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Title Page

GPCR expression in native cells: "Novel" endoGPCRs as physiologic regulators and therapeutic targets.

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Running Title Page

Running Title: Assessing GPCR expression in native cells.

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Abstract

G protein-coupled receptors (GPCRs), the largest family of signaling receptors in the human genome, are also the largest class of targets of approved drugs. Are the optimal GPCRs (in terms of efficacy and safety) currently targeted therapeutically? Especially given the large number (~120) of orphan GPCRs (which lack known physiologic agonists), it is likely that previously unrecognized GPCRs, especially orphan receptors, regulate cell function and can be therapeutic targets. Knowledge is limited regarding the diversity and identity of GPCRs that are activated by endogenous ligands (endoGPCRs) and that native cells express. Here, we review approaches to define GPCR expression in tissues and cells and results from studies using these approaches. We identify problems with the available data and suggest future ways to identify and validate the physiologic and therapeutic roles of previously unrecognized GPCRs. We propose that a particularly useful approach to identify functionally important GPCRs with therapeutic potential will be to focus on receptors that show selective increases in expression in diseased cells from patients and experimental animals.

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Introduction

G protein-coupled receptors (GPCRs, also termed 7-transmembrane, 7-TM or heptahelical receptors) have been of major interest for investigators in many disciplines, including molecular pharmacology. Early studies on GPCRs assessed the action in cells and plasma membrane preparations of neurotransmitters, hormones and pharmacological agents in terms of their ability to regulate the generation of second messengers (e.g., cAMP, Ca^{2+}) and in turn, cellular events via enzymes (e.g., protein kinases) and ion channels. Results obtained by the Human Genome Project and for the genomes of other eukaryotes have revealed that GPCRs are the largest family of signaling receptors in humans and other species (Fredriksson et al., 2003; Vassilatis et al., 2003; Insel et al., 2012; Foster et al., 2014b). The receptors include ones that interact with endogenous ligands (endoGPCRs), GPCRs regulated by exogenous factors, for example, photons of light, odorants, and tastants (chemosensory receptors) and GPCRs that lack known physiological ligands (termed orphan receptors). It is estimated that among the approximately 800 GPCRs in humans, ~380 are endoGPCRs, of which about a third are orphan receptors even though there have been substantial efforts at deorphanization (Fredriksson et al., 2003; Kroeze et al., 2003; Ozawa et al., 2010; Amisten et al., 2013; Civelli et al., 2013).

In parallel with the work that has involved the cloning, genomic characterization, heterologous expression, and studies of GPCR actions and regulation, other efforts have emphasized the utility of GPCRs as therapeutic targets. Indeed, GPCRs are the largest class (~30%) of the targets of approved drugs (Overington et al., 2006; Lundstrom, 2009; Rask-Andersen et al., 2014). Reasons for the utility of GPCRs as therapeutic targets include the many different types of chemical entities with which they interact, the accessibility of GPCRs on the plasma membrane from the extracellular milieu, their ability to initiate signaling pathways that undergo

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amplification in target cells, and the selectivity in their expression by different types of cells. This latter property aids in facilitating tissue- and cell-selective actions of GPCR-targeted drugs.

In spite of the widespread use of GPCRs as therapeutic targets, one can ask: *Are the optimal GPCRs (in terms of efficacy and safety) targeted by current therapeutic approaches?* This question derives in part from the current therapeutic targeting of only a fraction of the endoGPCRs. Moreover, orphan GPCRs have largely been ignored as therapeutic targets. It is thus necessary to identify the repertoire of GPCRs, in particular endoGPCRs, expressed by individual tissues and more importantly, native cells. Studies to assess this gap in knowledge test the hypothesis that certain GPCRs are enriched in native cells, regulate cellular (and tissue) function and can be targeted therapeutically. In this article, we review the approaches and data that have begun to provide data to test this hypothesis. In addition, we discuss problems and limitations of available data and future directions that may help definitively answer the question posed above.

