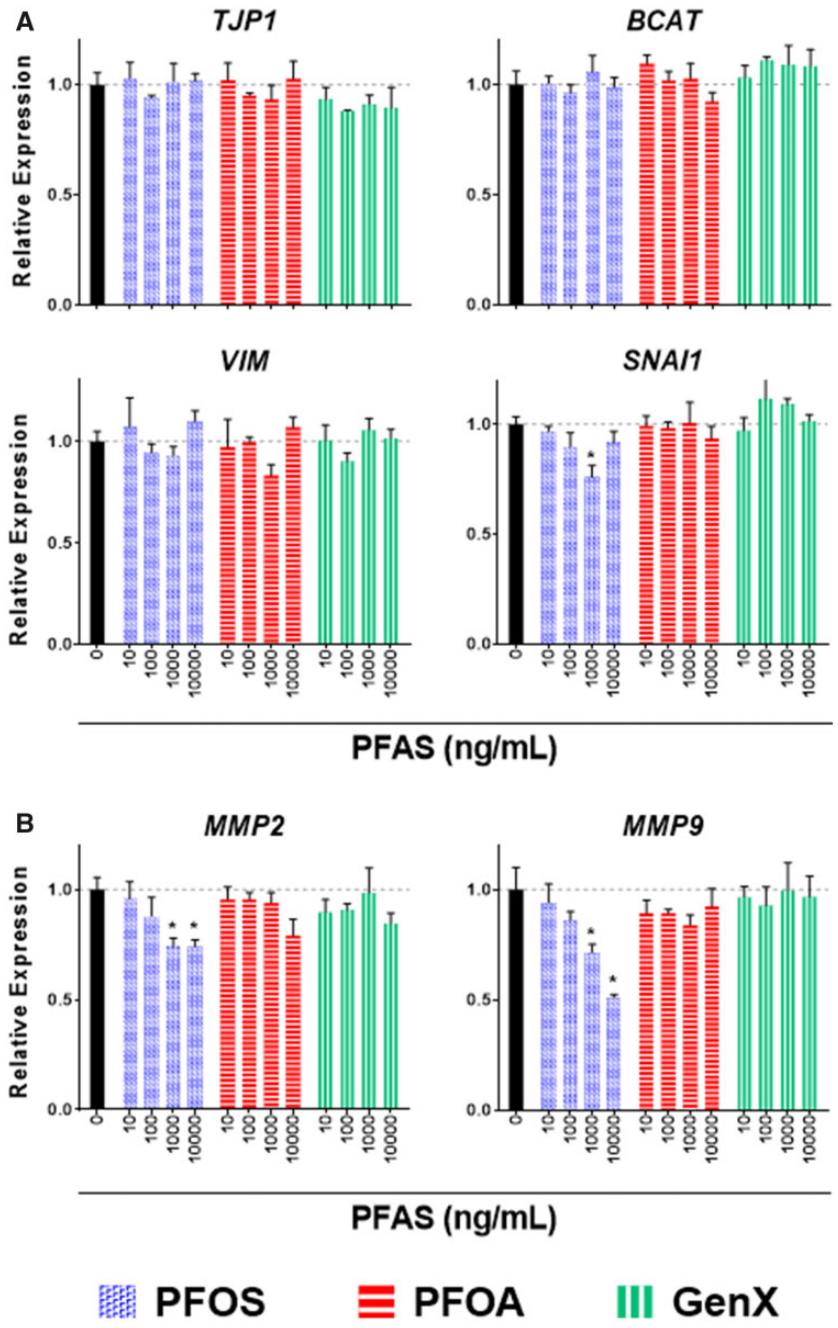

Figure 4. Effect of per- and polyfluoroalkyl substances on invasion-related gene expression in HTR-8/SVneo cells. Quantitative PCR results of invasion-related genes in HTR-8/SVneo cells following treatment with PFOS, PFOA, and GenX (10–10 000 ng/ml). Genes displayed include epithelial-mesenchymal transition-related genes (A) and matrix metalloproteinases (B) bars and lines represent the mean expression ± SEM relative to control. $N = 4$ experiments, for genes that were significant with FDR correction ($q < .2$), asterisks (*) denote significant changes ($p < .05$) for pairwise comparisons versus control.

migration. To conclude the involvement of CCRs in the changes observed here, additional studies will individually investigate each CCR-ligand pair. Moreover, the HTR-8/SVneo cell line used may be less sensitive to the effects of PFAS than extravillous trophoblasts *in vivo*, as we are measuring a population of trophoblasts that has already differentiated. It is therefore critical for future studies to utilize primary trophoblasts or stem cell-derived trophoblasts to measure the effect of PFAS on EMT. There also exist differences in efflux and uptake transport between trophoblasts *in vivo* and *in vitro*. Although studies have demonstrated the presence of PFAS in both maternal serum, comparable to those level observed in NHANES 2013–2014 as discussed above, and placenta (approximately 0.1–10 ng PFAS/g wet weight) (Mamsen *et al.*, 2019; NHANES, 2014), the mechanisms regulating their transport have not been clearly elucidated. Although the doses utilized here were representative of average and occupational serum levels, the internal dose of PFAS may not be representative of conditions *in vivo*. Lastly, this cell model only includes trophoblasts, but the process of trophoblast invasion involves trophoblast-immune crosstalk (Hsu and

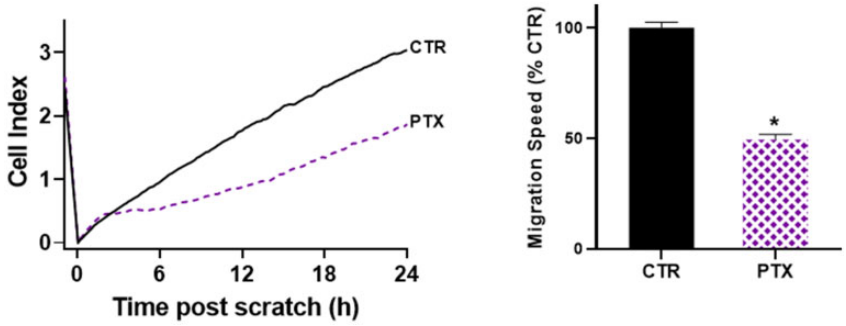

Figure 5. Inhibition of G-proteins decreases HTR-8/SVneo migration. Cells were treated with 10 ng/ml PTX immediately following initiation of scratch assay. Line plot (left) depicts real-time data each from 1 representative experiment; bar plot (right) represents the average rate of migration ± SEM from N = 3 experiments, $^{*}p < .05$.

Nanan, 2014). Future studies should examine the impact of PFAS on migration and invasion in a co-culture model of trophoblasts and immune cells, such as uterine natural killer cells.

In summary, the results of this study demonstrate that PFAS inhibit trophoblast migration and invasion. This is among the first to highlight the effects on migration and invasion and provide a potential hitherto unknown mechanism. These observations provide mechanistic evidence supporting prior reports that demonstrate the association between placental PFAS content and the incidence of preeclampsia (Darrow *et al.*, 2013; Huang *et al.*, 2019; Stein *et al.*, 2009; Wikström *et al.*, 2019). Moreover, the experiments performed here suggest that PFAS may modulate placental-immune signaling. These data warrant additional studies that investigate how PFAS change the molecular communication between trophoblasts and maternal-immune cells. This system of interactions is central to regulating trophoblast invasion and may therefore be a critical mechanistic target of environmental toxicants that contribute to preeclampsia.

SUPPLEMENTARY DATA

Supplementary data are available at *Toxicological Sciences* online.

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DECLARATION OF CONFLICTING INTERESTS

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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