Published in final edited form as:

Neurobiol Dis. 2015 October ; 82: 132–140. doi:10.1016/j.nbd.2015.05.019.

RNA-sequencing reveals transcriptional up-regulation of Trem2 in response to bexarotene treatment

Iliya Lefterov^{a,*}, Jonathan Schug^{b,c}, Anais Mounier^a, Kyong Nyon Nam^a, Nicholas F. Fitz^a, and Radosveta Koldamova^{a,*}

^aDepartment of Environmental & Occupational Health, University of Pittsburgh, Pittsburgh, PA 15219

^bInstitute for Diabetes, Obesity and Metabolism, University of Pennsylvania, Philadelphia, PA 19104

^cFunctional Genomics Core, Department of Genetics, University of Pennsylvania, Philadelphia, PA 19104

Abstract

We have recently demonstrated that short term bexarotene treatment of APP/PS1 mice significantly improves their cognitive performance. While there were no changes in plaque load, or insoluble A β levels in brain, biochemical analysis strongly suggested improved clearance of soluble A β , including A β oligomers. To get further insight into molecular mechanisms underlying this therapeutic effect, we explored genome-wide differential gene expression in brain of bexarotene and control treated APP/PS1 mice.

We performed high throughput massively parallel sequencing on mRNA libraries generated from cortices of bexarotene or vehicle treated APP/PS1 mice and compared the expression profiles for differential gene expression. Gene Ontology (GO) Biological Process categories with the highest fold enrichment and lowest False Discovery Rate (FDR) clustered in GO terms immune response, inflammatory response, oxidation-reduction and immunoglobulin mediated immune response. Chromatin immunoprecipitation (ChIP) followed by ChIP-QPCR, and RT-QPCR expression assays were used to validate select genes, including *Trem2*, *Tyrobp*, *Apoe* and *Ttr*, differentially expressed in response to Retinoid X Receptor (RXR) activation. We found that Bexarotene significantly increased the phagocytosis of soluble and insoluble A β in BV2 cells.

The results of our study demonstrate that in AD model mice expressing human APP, gene networks up-regulated in response to RXR activation by the specific, small molecule, ligand bexarotene may influence diverse regulatory pathways that are considered critical for cognitive performance, inflammatory response and A β clearance, and may provide an explanation of the bexarotene therapeutic effect at the molecular level. This study also confirms that unbiased

Correspondence to: Iliya Lefterov, MD, PhD, Tel: 1-412-383-6906; iliyal@pitt.edu; Radosveta Koldamova, MD, PhD, Tel: 1-412-383-7197; radak@pitt.edu; Department of Environmental and Occupational Health, University of Pittsburgh, BRIDG Building, 100 Technology Dr., Pittsburgh, PA 15219, USA.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

massive parallel sequencing approaches are useful and highly informative for revealing brain molecular and cellular mechanisms underlying responses to activated nuclear hormone receptors in AD animal models.

Keywords

Alzheimer's disease; APP/PS1 transgenic mice; mRNA-seq; ChIP; RXR; Bexarotene; Trem2; Tyrobp; A β phagocytosis

Introduction

The transcription factor Retinoid X Receptor (RXR) is a member of nuclear receptor superfamily and has an unusually complicated biology. In fact, the precise molecular mechanisms used by RXR to interact with the genome and to regulate gene expression are still not known (Daniel et al., 2014). Regardless of the mechanisms, however, silent or ligand activated RXR (including activation through permissive heterodimerization with other nuclear receptors) together with a network of RXR enhancers, are involved in the development of phenotypes that can be disease associated. Through RXR activation, these phenotypes can be influenced by synthetic small molecule RXR receptor specific agonists.

The role of activated RXR through LXR/RXR and RXR/PPAR γ permissive heterodimers in *in vivo* and *in vitro* models of Alzheimer's disease (AD) was first demonstrated a decade ago using partially specific LXR or PPAR γ agonists (Heneka et al., 2005; Koldamova et al., 2003; Koldamova et al., 2005) and later confirmed in numerous studies (Koldamova et al., 2014a). A promising therapeutic effect of a synthetic small molecule and specific RXR agonist - bexarotene, in AD model mice has only been recently reported (Cramer et al., 2012). Other laboratories failed to reproduce the beneficial effect of bexarotene on amyloid plaques (Fitz et al., 2013; 2013; Price et al., 2013; Tesseur et al., 2013; Veeraraghavalu et al., 2013). Fitz et al., however, reported a significant decrease in soluble A β species, including A β oligomers by 50%, following bexarotene treatment of APP/PS1 mice (Fitz et al., 2013). Veeraraghavalu et al. demonstrated even stronger reducing effect of bexarotene on soluble brain A β levels (Veeraraghavalu et al., 2013). Behavioral improvement in APP/PS1 mice in response to bexarotene treatment was found also by the De Strooper's lab (Tesseur et al., 2013). Notably, Fitz et al., (Fitz et al., 2013) and Boehm-Cagan et al., (Boehm-Cagan and Michaelson, 2014), found that bexarotene restores cognitive deficits in mice expressing human APOE4, and Tai et al. (Tai et al., 2014) demonstrated that bexarotene increases the level of APOE4 lipoprotein association/lipidation in 5xFAD mice with *APOE4* targeted replacement.

Bexarotene has been used so far in two clinical trials (one open-labeled and another, double-blind, placebo controlled) in patients with schizophrenia or schizoaffective disorders (Lerner et al., 2013). In both of them the scores recorded on Positive and Negative Syndrome Scale in bexarotene treated patients were significantly lower compared to those in placebo treated ones. While it is clear that the plaque lowering effects of bexarotene are difficult to reproduce and should be reconsidered in terms of the overall therapeutic effect of the drug, a striking conclusion is that in the context of AD and AD-like phenotype in AD model mice

there is a substantial lack of understanding of the effects of bexarotene at the molecular and cellular levels. To get further insight into molecular mechanisms underlining the therapeutic effect of bexarotene, demonstrated so far in numerous *in vivo* model systems and also in patients with cognitive impairment, we explored genome-wide differential gene expression in brain of bexarotene treated APP/PS1 mice.

Materials and Methods

Mice

All experiments involving mice in this study have been approved by the University of Pittsburgh IACUC. Breeding strategies, genotyping, and maintaining of APP/PS1 and APOE4 colonies have been published previously (Fitz et al., 2013; Koldamova et al., 2014c). WT C57BL/6 mice were purchased from Hilltop Laboratory Animals (Scottsdale, PA). Animals were randomly assigned to either Bexarotene (100 mg/kg/day; oral gavage; Targretin, Eisai Inc., WoodCliff Lake, NJ) or vehicle (0.2 mg/kg glycerol) treatment groups. Bexarotene treatment was for 10 days except otherwise indicated. Gender- and age-matched mice were used for bexarotene treatment and control. Tissue processing was according to the previously published protocol (Cronican et al., 2013; Koldamova et al., 2014c). Brains of euthanized mice were rapidly removed, divided into hemispheres, and cortices and hippocampi were separated from the olfactory bulbs, subcortical structures and cerebellum. All brain structures were snap-frozen on dry ice and stored at -80°C . Pieces of cortex (20–30 mg) were used for RNA isolation using Qiagen RNeasy mini kit (Qiagen Inc., Valencia, CA) as in the manufacturer's protocol. Briefly, tissue was lysed in RLT buffer by passing through a 25G x 5/8 needle on a 1cc syringe. An equal volume of 70% ethanol was added to the lysate and RNA purified using a spin column. Total RNA was eluted with 40 μl of EB buffer.