Methods and approaches to assess GPCR expression

Analyses of functional responses, second messengers or other signaling events represent hypothesis-testing approaches by asking if a particular receptor is biologically active and provide indirect ways to assess GPCR expression by tissues and cells. Radioligand binding assays facilitate the direct identification and quantification of GPCRs. However, functional, signaling and radioligand binding assays are biased approaches: one chooses a GPCR of interest and then uses agonists, antagonists and radioligands for the receptor being assessed. Thus, one can only study receptors for which appropriate reagents are available.

By contrast, hypothesis-generating approaches are not based on prior knowledge of a GPCR being present but instead rely on unbiased analyses of the expression of receptor mRNA or

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protein. Such approaches can define the GPCR expression profile/repertoire and can quantify receptor expression. Table 1 lists several approaches used to assess GPCR expression.

Numerous studies have used DNA microarrays ("DNA chips") to define the transcriptomes of cells and tissues. Such microarrays contain probes (specific DNA sequences) that hybridize with the genes of humans, mice or other species. Hybridization of the probes to target genes is quantified by chemiluminescence, fluorescence or other method, facilitating quantitation of the abundance of individual mRNAs/cDNAs. Commercially available microarrays that assess most or all genes in a transcriptome are not optimized to detect GPCRs but such arrays have been used to characterize GPCR expression.

Proprietary and commercial GPCR microarrays, to be discussed below, and real-time PCR analyses for individual GPCRs offer an alternative approach to identify chemosensory and non-chemosensory GPCRs. For example, Regard et al used TaqMan quantitative real-time PCR to quantify the transcripts of 353 non-odorant GPCRs in 41 adult mouse tissues and predicted previously unanticipated roles for less well-studied receptors—an idea consistent with our hypothesis that previously unrecognized GPCRs are enriched in native cells, contribute to physiology and are potential therapeutic targets (Regard et al., 2008).

With improvement in the ability to perform sequencing and a rapid decrease in its cost, new techniques such as high resolution RNA sequencing (RNA-seq) have begun to be used to identify and quantify expression of GPCRs and other members of the transcriptome. Such studies have recently included the profiling of GPCRs expressed in single cells (Manteniotis et al., 2013; Spaethling et al., 2014).

An alternative to assessing the expression of GPCR mRNA is the use of unbiased proteomic approaches, such as mass spectrometry. Although such technology has not as-yet been used

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to define and quantify overall GPCR expression in cells and tissues, initial results suggest this may be a feasible approach (Eisen et al., 2013; Feve et al., 2014).

Microarrays for the detection of GPCRs

Commercial microarrays have been created that are optimized to detect and quantify the mRNA of ~350 non-chemosensory GPCRs of mice, rats and humans. We and others have found these arrays to be quite useful to assess GPCR expression. Figure 1 shows a comparison of the detection of GPCRs by such a targeted array (a Taqman GeneSignature array from Life Technologies) with results obtained using an Affymetrix Mouse Genome 430A microarray (which detects ~14,000 mouse genes). We found that highly expressed GPCRs on the latter microarray show a positive correlation (and an R^2 of 0.37) with the GPCR array data. However, assessment of GPCRs expressed at lower levels reveals that the Affymetrix array has many false-positive and false-negative results (and R^2 = .01). GPCR arrays thus seem to be more useful to detect and quantify GPCRs than are non-targeted arrays. Data from others support this conclusion (Maurel et al., 2011).

What do available data reveal about GPCR expression in tissues and native cells?

GPCR expression has thus far been determined in a number of tissues (Table 2) and a smaller number of individual cell types (Table 3). Our laboratory has assessed the expression of non-chemosensory GPCRs by numerous individual cell types, including lymphoid cells, dendritic cells, neutrophils, vascular smooth muscle cells, several types of fibroblasts, adipocytes, renal epithelial cells, trigeminal neurons and several types of cancer cells.