RNA isolation, RNA-sequencing and data processing

4 mice per group were used for RNA-seq (2 X male and 2 X female per group, 6 mo old). Pieces of cortex (20–30 mg) were used for RNA isolation using Qiagen RNeasy mini kit (Qiagen Inc., Valencia, CA) as in the manufacturer's protocol. Briefly, tissue pieces (20–30 mg) were lysed in RLT buffer by passing through a 25G x 5/8 needle on a 1cc syringe. An equal volume of 70% ethanol was added to the lysate and RNA purified using a spin column. Total RNA was eluted with 40 μl of EB buffer. The quality control of all RNA samples was performed on an Agilent 2100 Bioanalyzer instrument and samples with RIN > 8 were further used for RT-QPCR and library construction using mRNA Library Prep Reagent Set (Illumina, San Diego, CA). Libraries were generated by PCR enrichment including incorporation of barcodes to enable multiplexing. RNA-seq libraries (4 per condition, bexarotene or vehicle treated animal) were sequenced on Illumina HiSeq 2000. Briefly, sequencing was 100bp single-read in 2 lanes using a single pool of 8 multiplexed samples. Reads were trimmed to eliminate poor quality bases and reads with runs of 5 or more Ns followed by removal of reads that aligned to ribosomal sequences. Alignment and transcript quantification was done using RUM against its mm9 genome using the default parameters for a non-stranded library (Grant et al., 2011). Only the uniquely-aligning reads were used for differential analysis. Differential analysis was done using RefSeq transcripts

as the units of count-level data in the comparison. CG bias and gene length were not corrected. A spreadsheet with sequencing statistics is provided as Supplemental Table 1. Fastq files are available at NCBI GEO. Additional downstream analyses of the aligned sequencing reads were performed using edgeR and Rsubread packages available through Bioconductor (Law et al., 2014). Functional Pathway analysis and functional annotation clustering were performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID, <http://david.abcc.ncifcrf.gov/version> 6.7) using as a background list the “expressed” genes in our RNA-seq dataset; the list was generated by excluding those genes that did not have at least 2 reads in at least 2 libraries.

For validation tests first strand cDNA was synthesized from 1 µg of total RNA using EcoDry™ Premix (Clontech, Mountain View, CA). RT-QPCR was performed using TaqMan® Universal Master Mix II, no UNG and expression assays for the corresponding genes (Life Technologies, Grand Island, NY). Transcript levels were normalized to Gapdh and amplification plots were analyzed by comparative Ct method. RT-QPCR for validation was performed on gender-matched 6 mo old mice treated 10 days with bexarotene.

Chromatin immunoprecipitation and ChIP-QPCR

For ChIP-QPCR (shown on Fig 2D) we used WT mice treated for 24 hours with bexarotene at 2 months of age. Chromatin for ChIP was prepared as described previously (Cronican et al., 2013; Koldamova et al., 2014c; Lefterova et al., 2008) with some modifications. After cross-linking with 2% formaldehyde in PBS and nuclear extraction, chromatin was sheared by 3 pulses of 15 sec at 30 amplitude, a 120 sec pause and 3 pulses of 15 sec at 40 amplitude using a Fisher Scientific™ Model 705 Sonic Dismembrator (Fisher Scientific, Pittsburgh, PA) to obtain fragments of 200–600 bp. Lysates were cleared by centrifugation, and supernatant was used for immunoprecipitation. Immunoprecipitation was performed on 50 µg chromatin with rabbit polyclonal anti-RXR antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Validation assays were performed using Power SYBR Green PCR Master mix on ViiA 7 instrument (Applied Biosystems, Foster City, CA). Analysis was performed by the standard curve method and % input was calculated for each examined region. For comparison Insulin (Ins) proximal promoter was used as negative control.

In vitro Phagocytosis

BV2 cells (mouse microglial cell line) were maintained in DMEM/F12 containing 5% heat-inactivated FBS, 1% penicillin/streptomycin and 2mM L-glutamine. For *in vitro* phagocytosis assays cells were plated at a density of 30,000 cells/well in 8-well chamber slides (Nalge Nunc International, Naperville, IL) and incubated with different concentrations of bexarotene in serum-free media for 24 hours.

Uptake of soluble Aβ—The preparation of soluble Cy3-labeled Aβ1-42 (AnaSpec, Fremont, CA, USA) was performed as before (Lee et al., 2014) with slight modifications. Briefly, lyophilized Cy3-labeled Aβ1-42 was dissolved to 1mM in hexafluoroisopropanol, the solvent evaporated to produce film and the stock solution prepared in DMSO to 5mM; further dilutions were made in serum-free medium. This protocol yields mostly monomeric

A β as determined by Western blotting (not shown). Following drug pretreatment, cells were treated with soluble Cy3-labeled A β 1-42 at 1 μ M for 1 hour. Cells were washed with PBS, fixed in 4% Paraformaldehyde in PBS and stained with nuclear stain DAPI. Images were taken using Nikon Eclipse 90i microscope (Nikon, Tokyo, Japan) and % soluble A β uptake was counted by NIS Elements (Nikon, Tokyo, Japan) on 2 randomly selected fields per well (or on at least 400 cells per well) as number of cells with Cy3-A β divided by the total number of cells with DAPI.

Phagocytosis—Hilyte-fluor-488-labeled A β 1-42 was purchased from AnaSpec and dissolved according to manufacturer's instruction. A β diluted in serum-free medium was aggregated at 80 μ M for 24 hours at 37°C and following bexarotene pre-treatment applied to BV cells at 1 μ M for 1 hour. Cells were fixed in 4% Paraformaldehyde in PBS, incubated with the Alexa fluor 647[®] CD11b antibody (Biolegend, San Diego, CA) at 1:100 dilution overnight. The phagocytosis was counted using Nikon Eclipse 90i microscope as percentage of cells with Hilyte-A β divided by the total number of cells stained with CD11b.

Cell viability assay

BV2 cells were plated at a density of 30,000 cells/well in 96-well plates and incubated with 10, 20, 50 or 100 nM of bexarotene in serum-free medium for 24 hours. For cell viability assay the cells were incubated in 10 % AlamarBlue[®] Cell Viability Reagent (Life Technologies, Grand Island, NY) diluted in culture medium, for 2 hours at 37 °C. The optical density of the samples was measured at 570 nm in a *Spectramax 340* microplate reader (Molecular Devices, Sunnyvale, CA) or in a *Synergy2* Multi-Mode fluorescence spectrophotometer at 540 nm fluorescence excitation and 590 nm emission wavelengths.

Results

Differential gene expression

To examine how bexarotene affects the transcriptome in the brain of APP mice, we treated 6 month old APP/PS1 mice for 10 days with bexarotene and vehicle. Using total RNA isolated from the cortex of these mice for library preparation and high-throughput sequencing, we found 157 genes differentially affected ($p < 0.003$) by bexarotene treatment. In brains of treated mice the majority of those genes were up-regulated, while less than 10% were down-regulated (Figure 1A). Gene Ontology (GO) analysis revealed Biological Process (BP) terms with a very low False Discovery Rate (FDR) clustered in groups that are highly significant for AD pathogenesis and presumably therapeutic response in AD and other neurodegenerative disorders (Figure 1C). Thus, each of the top 9 categories including immune response, inflammatory response, immunoglobulin mediated immune response and oxidation reduction had an enrichment value (Fold enrichment) between 3 and 15 at FDR less than 0.2% (Figure 3C). Importantly KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway mapping of the entire set of differentially expressed genes revealed highly significant molecular interactions for KEGG entries Parkinson's, Alzheimer's and Huntington's disease (Figure 1D).

Validation

The regulatory role of activated RXR on the expression level of genes in bexarotene treated mice was validated independently *in vivo* and *in vitro*. Using IGV browser (<http://www.broadinstitute.org/igv>) we first visualized and reexamined the expression level/number of reads per exon of all annotated and differentially expressed genes within GO clusters listed in Figure 1C. In Figure 2A we demonstrate quantile normalized Log2 values of select differentially affected genes in bexarotene treated mice and identified by DAVID in the category immune response (*GO:0006955 immune response*) and other select genes known as associated with immune response and inflammation. Importantly, among those were Transthyretin (*Ttr*), Triggering receptor expressed on myeloid cells 2 (*Trem2*) and Tyrosine kinase binding protein (*Tyrobp*) - genes known to be associated with immune response and inflammation.

Next, we performed RT-QPCR expression assays for *Trem2* and *Tyrobp* (Figure 2B). We rationalized *Trem2* validation assays (up-regulated in bexarotene treated mice) by the recent discoveries that a common genetic variant of human *TREM2* is associated with a high risk (maybe as high as *APOEε4* carrier status) (Jonsson et al., 2013) for late-onset AD (LOAD). We also validated the expression level of *Tyrobp* (*Dab12*) and *Ttr* - both differentially up-regulated in bexarotene treated mice. TYROBP (Tyrosine Kinase Binding Protein) is a required partner for TREM2 mediated intracellular signaling and its physiological effects (Lue et al., 2014). Transthyretin (*Ttr*), although primarily secreted by liver, is also expressed in neurons and glia, and has been identified differentially expressed in our bexarotene treated mice (Figure 2B, $p < 0.05$). In addition, we also measured mRNA expression of two apolipoproteins, namely *ApoE* and *ApoD* which have been differentially expressed. As shown on Figure 2B, *ApoE* mRNA was increased in statistically significant manner and *ApoD* showed a strong trend towards an increase.

Since recent studies have shown that TREM2 level increases in AD patients (Lue et al., 2014), we also measured its level in age-matched wild type non-APP mice that express mouse *ApoE* (WT) as well as in non-APP mice expressing human APOE4. Figure 2C shows that in both, WT and APOE4 mice the expression level of *Trem2* and *Tyrobp* in response to bexarotene treatment remained unchanged. These results were confirmed by subsequent RNA-Seq on independently generated libraries from cortices of those APOE4 mice (manuscript under review).

To validate the effect of bexarotene in an *in vitro* test system we used mouse embryonic stem (ES) cells. We chose these cells because they spontaneously differentiate into multiple cell types (including hematopoietic progenitors) after LIF removal and have been extensively used as a model system to assess the biology of numerous transcription factors. In addition, stimulation of *Trem2* expression has been demonstrated in mixed cell cultures, but not in pure microglia or BV2 cell cultures (Melchior et al., 2010). We used cellular aggregates (CA) derived from ES cells (line R1) (Bibel et al., 2004), maintained in suspension culture 4 days before bexarotene treatment. We evaluated the expression level of *Trem2* and *Tyrobp* 4 and 24 hr following treatment. On Figure 2D, we show that *Trem2* and *Tyrobp* expression level is increased 24 hr post-treatment, but not at the 4 hr time-point.

In a recent study (Daniel et al., 2014) using murine macrophages derived from bone marrow hematopoietic cells demonstrated that ligand activated-RXR binds upstream (at a distance of 2713 bp) of *Trem2* Transcription Start Site, suggesting that *Trem2* is a direct RXR target. To further confirm these results we treated WT C57BL/6J mice with bexarotene for 24 hours and performed chromatin immunoprecipitation (IP) followed by QPCR (ChIP-QPCR). As shown in Figure 2E, bexarotene treatment significantly increased RXR binding. Although the binding site is within a *Trem2* canonical promoter, and up-regulation of *Trem2* is detected in response to specific RXR agonist, our mRNA-seq and ChIP-QPCR data do not rule out an effect mediated by RXR heterodimerization with another NR. Daniel et al., identified also a second RXR binding site upstream of the entire *Trem* cluster (not tested in our study), which emphasizes the complexity of the regulatory mechanisms mediated by activated RXR and supports the idea that other transcription factors - direct RXR targets, as well as, changes in chromatin structure induced by liganded RXR could provide an explanation for the final outcome of Bexarotene treatment.

Increased phagocytosis in response to bexarotene treatment

To examine the effect of bexarotene on phagocytosis, we used BV2 mouse microglial cell line treated with increasing concentrations for 24 hours. As shown on Figure 3C, bexarotene was not toxic to BV2 cells as it did not significantly affect cell death. We first examined if pre-treatment of bexarotene affects the uptake of soluble A β ₁₋₄₂ in BV2 cells incubated with soluble Cy3-labeled A β for 1 or 2 hours. As visible from Figure 3A and B, bexarotene treatment significantly increased A β uptake (red color on Figure 3A) in a dose-dependent manner. It should be noted, however, that because of the high aggregation rate of A β ₁₋₄₂, even if we added it to the cells in a monomeric form, it was impossible at these experimental conditions (37°C) to rule out A β aggregation and thus formation of oligomers and proto-fibrils. We did not find a difference in response depending on the A β incubation time - 1 or 2 hours (data not shown). To examine the effect of bexarotene on the phagocytosis of aggregated A β , we used aggregated Hilyte-labeled A β ₁₋₄₂ (green color on Figure 3D, a and b) applied to bexarotene pre-treated BV2 cells. To ensure that aggregated A β is within the cells we used non-nuclear staining with CD11b (purple color on Fig 3Da and b). As visible from Figure 3D and E, bexarotene treatment significantly increased the phagocytosis of aggregated A β ₁₋₄₂.