The notion that GPCRs and GPCR signaling pathways are altered (and are potential therapeutic targets) in cancer in addition to various endocrine tumors (for which GPCR-targeted drugs are commonly used) has been largely ignored by investigators in oncology. Even so, several recent reviews have emphasized the importance of GPCRs and GPCR signaling in cancer (Lappano

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and Maggiolini, 2011; Feigin, 2013; O'Hayre et al., 2013; O'Hayre et al., 2014). O'Hayre and colleagues noted that nearly 20% of human tumors have GPCR mutations, 4% of tumors have activating mutations of the Gαs gene and activating mutations of Gαq family members occur in melanomas (O'Hayre et al., 2013). GPCRs may contribute to cancer not only by effects on the growth, death, metabolism and function of malignant cells but also by actions on cells of the tumor microenvironment, including vascular cells, immune cells and cancer-associated fibroblasts. Although not widely explored, such actions may have functional importance for the malignant phenotype (Hanahan and Weinberg, 2011) and thus have therapeutic potential.

Several general conclusions regarding GPCR expression in tissues and cells can be drawn from published findings and our unpublished results:

1) Most tissues and individual cell types express at least 100 different GPCRs, including GPCRs that link to each of the major classes of heterotrimeric G proteins (G_s, $G_{i/o}$, $G_{q/11}$, and $G_{12/13}$).

2) Among the GPCRs with the highest expression are ones not previously known to be expressed in those tissues and cells and thus, not the subject of prior studies.

3) Many of the highest expressed GPCRs are orphan receptors.

4) Cells from animals or patients with particular diseases show prominent changes (increases and decreases) in expression of numerous GPCRs, thus revealing disease-specific changes in GPCR expression.

5) Additional methods (quantitative real-time PCR, antibody-based and functional [including signal transduction and cellular response] assays) confirm data obtained with GPCR arrays, including for example, evidence that the expression of several G_s-coupled GPCRs correlates with their ability to maximally stimulate cellular cAMP production.

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Such findings thus indicate that analysis of GPCR expression of tissues, and especially of individual cell types, appears to be a highly useful means to identify previously unrecognized GPCRs that may be functional, contribute to pathophysiology and serve as novel drug targets.

What are some of the problems and limitations of efforts to define GPCR expression in tissues and cells?

In spite of the potential importance of the findings related to the discovery of "new GPCRs", i.e., endoGPCRs not previously known to be expressed, in individual tissues and cell types, a number of issues must be considered in studies of GPCR expression:

1) *The source of material analyzed for GPCR expression:* The results in Table 2 were primarily derived from studies of whole organs, tissues and tissue biopsies, all of which are heterogeneous cellular preparations. The contribution of the different cell types in such preparations is thus not clear. Studies of individual cell types (Table 3) obviate this concern. Even in such studies, though, one analyzes a population of cells. The use of techniques such as RNA-seq (Manteniotis et al., 2013; Spaethling et al., 2014) and perhaps proteomic methods (Davies et al., 2007; Wu et al., 2012; Eisen et al., 2013) to assess GPCRs in individual cells will thus be an important future direction for defining the profile and variability in GPCR expression in particular cell types.

2) *Reproducibility and consistency of methods used to prepare mRNA, cDNA or protein and to assess gene and protein expression:* Standards have been developed to facilitate reproducibility in microarray (Brazma et al., 2001; Hansen et al., 2007; Rung and Brazma, 2013) and proteomic studies (Davies et al., 2007; Taylor et al., 2007) but results from different laboratories can differ. Such differences are generally attributed to variations in the methods used for the preparation and processing of samples. Primer design may not be ideal for the detection of all relevant GPCRs by arrays. For example,