Discussion

While changes in the levels of brain soluble A β , oligomeric A β in ISF, and a modulatory effect on microglia phenotype were important outcomes when assessing the therapeutic effect of bexarotene, no molecular explanation was provided by any of the studies where bexarotene was applied to APP expressing mice (Boehm-Cagan and Michaelson, 2014; Cramer et al., 2012; Fitz et al., 2013; Price et al., 2013; Tai et al., 2014; Tesseur et al., 2013; Veeraghavalu et al., 2013). No explanation has been suggested for the observed effect in bexarotene treated patients in the clinical trials, either (Lerner et al., 2008; Lerner et al., 2013). The results of unbiased molecular, biochemical and computational approaches, presented here, demonstrate that some of the bexarotene therapeutic effects in APP/PS1 mice may be mediated by transcriptional regulation of networks of genes through direct and

enhanced RXR binding to their response elements. The analysis of the sequencing datasets and subsequent *in vitro* and *in vivo* validation assays suggest molecular mechanisms affecting major aspects of AD phenotype, possibly acting through enhanced immune response, inhibition of inflammatory reactions and neuronal protection.

Microglia mediated clearance of A β is considered one of the major pathways contributing to the reduction of A β in the brain of AD model mice during early stages of the developing AD phenotype (Hickman and El Khoury, 2014). Thus, up-regulation of genes that are part of the GO terms immune response and inflammatory response, as found in this study (see Figure 1C) and the increased phagocytosis of aggregated A β by BV2 cells (Figure 3), provide an explanation, to some extent, of the molecular mechanisms driving the effects of bexarotene treatment. Thus we found that bexarotene differentially affected genes that have a potential role in A β phagocytosis such as Trem2, Tyrobp, C1q and Apoe.

The increased expression of C1q genes in response to bexarotene activated RXR provides additional molecular explanation of a therapeutic effect mediated by M2 microglia. It is now well established that C1q plays a prominent role in clearance of apoptotic cells – complement components readily coat the surface of apoptotic cells and facilitate ingestion by macrophages. In fact, activated C1q triggers relatively well understood metabolic pathway and elicits a macrophage phenotype that is tailored specifically for clearance of apoptotic cells (Bohlsón et al., 2014; Galvan et al., 2012). Although the transcriptional activation of C1q is not fully understood it has been shown that PPAR δ – a permissive heterodimeric RXR partner regulates a transcriptional program for sensing and silent disposal of apoptotic cells by phagocytosis through increased expression of opsonins (Mukundan et al., 2009). Apoptotic cell engulfment activates also LXR, and once activated, LXRs induce the expression of a network of genes that, promote clearance of apoptotic debris and inhibit inflammatory pathways (A-Gonzalez et al., 2009). It has also been demonstrated that if up-regulated *in vivo*, C1q exerts a strong neuroprotective effect in the early stages of AD through the inhibition of fibrillar A β induced neuronal death (Benoit et al., 2013). While these candidate molecular mechanisms underlie neuroprotective effects of C1q that are possibly stimulated as a result of bexarotene treatment, the role of C1q in the development of AD-like phenotype in APP expressing mice is poorly understood and the results vary depending on the animal model (Zabel and Kirsch, 2013). Moreover, it has been demonstrated recently, that IL-10 overexpression or deletion affects amyloid load, cognitive behavior and microglial A β phagocytosis (Chakrabarty et al., 2015; Guillot-Sestier et al., 2015). IL-10 is an anti-inflammatory cytokine and its release, following C1q activation, accompanies engulfment of apoptotic cells by resolving M2 macrophages and microglia, with a strong anti-inflammatory effect in the early stages of chronic inflammation (Galvan et al., 2014; Spivia et al., 2014). How to interpret the effect of activated RXR and C1q up-regulation in the context of these seemingly controversial findings is a difficult question. It is possible, however, that if there is a significant amount of aggregated A β deposited in amyloid plaques, C1q/IL-10 promoted clearing and anti-inflammatory mechanisms, and the effect of increased release of anti-inflammatory cytokines TGF β and IL-10, may change. Therefore, further research is required with AD model mice at ages before A β deposition, when C1q regulated microglia polarization and clearing mechanisms of soluble A β are not

perturbed and may be easier to measure. Another gene that can contribute to the effect of bexarotene is *Ttr*. There is evidence supporting the hypothesis that Transthyretin has the capacity to inhibit formation of oligomeric A β , to enhance clearance of soluble A β and ultimately to neutralize the neurotoxic effects of fibrillar A β (Cascella et al., 2013).

Of particular interest in relation to microglial activation and inflammatory response in bexarotene treated mice was the effect of bexarotene on the expression level of *Trem2*, subsequently validated *in vivo* and *in vitro*. Since TREM2 participates in signaling cascades central to initiation and execution of innate immune response, induces phagocytosis of apoptotic neurons without inflammatory response and (at least in *in vitro* models) facilitates A β phagocytosis, it is possible that some, or a significant part, of the therapeutic effects of bexarotene could be mediated by up-regulation of *Trem2*. The significant *in vitro* and *in vivo* up-regulation of *Tyrobp* in response to bexarotene treatment, as demonstrated in our study, brings another level of support to the explanation of the molecular mechanisms underlying at least part of the therapeutic outcome in bexarotene-treated APP-expressing mice. In this regard the results of a recent study (Zhang et al., 2013) conducted with postmortem brain samples from LOAD patients and non-demented individuals seem relevant and very important: using integrative network based approach the authors constructed and rank-ordered molecular interaction structures for relevance to LOAD pathology. The highest ranked immune and microglia-specific modules, were dominated by genes involved in pathogen phagocytosis and contained up-regulated *TYROBP*. The precise biological significance of up-regulated *TYROBP* and *TREM2* in the context of AD and particularly early stages of the disease in humans, and in rodents, is not entirely understood. However, the fact that both genes and very similar regulatory networks have been identified in the above cited and our study, and since *TYROBP* (DAP-12) is the only identified adaptor associated with TREM2, parallel changes in expression level of both genes following bexarotene treatment reinforce our hypothesis that transcriptional up-regulation of *Trem2* and *Tyrobp* may well be a mediator of the bexarotene therapeutic effect.