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Taqman-based GPCR arrays may not detect certain, including functionally relevant, GPCRs. The use of different approaches for data analysis and statistics by different laboratories can contribute to discordant results (Pan, 2002; Motulsky, 2014). The dearth of well-validated antibodies to detect native GPCRs, including for use in proteomic studies, is an important problem in GPCR research (Hutchings et al., 2010; Talmont et al., 2012; Eisen et al., 2013; Marchalant et al., 2014). Use of antibodies to study GPCRs is challenging because the receptors are typically expressed in cells at lower levels than many other cellular proteins. Criteria, including studies with cells or animals engineered to have a knockout of a particular GPCR, have been proposed to help validate GPCR-targeted antibodies (Michel et al., 2009). Moreover, since protein levels in cells are not necessarily predicted by mRNA abundance, differences in protein expression could derive from factors that include altered protein translation and/or degradation (de Sousa et al., 2009; Maier et al., 2009). Especially given the difficulties in obtaining well-validated GPCR antibodies, a possible solution is to measure levels of actively translated mRNA using polysome profile analysis together with DNA high density arrays or GPCR-specific qPCR (Masek et al., 2011; Gandin et al., 2014).

3) *Normalization of results for GPCR expression:* Most assays used to determine mRNA and protein expression rely on normalization to “housekeeping” genes/proteins. An extensive literature has discussed problems related to normalization in such studies. One such problem is a change in expression of the gene/protein used for normalization--a particular concern in studies of development, differentiation or disease (Khimani et al., 2005; Brattelid et al., 2007). Two issues in the use of arrays to assess GPCR expression are how to define the limit of detection of a receptor in terms of ΔCycle-threshold (ΔC(t)) relative to the reference used for normalization and the most appropriate statistical tests for data analysis (Khimani et al., 2005; Rubie et al., 2005).

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4) *Approaches used to classify GPCRs in profiles of their expression in tissues and cells:* One approach is to cluster results for GPCR expression is on the basis of the coupling of receptors to heterotrimeric G-proteins. Such information is available in articles and databases, such as The International Union of Basic and Clinical Pharmacology/British Pharmacological Society (IUPHAR/BPS) Guide to Pharmacology (Pawson et al., 2014). IUPHAR/BPS systematically annotates each GPCR and provides reviews from expert subcommittees for each of the target families in the database. Results that supersede such data are published on an ongoing basis, therefore one must be vigilant in updating conclusions regarding the coupling of GPCRs to G-proteins. Problems associated with classifying results on this basis include the evidence that some GPCRs couple to multiple G proteins, the limited data regarding the G-protein linkage of orphan GPCRs, and the ability of GPCRs to act via β-arrestin instead of (or in addition to) G-proteins. Other ways to classify GPCRs include the GRAF system, evolutionary relationships, ligand interactions, structural data and susceptibility to post-translational modifications (Fredriksson et al., 2003; Davies et al., 2007; Secker et al., 2010; Lin et al., 2013; Venkatakrishnan et al., 2013).

5) *Which types of GPCRs should be studied?* As shown by the studies in Tables 2 and 3, most efforts have focused on the use of array- or PCR-based methods and have emphasized the expression of non-chemosensory GPCRs. Recent studies, however, have identified chemosensory receptor expression in tissues not typically thought to be in involved in sensation (Reimann et al., 2012; Pronin et al., 2014; Rajkumar et al., 2014; Foster et al., 2014a; Foster et al., 2014b; Foster et al., 2014c; Malki et al., 2015). With the discovery of small molecule metabolites (in some cases, products of microbiota [e.g., (Natarajan and Pluznick, 2014)]) that interact with GPCRs and that may have been considered odorants or tastants, the distinction between chemosensory and non-

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chemosensory GPCRs is blurring. Thus, future studies may need to incorporate analyses of both types of GPCRs.

6) *Prioritization of results regarding GPCR expression so as to guide subsequent studies that validate the expression and evaluate the functional role of individual receptors:* The discovery that native cells typically express >100 GPCRs creates an embarrassment of riches but also, a challenge in terms of choosing individual GPCRs for subsequent studies. A key goal is to identify GPCRs that are important for cellular function and that may be therapeutic targets. The use of RNA interference and gene editing approaches (i.e., CRISPR/Cas9) to knockdown receptor expression in cells of interest and then to assess the impact of receptor knockdown on functional activity (signal transduction or cellular responses) provides a way to survey a population of GPCRs and identity physiologically (and potentially pharmacologically) important GPCRs (Willets and Nash, 2013). Additional features to help such prioritization include: a) the level of expression (more highly expressed GPCRs are predicted to contribute to a greater extent to cell function and may be easier to study); b) knowledge of other cell types that express receptors of potential interest (so as to achieve greater selectivity in the site of action, including by comparing expression in cells that are related to one another, such as, for example, in different types of macrophages (Lattin et al., 2008; Groot-Kormelink et al., 2012; Hohenhaus et al., 2013); c) choosing GPCRs whose expression is shared in a cell type found in humans and experimental animals (e.g., mice, rats); and d) availability of reagents (e.g., agonists, antagonists, antibodies) to conduct signaling and functional studies. A useful feature, especially for studies of disease settings, is to focus efforts on GPCRs with prominent differences in expression between the normal cells and cells from diseased animals or patients. Differential GPCR expression can also be helpful in choosing GPCRs for studies of development and differentiation of tissues and cells.

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7) *Determining the function of highly expressed orphan GPCRs. S*ome of the most exciting (and unexpected) data that we have obtained from our studies of GPCR expression in native cells is the high expression of a variety of orphan GPCRs. This is perhaps not surprising since orphan receptors represent about a third of the endoGPCRs and limited reagents have been available to assess function mediated by most orphan GPCRs. Since the endogenous ligands for such receptors are unknown, investigating their physiological roles can be difficult (Ahmad et al., 2014). Molecular approaches, such as siRNA or overexpression in heterologous cells can help define signaling mechanisms, especially if a GPCR is constitutively active. Antibodies to orphan GPCRs might be used to block signaling and obviate the need for a ligand, although, as noted above, validation of GPCR antibodies can be difficult. Another challenge with orphan GPCRs has been the difficulty in setting up screens for ligands as the G-protein coupling of an orphan may not be known; chimeric G proteins may be a way to develop high-throughput screens (Yin et al., 2004).

Summary and conclusions

Even though they have been widely studied and highly useful as therapeutic targets, GPCRs continue to be very important molecular entities. The recognition from genomic studies that there are many more GPCRs than were previously known or characterized and that many of these are orphan receptors, provides opportunities to discovery physiological and therapeutic roles for newly recognized GPCRs. Studies of GPCR expression in tissues, but especially in native cells, can reveal that previously unrecognized GPCRs contribute to cell function in health and disease. Numerous challenges exist in studies of GPCR expression but if “new” GPCRs can be validated and shown to be functionally active, we anticipate that such GPCRs may prove to be as important—and perhaps even more so—than the GPCRs that have been the focus of efforts in physiology and pharmacology and that have so valuable as therapeutic targets.

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Authorship Contributions

Wrote or contributed to writing of the manuscript: Insel, Wilderman, Zambon, Snead, Murray, Aroonsakool, McDonald, Zhou, McCann, Zhang, Sriram, Chinn, Michkov, Lynch, Overland, Corriden

Conducted experiments: Wilderman, Zambon

Performed data analysis: Wilderman, Zambon

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Footnotes

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Legends for Figures

Figure 1: Comparison of GPCR expression data obtained using a GPCR-specific array and a generic cDNA array. The data were collected in two experiments with data obtained using cDNA prepared from murine wild-type S49 lymphoma cells and that could be compared for GPCRs present on an Affymetrix Mouse Genome 430A microarray and a Taqman GeneSignature array (Life Technologies). The 32 GPCRs highly expressed (>$\log2^6$) on the Affymetrix array) show a positive correlation (R^2= 0.37) between the two arrays. GPCRs expressed at lower levels (<$\log2^6$) show numerous false positive and false negative results in the detection of GPCRs by the Affymetrix array compared to the GPCR array (R^2= 0.01). GCRMA = Guanine cytosine robust multi-array analysis. ΔCT = cycle threshold relative to the mRNA used to normalize expression of each GPCR.