The increased A β phagocytosis in our experiments with BV2 cells, further supports the idea that the overall effect in bexarotene treated mice may be significantly mediated by microglia/mononuclear phagocytes. Phagocytosis of A β , particularly in the early stages of the developing AD-like phenotype in model mice, and presumably in AD patients, depends on the expression and receptor-ligand type of interactions between numerous proteins. TREM2/DAP-12 association with positive regulation of phagocytosis by myeloid, innate immune cells is just one of those (Hickman et al., 2013). At this time it is important to remember that a conflicting reports have been published showing a beneficial effect of global deletion of *Trem2*, with reduced inflammation and ameliorated amyloid and tau pathologies (Jay et al., 2015; Wang et al., 2015b). Nevertheless, since RXR, as a pharmacological target of bexarotene is a transcription factor, it is possible that RXR modulatory effect on the expression level of other genes and proteins, in addition to TREM2 and DAP-12, ultimately promotes microglia mediated phagocytosis and A β clearance. One such candidate mouse brain is *ApoE*. Previous data from several groups demonstrated that ApoE is important for A β phagocytosis (Jiang et al., 2008; Terwel et al., 2011). Moreover, liganded-LXRs, heterodimeric partners of RXR, increase *ApoE* expression and A β

degradation by microglia (Fitz et al., 2010; Jiang et al., 2008; Terwel et al., 2011). Interestingly, a recent study demonstrated that TREM2 recognizes negatively charged phospholipids, which are bound to A β (Wang et al., 2015b). It should be noted that negatively charged phospholipids such as phosphatidylserine, are part of HDL (Camont et al., 2013) and A β was shown to bind HDL and other lipoproteins (reviewed in (Lefterov et al., 2010; Tai et al., 2014). In brain HDL-like lipoproteins contain ApoE as a major apolipoprotein (Hottman et al., 2014; Koldamova et al., 2014b) advocating the role of ApoE as a lipid carrier important for A β phagocytosis or other molecules prone to lipid binding. Additional research, however, is required to precisely reveal the molecular mechanisms leading to up-regulation of *Trem2*, *Tyrobp* and *ApoE* and other genes in bexarotene treated mice, as well as the spectrum of downstream signaling cascades initiated by A β secretion and deposition in AD brain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Supported by NIH: AG037481, AG037919, ES024233, ES021243, K01AG044490, and DOD: W81XWH-13-1-0384.

References

- Gonzalez A-N, et al. Apoptotic cells promote their own clearance and immune tolerance through activation of the nuclear receptor LXR. *Immunity*. 2009; 31:245–58. [PubMed: 19646905]
- Bailey CC, et al. IFITM-Family Proteins: The Cell's First Line of Antiviral Defense. *Annu Rev Virol*. 2014; 1:261–283. [PubMed: 25599080]
- Benoit ME, et al. C1q-induced LRP1B and GPR6 proteins expressed early in Alzheimer disease mouse models, are essential for the C1q-mediated protection against amyloid-beta neurotoxicity. *J Biol Chem*. 2013; 288:654–65. [PubMed: 23150673]
- Bibel M, et al. Differentiation of mouse embryonic stem cells into a defined neuronal lineage. *Nat Neurosci*. 2004; 7:1003–9. [PubMed: 15332090]
- Boehm-Cagan A, Michaelson DM. Reversal of apoE4-driven brain pathology and behavioral deficits by bexarotene. *J Neurosci*. 2014; 34:7293–301. [PubMed: 24849361]
- Bohlson SS, et al. Complement, c1q, and c1q-related molecules regulate macrophage polarization. *Front Immunol*. 2014; 5:402. [PubMed: 25191325]
- Brass AL, et al. The IFITM proteins mediate cellular resistance to influenza A H1N1 virus, West Nile virus, and dengue virus. *Cell*. 2009; 139:1243–54. [PubMed: 20064371]
- Camont L, et al. Small, dense high-density lipoprotein-3 particles are enriched in negatively charged phospholipids: relevance to cellular cholesterol efflux, antioxidative, antithrombotic, anti-inflammatory, and antiapoptotic functionalities. *Arterioscler Thromb Vasc Biol*. 2013; 33:2715–23. [PubMed: 24092747]
- Cascella R, et al. Transthyretin suppresses the toxicity of oligomers formed by misfolded proteins in vitro. *Biochim Biophys Acta*. 2013; 1832:2302–14. [PubMed: 24075940]
- Chakrabarty P, et al. IL-10 alters immunoproteostasis in APP mice, increasing plaque burden and worsening cognitive behavior. *Neuron*. 2015; 85:519–33. [PubMed: 25619653]
- Chen G, et al. Molecular mechanisms for synchronized transcription of three complement C1q subunit genes in dendritic cells and macrophages. *J Biol Chem*. 2011; 286:34941–50. [PubMed: 21862594]

- Choubey D, et al. Interferon-inducible p200-family proteins as novel sensors of cytoplasmic DNA: role in inflammation and autoimmunity. *J Interferon Cytokine Res.* 2010; 30:371–80. [PubMed: 20187776]
- Corsetti JP, et al. Plasminogen activator inhibitor-2 polymorphism associates with recurrent coronary event risk in patients with high HDL and C-reactive protein levels. *PLoS One.* 2013; 8:e68920. [PubMed: 23874812]
- Cramer PE, et al. ApoE-directed therapeutics rapidly clear beta-amyloid and reverse deficits in AD mouse models. *Science.* 2012; 335:1503–6. [PubMed: 22323736]
- Cronican AA, et al. Genome-wide alteration of histone H3K9 acetylation pattern in mouse offspring prenatally exposed to arsenic. *PLoS One.* 2013; 8:e53478. [PubMed: 23405071]
- Daniel B, et al. The active enhancer network operated by liganded RXR supports angiogenic activity in macrophages. *Genes Dev.* 2014; 28:1562–77. [PubMed: 25030696]
- Dassati S, et al. Apolipoprotein D takes center stage in the stress response of the aging and degenerative brain. *Neurobiol Aging.* 2014; 35:1632–42. [PubMed: 24612673]
- Dominguez-Soto A, et al. Intravenous immunoglobulin promotes antitumor responses by modulating macrophage polarization. *J Immunol.* 2014; 193:5181–9. [PubMed: 25326025]
- Feenstra B, et al. Common variants associated with general and MMR vaccine-related febrile seizures. *Nat Genet.* 2014; 46:1274–82. [PubMed: 25344690]
- Fitz NF, et al. Liver X receptor agonist treatment ameliorates amyloid pathology and memory deficits caused by high-fat diet in APP23 mice. *J Neurosci.* 2010; 30:6862–6872. [PubMed: 20484628]
- Fitz NF, et al. Comment on “ApoE-directed therapeutics rapidly clear β -amyloid and reverse deficits in AD mouse models”. *Science.* 2013; 340:924–92c. [PubMed: 23704552]
- Flex A, et al. Effect of proinflammatory gene polymorphisms on the risk of Alzheimer’s disease. *Neurodegener Dis.* 2014; 13:230–6. [PubMed: 24022074]
- Fukushima Y, et al. Molecular cloning of chicken interleukin-5 receptor alpha-chain and analysis of its binding specificity. *Dev Comp Immunol.* 2012; 37:354–62. [PubMed: 22391510]
- Galvan MD, et al. Complement component C1q regulates macrophage expression of Mer tyrosine kinase to promote clearance of apoptotic cells. *J Immunol.* 2012; 188:3716–23. [PubMed: 22422887]
- Galvan MD, et al. Complement protein C1q and adiponectin stimulate Mer tyrosine kinase-dependent engulfment of apoptotic cells through a shared pathway. *J Innate Immun.* 2014; 6:780–92. [PubMed: 24942043]
- Grant GR, et al. Comparative analysis of RNA-Seq alignment algorithms and the RNA-Seq unified mapper (RUM). *Bioinformatics.* 2011; 27:2518–28. [PubMed: 21775302]
- Guillot-Sestier MV, et al. I110 deficiency rebalances innate immunity to mitigate Alzheimer-like pathology. *Neuron.* 2015; 85:534–48. [PubMed: 25619654]
- Heneka MT, et al. Acute treatment with the PPAR γ agonist pioglitazone and ibuprofen reduces glial inflammation and A β 1–42 levels in APPV717I transgenic mice. *Brain.* 2005; 128:1442–53. [PubMed: 15817521]
- Hickman SE, El Khoury J. TREM2 and the neuroimmunology of Alzheimer’s disease. *Biochem Pharmacol.* 2014; 88:495–8. [PubMed: 24355566]
- Hickman SE, et al. The microglial sensome revealed by direct RNA sequencing. *Nat Neurosci.* 2013; 16:1896–905. [PubMed: 24162652]
- Hottman DA, et al. HDL and cognition in neurodegenerative disorders. *Neurobiol Dis.* 2014; 72(Pt A): 22–36. [PubMed: 25131449]
- Hwang Y, et al. Gene expression profiling by mRNA sequencing reveals increased expression of immune/inflammation-related genes in the hippocampus of individuals with schizophrenia. *Transl Psychiatry.* 2013; 3:e321. [PubMed: 24169640]
- Jay TR, et al. TREM2 deficiency eliminates TREM2+ inflammatory macrophages and ameliorates pathology in Alzheimer’s disease mouse models. *J Exp Med.* 2015; 212:287–95. [PubMed: 25732305]
- Jiang Q, et al. ApoE promotes the proteolytic degradation of A β . *Neuron.* 2008; 58:681–693. [PubMed: 18549781]