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Tables

Table 1: Unbiased methods to assess expression of GPCRs

DNA Microarrays that assess entire transcriptomes
Real-time PCR analysis with primers for each GPCR
Targeted DNA microarrays that assess expression of non-chemosensory GPCRs
High resolution RNA sequencing (RNA-seq)
Proteomic approaches

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Table 2: GPCR expression in tissues

Tissue	Species	Methods & Results	Reference
Various: neurons, pancreas, liver etc.	Human, Mouse	Proprietary arrays; assessed known, orphan and odorant receptors in human tissues and blood cells	(Hakak et al., 2003)
Various: neurons, kidney, liver, etc.	Human, Mouse	RT-PCR; assessed 100 endoGPCRs (as no,low, moderate or strong expression) in 17 tissues and 9 brain regions	(Vassilatis et al., 2003)
Lung, breast, prostate, melanoma, and gastric cancers; B cell lymphoma	Human	*In silico* analysis of GPCR expression in human tumors (8 microarray data sets of non-small cell lung cancer, breast cancer, prostate cancer, melanoma, gastric cancer and diffuse B cell lymphoma); found several GPCRs over-expressed in the cancers	(Li et al., 2005)
Various: eye, cardiovascular, pulmonary etc.	Mouse	Transcript analysis of 353 non-odorant GPCRs in 41 mouse tissues; predicted functional roles for previously unrecognized GPCRs	(Regard et al., 2008)
Squamous cell carcinoma and adenocarcinoma	Human	Laser capture microdissection and GPCR-focused DNA microarrays; assessed 929 GPCR transcripts in patient-derived squamous cell lung carcinoma or adenocarcinoma; found 5l GPCRs over-expressed plus many with decreased expression.	(Gugger et al., 2008)
Cardiac Chambers	Mouse	Real-time RT-PCR; evaluated non-chemosensory endoGPCRs in the 4 cardiac chambers; focused on 128 GPCRs and chamber-specific expression	(Moore-Morris et al., 2009)

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Adrenal gland	Human	cDNA chip with nucleotide sequences of 865 GPCRs to assess adrenals and adrenal cortical tumors; tumors had higher expression of several GPCRs	(Assie et al., 2010)
Melanoma metastases and nevi	Human	Quantitative PCR; assessed expression of 75 orphan and 19 chemokine GPCRs in melanoma metastases and benign nevi; found several orphan GPCRs with higher expression in the metastases	(Qin et al., 2011)
Urothelial tissue	Human	RT-PCR array; evaluated 40 GPCRs in human urothelium and urothelium-derived cell lines; cell lines had lower expression of most GPCRs	(Ochodnicky et al., 2012)
Pancreas, Small Intestine	Human	Taqman GPCR arrays; compared small bowel and pancreatic neuroendocrine tumors (SMNET, PNET) and normal tissue; found altered expression of 28 and 18 GPCRs in SMNET and PNET, respectively	(Carr et al., 2012)
Pancreatic islets	Human	Data from the IUPHAR GPCR database; GeneCards.org, ingenuity.com, PubMed.gov used to define a human GPCRome and then qPCR primers for these 384 GPCRs; found 293 GPCRs expressed predicted to activated by 271 ligands and identified 107 drugs predicted to stimulate and 184 drugs predicted to inhibit insulin secretion.	(Amisten et al., 2013)
Cerebellum	Human	GPCR RT-PCR arrays; 4 normal pediatric cerebellums and 41 medulloblastomas; numerous GPCRs had increased expression and clusters of tumors had particular patterns of GPCR expression	(Whittier et al., 2013)
Eye	Human, Mouse	RNA sequencing of cDNA of eyes and retinas of mice and a human donor eye; 165 GPCRs identified	(Chen et al., 2013)

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Adipose tissue	Human	Quantitative PCR of 384 GPCRs; Found 163 GPRs in subcutaneous adipose tissue, 119 drugs (acting on 23 GPCRs) that may stimulate lipolysis and 173 drugs (acting on 25 GPCRs), that may inhibit lipolysis	(Amisten et al., 2014)
Hypothalamus	Mouse	Taqman GPCR arrays; examined hypothalamic arcuate nucleus of female mice; identified 292 GPCRs (including 109 orphan GPCRs)	(Ronnekleiv et al., 2014)

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Table 3: GPCR expression in cells

Cell	Species	Methods & Results	Reference
Bone Marrow Stromal Cell lines	Human	GPCR RT-PCR array; showed relationship between number of GPCRs detected vs amount of total RNA in samples; detected mRNA for 199 GPCRs; highly expressed GPCRs detected with ~1ng total RNA.	(Hansen et al., 2007)
Macrophages (Bone marrow and peritoneal)	Mouse	DNA microarray data from NCBI (accession number GSE10246); assessed expression in bone marrow- and peritoneal-derived macrophages, microglia and a macrophage-like cells,RAW264; 67 GPCRs expressed constitutively or induced by lipopolysaccharide	(Lattin et al., 2008)
Embryonic Stem Cells (ESCs)	Mouse	GPCR RT-PCR arrays; examined ESCs cultured 4 or 20 days; ~200 GPCRs had low, high or moderate expression	(Layden et al., 2010)
BV2 **N18** **HEK 293, AtT20**	Rat Mouse Human	DNA high density arrays and GPCR-specific qPCR; assessed 20,000 genes of HEK293, AtT20 (pituitary), BV2 (microglial) and N18 (neuroblastoma) cells; found 73, 79, 108, and 105 non-chemosensory GPCRs, respectively, in those cells and related signaling proteins	(Atwood et al., 2011)
Cerebral Neurons	Mouse	Customized quantitative real-time RT-PCR; studied cerebellar granule neurons (CGN); identified 38 highly and 46 intermediately expressed GPCRs; expression of some GPCRs changed during CGN development	(Maurel et al., 2011)