- Jonsson T, et al. Variant of TREM2 associated with the risk of Alzheimer's disease. *N Engl J Med*. 2013; 368:107–16. [PubMed: 23150908]
- Jordanovski D, et al. The hypoxia-inducible transcription factor ZNF395 is controlled by I κ B kinase-signaling and activates genes involved in the innate immune response and cancer. *PLoS One*. 2013; 8:e74911. [PubMed: 24086395]
- Khondoker M, et al. Linking Genetics of Brain Changes to Alzheimer's Disease: Sparse Whole Genome Association Scan of Regional MRI Volumes in the ADNI and AddNeuroMed Cohorts. *J Alzheimers Dis*. 2015; 45:851–64. [PubMed: 25649652]
- Khorooshi R, Owens T. Injury-induced type I IFN signaling regulates inflammatory responses in the central nervous system. *J Immunol*. 2010; 185:1258–64. [PubMed: 20562259]
- Kleinberger G, et al. TREM2 mutations implicated in neurodegeneration impair cell surface transport and phagocytosis. *Sci Transl Med*. 2014; 6:243ra86.
- Koldamova R, et al. ATP-binding cassette transporter A1: From metabolism to neurodegeneration. *Neurobiol Dis*. 2014a
- Koldamova R, et al. ATP-binding cassette transporter A1: from metabolism to neurodegeneration. *Neurobiol Dis*. 2014b; 72(Pt A):13–21. [PubMed: 24844148]
- Koldamova R, et al. Genome-wide approaches reveal EGR1-controlled regulatory networks associated with neurodegeneration. *Neurobiol Dis*. 2014c; 63:107–14. [PubMed: 24269917]
- Koldamova RP, et al. 22R-hydroxycholesterol and 9-cis-retinoic acid induce ATP-binding cassette transporter A1 expression and cholesterol efflux in brain cells and decrease amyloid beta secretion. *J Biol Chem*. 2003; 278:13244–56. [PubMed: 12547833]
- Koldamova RP, et al. The Liver X Receptor Ligand T0901317 Decreases Amyloid {beta} Production in Vitro and in a Mouse Model of Alzheimer's Disease. *Journal of Biological Chemistry*. 2005; 280:4079–4088. [PubMed: 15557325]
- Law CW, et al. voom: Precision weights unlock linear model analysis tools for RNA-seq read counts. *Genome Biol*. 2014; 15:R29. [PubMed: 24485249]
- Lee L, et al. Regulation of synaptic plasticity and cognition by SUMO in normal physiology and Alzheimer's disease. *Sci Rep*. 2014; 4:7190. [PubMed: 25448527]
- Lefterov I, et al. Apolipoprotein A-I deficiency increases cerebral amyloid angiopathy and cognitive deficits in APP/PS1DeltaE9 mice. *Journal of Biological Chemistry*. 2010; 285:36945–36957. [PubMed: 20739292]
- Lefterova MI, et al. PPARgamma and C/EBP factors orchestrate adipocyte biology via adjacent binding on a genome-wide scale. *Genes Dev*. 2008; 22:2941–52. [PubMed: 18981473]
- Lerner V, et al. Bexarotene as add-on to antipsychotic treatment in schizophrenia patients: a pilot open-label trial. *Clin Neuropharmacol*. 2008; 31:25–33. [PubMed: 18303488]
- Lerner V, et al. The retinoid X receptor agonist bexarotene relieves positive symptoms of schizophrenia: a 6-week, randomized, double-blind, placebo-controlled multicenter trial. *J Clin Psychiatry*. 2013; 74:1224–32. [PubMed: 24434091]
- Li Y, et al. Promotion of expression of interferon-stimulated genes in U937 monocytic cells by HIV RNAs, measured using stable isotope labeling with amino acids in cell culture (SILAC). *Arch Virol*. 2015
- Liu B, et al. Irgm1-deficient mice exhibit Paneth cell abnormalities and increased susceptibility to acute intestinal inflammation. *Am J Physiol Gastrointest Liver Physiol*. 2013; 305:G573–84. [PubMed: 23989005]
- Lue LF, et al. What happens to microglial TREM2 in Alzheimer's disease: Immunoregulatory turned into immunopathogenic? *Neuroscience*. 2014
- Ma J, et al. TYROBP in Alzheimer's Disease. *Mol Neurobiol*. 2015; 51:820–6. [PubMed: 25052481]
- McDermott JE, et al. Controlling the response: predictive modeling of a highly central, pathogen-targeted core response module in macrophage activation. *PLoS One*. 2011; 6:e14673. [PubMed: 21339814]
- Melchior B, et al. Dual induction of TREM2 and tolerance-related transcript, Tmem176b, in amyloid transgenic mice: implications for vaccine-based therapies for Alzheimer's disease. *ASN Neuro*. 2010; 2:e00037. [PubMed: 20640189]

- Mukundan L, et al. PPAR-delta senses and orchestrates clearance of apoptotic cells to promote tolerance. *Nat Med*. 2009; 15:1266–72. [PubMed: 19838202]
- Nguyen PD, et al. Haematopoietic stem cell induction by somite-derived endothelial cells controlled by meox1. *Nature*. 2014; 512:314–8. [PubMed: 25119043]
- Poh KW, et al. Comprehensive gene expression profiling in the prefrontal cortex links immune activation and neutrophil infiltration to antinociception. *J Neurosci*. 2012; 32:35–45. [PubMed: 22219268]
- Price AR, et al. Comment on “ApoE-directed therapeutics rapidly clear beta-amyloid and reverse deficits in AD mouse models”. *Science*. 2013; 340:924–d. [PubMed: 23704553]
- Ramsland PA, et al. Structural basis for Fc gammaRIIa recognition of human IgG and formation of inflammatory signaling complexes. *J Immunol*. 2011; 187:3208–17. [PubMed: 21856937]
- Saba K, et al. A C-type lectin MGL1/CD301a plays an anti-inflammatory role in murine experimental colitis. *Am J Pathol*. 2009; 174:144–52. [PubMed: 19095961]
- Saiman Y, et al. Inhibition of the CXCL12/CXCR4 chemokine axis with AMD3100, a CXCR4 small molecule inhibitor, worsens murine hepatic injury. *Hepatology*. 2014
- Schaffler A, Buechler C. CTRP family: linking immunity to metabolism. *Trends Endocrinol Metab*. 2012; 23:194–204. [PubMed: 22261190]
- Sekar S, et al. Alzheimer’s disease is associated with altered expression of genes involved in immune response and mitochondrial processes in astrocytes. *Neurobiol Aging*. 2015; 36:583–91. [PubMed: 25448601]
- Skov V, et al. Whole-blood transcriptional profiling of interferon-inducible genes identifies highly upregulated IFI27 in primary myelofibrosis. *Eur J Haematol*. 2011; 87:54–60. [PubMed: 21447007]
- Spivia W, et al. Complement protein C1q promotes macrophage anti-inflammatory M2-like polarization during the clearance of atherogenic lipoproteins. *Inflamm Res*. 2014; 63:885–93. [PubMed: 25091012]
- Tai LM, et al. Amyloid-beta Pathology and APOE Genotype Modulate Retinoid X Receptor Agonist Activity in Vivo. *J Biol Chem*. 2014; 289:30538–55. [PubMed: 25217640]
- Tantawy MA, et al. The interferon-induced gene Ifi2712a is active in lung macrophages and lymphocytes after influenza A infection but deletion of Ifi2712a in mice does not increase susceptibility to infection. *PLoS One*. 2014; 9:e106392. [PubMed: 25184786]
- Terwel D, et al. Critical role of astroglial apolipoprotein E and liver X receptor-alpha expression for microglial Abeta phagocytosis. *J Neurosci*. 2011; 31:7049–59. [PubMed: 21562267]
- Tesseur I, et al. Comment on “ApoE-directed therapeutics rapidly clear beta-amyloid and reverse deficits in AD mouse models”. *Science*. 2013; 340:924–e. [PubMed: 23704554]
- Veeraraghavalu K, et al. Comment on “ApoE-directed therapeutics rapidly clear beta-amyloid and reverse deficits in AD mouse models”. *Science*. 2013; 340:924–f. [PubMed: 23704555]
- Wang M, et al. Macrogliia-microglia interactions via TSPO signaling regulates microglial activation in the mouse retina. *J Neurosci*. 2014; 34:3793–806. [PubMed: 24599476]
- Wang X, et al. Effects of n-3 FA supplementation on the release of proresolving lipid mediators by blood mononuclear cells: the OmegaAD study. *J Lipid Res*. 2015a; 56:674–81. [PubMed: 25616438]
- Wang Y, et al. TREM2 Lipid Sensing Sustains the Microglial Response in an Alzheimer’s Disease Model. *Cell*. 2015b
- Zabel MK, Kirsch WM. From development to dysfunction: microglia and the complement cascade in CNS homeostasis. *Ageing Res Rev*. 2013; 12:749–56. [PubMed: 23419464]
- Zeldich E, et al. The neuroprotective effect of Klotho is mediated via regulation of members of the redox system. *J Biol Chem*. 2014; 289:24700–15. [PubMed: 25037225]
- Zhang B, et al. Integrated systems approach identifies genetic nodes and networks in late-onset Alzheimer’s disease. *Cell*. 2013; 153:707–20. [PubMed: 23622250]
- Zhou Y, et al. APOE Genotype Alters Immunoglobulin Subtypes in Knock-In Mice. *J Alzheimers Dis*. 2015

Zotova E, et al. Inflammatory components in human Alzheimer's disease and after active amyloid-beta42 immunization. *Brain*. 2013; 136:2677–96. [PubMed: 23943781]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Highlights

- We treated APP/PS1 mice with Bexarotene – a specific RXR agonist in clinical use;
- The response to Bexarotene treatment involves genes relevant to inflammation;
- Up-regulation of Term2, Tyrobp, C1q complex and Apoe could explain the effect;
- Ligand activation of RXR may have a therapeutic effect in early stages of AD.

Figure 1.

mRNA-seq data demonstrate genome-wide effects of bexarotene activated RXR. Six month old APP/PS1 mice were treated with 100 mg/kg/day bexarotene for 10 days. RNA was isolated from cortices and mRNA-seq libraries were sequenced as described in Methods. N=4 per group. The panels show differentially expressed genes and associated annotation terms. A. The heat map was generated using Log2 fold change of total reads per sample/mouse, with genes down-regulated by bexarotene (14 genes) on the bottom, and genes up-regulated following bexarotene treatment (143 genes) on the top. Each row represents a single gene and each column is a single mouse. B. Example sequence coverage of individual genes up-regulated in mice treated with bexarotene in comparison to vehicle treatment, as visualized in IGV. C. Statistically significant functional annotation (GOTERM "Biological process" BP, at FDR < 0.02%), and D. KEGG pathways at FDR < 0.001, as identified using DAVID for all 157 genes. Among the significantly changed BP terms were: "immune response" ($P = 6.95 \times 10^{-6}$); "inflammatory response" ($P = 2.9 \times 10^{-4}$) and "immunoglobulin mediated immune response" ($P = 5 \times 10^{-6}$); "Alzheimer disease" ($P = 9.7 \times 10^{-9}$) and "Parkinson's disease" ($P = 2.7 \times 10^{-9}$) were significantly affected, as well.

Figure 2.