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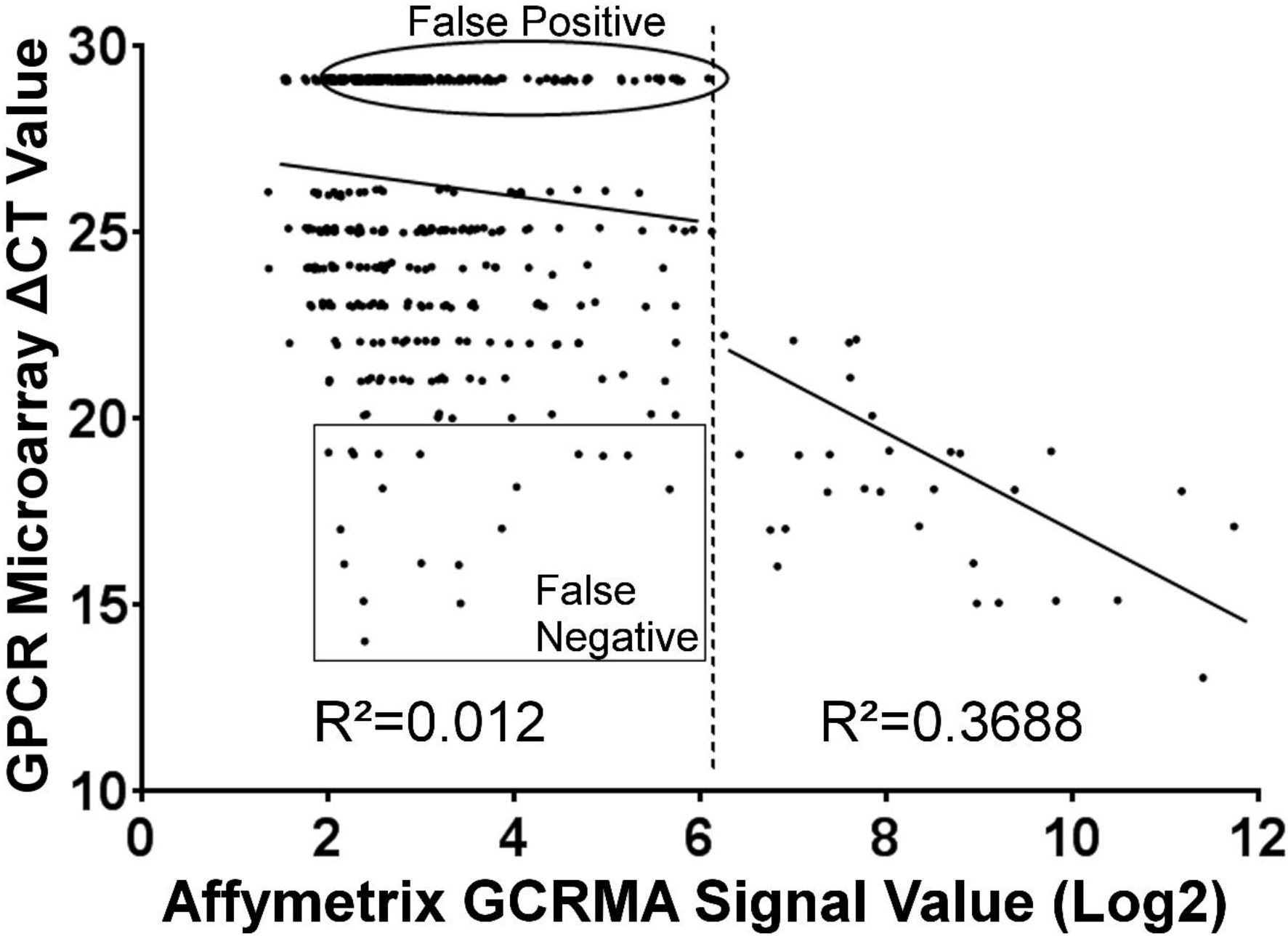
Cardiac Fibroblasts	Rat	GPCR RT-PCR arrays; assessed cardiac fibroblasts; identified 190 GPCRs; highest expressed, protease-activated receptor 1, was shown to be pro-fibrotic	(Snead and Insel, 2012)
Monocytes, Macrophages	Human	GPCR arrays; assessed alveolar macrophages (AM); phorbol ester (PMA)-treated THP-1, HL60 and, U937 cells; peripheral blood monocytes and monocyte-derived macrophages; 164 GPCRs identified; highest expressed in AM was complement 5a receptor (C5R1)	(Groot-Kormelink et al., 2012)
Lung fibroblasts	Human	GPCR RT-PCR array; defined GPCR expression differences in normal lung fibroblasts and a gefitinib-resistant non-small cell lung cancer cell line, H1975.	(Kuzumaki et al., 2012)
Monocytes, Macrophages	Human	GPCR RT-PCR arrays; evaluated GPCR expression changes during differentiation of monocytes to macrophages and by TLR4 activation, which altered expression of 101 GPCRs	(Hohenhaus et al., 2013)
Ghrelin cells	Mouse	GPCR arrays; found 90 GPCRs in gastric ghrelin cells; validated functional activity of several GPCRs.	(Engelstoft et al., 2013)
Glioblastoma Cancer stem-like cells, U-87 MG cells, astrocytes, Fetal Neural stem cells	Human	GPCR RT-PCR array; assessed glioblastoma cancer stem-like cells, glial tumor cells, a glioblastoma cell line (U-87 MG cells), astrocytes and fetal neural stem cells; 8 GPCRs were specific to glioblastoma cells; 17 GPCRs specific to cells with stem properties.	(Feve et al., 2014)

Figure 1


False Positive
GPCR Microarray ΔCT Value
30
25
20
15
10
False
Negative
R²=0.012
R²=0.3688
0
2
4
6
8
10
12
Affymetrix GCRMA Signal Value (Log2)