Validation of sequencing results in mice and *in vitro*. A. Quantile normalized Log2 values of select differentially expressed genes (at $p < 0.003$) in bexarotene treated mice identified by DAVID in GO category immune response (GO:0006955), and other select genes known as associated with immune response and inflammation. Feature summarization and statistical analysis for differential gene expression was performed using Bioconductor packages Rsubread, Edge-R and Limma. Statistical difference between vehicle and bexarotene for each gene (Edge p value) is shown on the right of the graph. B. mRNA of *Trem2*, *Tyrbp*, *ApoE* and *Ttr* are significantly increased in APP/PS1 mice treated with bexarotene. C. Trem2 and Tyrbp mRNA is not affected in non-APP mice expressing mouse *ApoE* (WT) or expressing human *APOE4* mice (APOE4). For B and C, Results of RT-QPCR with mRNA isolated from cortices of 6–7 months old APP/PS1 and age matched WT and APOE4 mice treated with bexarotene for 10 days. For APP/PS1, N=6–8 mice/group; WT, N=4 mice/group and APOE4, N=8–10 mice/group. D. Cultured mouse embryonic stem cells were

treated with 5 μ M bexarotene for 4 and 24 hours and mRNA expression level of Trem2 and Tyrobp examined by QPCR. Abca1 expression was used as a positive control for RXR target engagement. E. Bexarotene treatment increased binding of RXR to Trem2. Wild type mice were treated with 100 mg/kg bexarotene for 24 hours and ChIP-QPCR performed on lysates from cortices of those mice. The data are presented as % input and normalized to the negative control, mouse Glg. N=3. For B, C, D and E the statistical analysis is by *t*-test. ***, $p < 0.001$ and **, $p < 0.01$, *, $p < 0.05$.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 3.

Bexarotene increases A β phagocytosis. BV2 cells were pretreated with 50 nM, 100 nM bexarotene or vehicle for 24 hours prior to addition of A β to determine % uptake or % phagocytotic cells. A and B, Bexarotene increases the uptake of soluble A β . Representative images of cells treated with vehicle (a) and pre-treated with 50 nM (b) or 100 nM bexarotene (c) prior to addition of 1 μ M soluble Cy3-labeled A β (red). Nuclear DAPI stain was used to label the nuclei. The arrows are pointing to Cy3-A β . B: Quantification of % uptake. Data are from 3 experiments in triplicate and analyzed by one-way ANOVA, followed by Tukey's post test. C. Bexarotene does not significantly affect cell viability (data from 3 experiments in triplicate). D and E, Bexarotene increases the phagocytosis of fibrillar A β . Hi-lite-labeled A β (green) was aggregated for 24 hours at 37°C and applied to BV2 cells at 1 μ M followed by incubation with CD11b antibody (purple). D, Representative images of cells treated with vehicle (a) or pre-treated with 100 nM (b) prior to addition of 1 μ M Hi-lite-labeled fibrillar A β . The arrows are pointing to example cells with phagocytosed A β . E: Quantification of % phagocytosis. Data were analyzed by *t*-test.

Table 1
Immune/inflammation-related genes

List of differentially affected in response to bexarotene treatment genes involved in immune response and relevant to inflammation.

Gene symbol	Gene name	Fold change (Bexa vs vehicle)	p-value	Reference
Ttr	Transthyretin	22	2.65808E-11	(Wang et al., 2015a)
C1qb	complement component 1, q subcomponent, beta	1.71	9.89336E-07	(Mukundan et al., 2009)
C1qa	complement component 1, q subcomponent, alpha	1.64	1.04728E-05	(Bohlson et al., 2014)
Apoe	apolipoprotein E	1.6	1.14993E-05	(Zhou et al., 2015)
Apod	apolipoprotein D	1.69	7.01748E-05	(Dassati et al., 2014)
Dbi	diazepam binding inhibitor	1.6	7.5548E-05	(Wang et al., 2014)
Ifitm3	interferon-induced transmembrane protein 3	2	8.0643E-05	(Bailey et al., 2014)
Ifit3	interferon -induced protein with tetratricopeptide repeats 3	2.3	8.76717E-05	(Brass et al., 2009)
Ifi44	interferon-induced protein 44 complement component 1, q subcomponent, C	4.8	0.00013348	(Jordanovski et al., 2013)
C1qc	chain	3.4	0.000136195	(Chen et al., 2011)
Ifi44l	interferon-induced protein 44 like	10	0.00015743	(Feenstra et al., 2014)
Kl	Klotho	3	0.000195299	(Zeldich et al., 2014)
Fcrls	Fc receptor-like S, scavenger receptor	1.55	0.000201735	(Ramsland et al., 2011)
Irf7	interferon regulatory factor 7	2.45	0.000318895	(Khorrooshi and Owens, 2010)
Cyba	cytochrome b-245, alpha polypeptide	1.82	0.0003226	(Corsetti et al., 2013)
Irgm1	immunity-related GTPase family M member	1.75	0.000368579	(Liu et al., 2013)
Ifitm6	interferon induced transmembrane protein 6	5.7	0.000390398	(Li et al., 2015)
Ifi27	interferon, alpha-inducible protein 27-like	1.53	0.000397104	(Skov et al., 2011)
Ifi16, p204	interferon activated gene 204	3.43	0.000447954	(Choubey et al., 2010)
Cd68	CD68 antigen	1.65	0.000531375	(Zotova et al., 2013)
Fcgr3	Fc receptor, IgG, low affinity III	1.55	0.000564984	(Dominguez-Soto et al., 2014)
Il5ra	interleukin 5 receptor, alpha CD74 antigen	11	0.000643726	(Fukushima et al., 2012)
Cd74		2.1	0.000660881	(Sekar et al., 2015)
C1qtnf5	C1q and tumor necrosis factor related protein 5	1.65	0.00090232	(Schaffler and Buechler, 2012)
Clec10a/CD301a	C-type lectin MGL1/CD301a	4	0.000969014	(Saba et al., 2009)
Oasl2	2'-5' oligoadenylate synthetase-like 2	1.98	0.001011562	(McDermott et al., 2011)
Mif	macrophage migration inhibitory factor	1.4	0.001320706	(Flex et al., 2014)
Fcgr1	Fc receptor, IgG, high affinity I	1.92	0.001457242	(Poh et al., 2012)
Ifitm2	interferon induced transmembrane protein 2	1.92	0.001471911	(Hwang et al., 2013)
LY86	lymphocyte antigen 86	3.7	0.001679332	(Nguyen et al., 2014)
Tyrobp/Dap12	TYRO protein tyrosine kinase binding protein	1.55	0.001705932	(Ma et al., 2015)
Cxcl12	chemokine (C-X-C motif) ligand 12	3.3	0.001954175	(Saiman et al., 2014)
Trem2	triggering receptor expressed on myeloid cells 2	1.52	0.002330373	(Kleinberger et al., 2014)
Meox1	mesenchyme homeobox 1	4	0.002523221	(Nguyen et al., 2014)
Ifi27l2a	interferon, alpha-inducible protein 27 like 2A	2.4	0.002881009	(Tantawy et al., 2014)

Gene symbol	Gene name	Fold change (Bexa vs vehicle)	p-value	Reference
Eomes	eomesodermin homolog	0.16	0.00141725	(Khondoker et al., 2015)

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript