Endogenous GPCR Transcript Profiling of Life Technologies' Primary Cell Offerings: Opening the Door to Physiologically Relevant Cell Systems for Pharmaceutical Screens

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ABSTRACT

The association of ligands with cell surface G protein-coupled receptors (GPCRs) elicits a myriad of physiological responses through activation of intracellular signaling cascades. Mutations in GPCRs and associated G-proteins have been implicated in a number of diseased states. Given the localization of these receptors and their role in pathogenesis, it is not surprising that approximately fifty percent of all pharmaceuticals target this family of receptors.

Heterologous over-expression of GPCRs is commonly utilized to screen the efficacy of GPCR-targeted drug candidates. While these engineered cell lines have certain utility, several significant problems have been noted: receptors (1) are expressed at levels far-exceeding physiologically relevant concentrations, (2) can hetero-oligomerize and/or cross talk with non relevant endogenous receptors, and (3) many times are not appropriately targeted to the plasma membrane. In addition, intellectual property places restrictions on the use of certain GPCR expressing cell lines. Together, these caveats underscore the need for alternative cellular assay systems that overcome these limitations in the discovery and development of GPCR-targeted pharmaceutics.

Primary cells provide physiologically relevant systems for the assessment of complex signaling events, with appropriately targeted endogenous receptors and without the restrictions of IP. Yet, use of primary cells in GPCR drug development has been limited due to several factors that can include relatively low endogenous receptor expression and/or presence of multiple related receptor isoforms that may complicate analysis. Additionally, donor-to-donor variability and issues of scale are important considerations. To address these issues, we undertook GPCR transcript profiling of normal neonatal human epidermal keratinocytes (HEKn) using TaqMan® arrays and quantified GPCR transcripts levels across a range of donors and through multiple passages.

The GPCR TaqMan® array available from Life Technologies/Applied Biosystems targets 367 GPCR transcripts representing 50 subfamilies. Our results show remarkable consistency in transcript levels both across HEKns from 2 donors and during expansion throughout 6 passages (18 population doublings). Among the subfamilies present within our primary keratinocytes are LPA receptors, adrenoreceptors, retinoic acid receptors, and proteinase-activated receptors. Many of these endogenous receptors have been, and continue to be, targets of pharmaceutical development to treat tumor metastasis, pain, and neurological and inflammatory disorders. Of particular interest are orphan receptors that are expressed within our primary keratinocytes, including receptors which have been implicated in tumorogenesis (GPR56 and GPR110). While the endogenous ligands of these receptors have yet to be identified, they represent interesting targets for basic research and pharma development. Taken together our data highlight the value of using physiologically relevant primary human cell models in drug discovery and development.

MATERIALS AND METHODS

Total RNA was isolated from primary cells grown in varying basal medium formulations available from Life Technologies. Using cDNA libraries, created by reverse transcription, the GPCR transcript levels were determined *via* TaqMan® array analysis and it was determined whether or not GPCR transcript levels change as a function of donor-to-donor variation, upon increasing passage, between epithelial cell types grown in various basal media and supplements, and between epithelial and mesenchymal cell types.

RESULTS

Figure 1. Transcript Levels Within 4' Neonatal Keratinocytes Were Assessed using the TaqMan® Gene Signature Array for Human GPCRs

ADCYAP1R1	ADMR	ADORA1	ADORA2A	ADORA2B	ADORA3	ADRA1A	ADRA1B	ADRA1D	ADRA2A	18S	18S
BAI3	BDKRB1	BDKRB2	BLR1	BRS3	GPR137	C3AR1	C5R1	CALCR	CALCRL	18S	18S
CCR9	CCRL1	CCRL2	CD97	CELSR1	CELSR2	CELSR3	CHRM1	CHRM2	CHRM3	CHRM4	CHRM5
DRD2	DRD3	DRD4	DRD5	EBI2	SIPR1	LPAR1	SIPR3	LPAR2	S1PR2	S1PR4	LPAR3
GPR174	FKSG83	OR7E5P	FPR1	FPRL1	FPRL2	FSHR	FY	FZD1	FZD10	FZD2	FZD3
GHSR	GIPR	GLP1R	GLP2R	GNRHR	GNRHR2	GPBAR1	GPR176	GPR1	GPR10	GPR101	GPR103
GPR125	GPR126	GPR128	GPR133	GPR135	UTS2R	GPR141	GPR142	GPR143	GPR145	GPR146	GPR148
GPR22	GPR23	GPR24	GPR25	GPR26	GPR27	GPR3	GPER	GPR31	GPR32	TRBV5	GPR34
GPR51	GPR52	GPR54	GPR55	GPR56	TAAR2	GPR6	GPR61	GPR62	GPR63	GPR64	GPR65
GPR82	GPR83	GPR84	GPR85	P2RY13	GPR87	GPR88	SUCNR1	LPAR5	GPR97	GPRC5B	GPRC5C
HCRTR1	HCRTR2	HDAC3	HRH1	HRH2	HRH3	HRH4	HTR1A	HTR1B	HTR1D	HTR1E	HTR1F
LGR7	LHCGR	SLC26A7	LPHN1	LPHN2	LPHN3	LTB4R	LTB4R2	MAS1	MAS1L	MASS1	MATK
MTNR1B	NMBR	NMUR2	NPY1R	NPY2R	NPY5R	NPY6R	NTSR1	NTSR2	RARS	GCNT2	OPN1SW
OR7C2	GPR147	OXTR	P2RY1	P2RY10	P2RY11	P2RY12	P2RY2	P2RY4	P2RY5	P2RY6	P2RY8
PTHR2	GPRC5A	RGR	RHO	RRH	RLN3R1	SCTR	SMO	GPR173	SSTR1	SSTR2	SSTR3
GPR137B	TAAR8	TRHR	TSHR	VIPR1	VIPR2	VN1R1	VN1R2	VN1R5	XCR1	MRGPRE	GAPDH

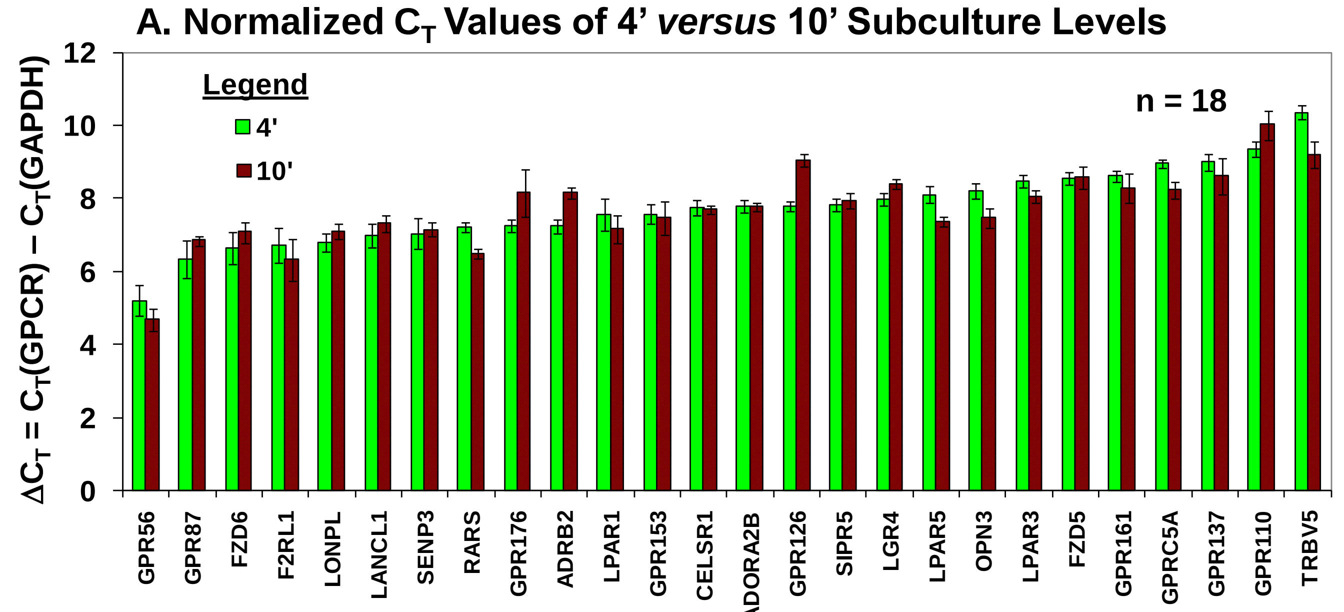
ADRA2B	ADRB1	ADRB2	ADRB3	AGTR1	AGTR2	AGTRL1	AVPR1A	AVPR1B	AVPR2	BAI1	BAI2
CASR	CCBP2	CCKAR	CCKBR	CCR1	CCR2	CCR3	CCR4	CCR5	CCR6	CCR7	CCR8
CMKLR1	CNR1	CNR2	CRHR1	CRHR2	CX3CR1	CXCR3	CXCR4	CXCR6	CYSLTR1	CYSLTR2	DRD1
S1PR5	EDNRA	EDNRB	ELTD1	EMR1	EMR2	EMR3	GPR37L1	F2R	F2RL1	F2RL2	F2RL3
FZD4	FZD5	FZD6	FZD7	FZD8	FZD9	GABBR1	GALR1	GALR2	GALR3	GCGR	GHRHR
P2RY14	GPR110	GPR111	GPR112	GPR113	GPR114	GPR115	GPR116	GPR12	GPR120	GPR123	GPR124
GPR15	GPR150	GPR152	GPR153	GPR160	GPR161	GPR17	GPR18	GPR19	CCR10	GPR20	GPR21
GPR35	GPR37	MLNR	GPR39	GPR4	GPR40	GPR43	GPR44	GPR45	LGR4	LGR5	GPR50
NMUR1	GPR68	GPR7	GPR73	GPR73L1	GPR74	GPR75	GPR77	GPR78	GPR8	OXGR1	GPR81
GPRC5D	GPRC6A	GRM1	GRM2	GRM3	GRM4	GRM5	GRM6	GRM7	GRM8	GRPR	GPR171
HTR2A	HTR2B	HTR2C	HTR4	HTR5A	HTR6	HTR7	IL8RA	IL8RB	LANCL1	LANCL2	LGR6
MC1R	MC2R	MC3R	MC4R	MC5R	MRGPRF	MRGPRD	MRGPRX1	MRGPRX2	MRGPRX3	MRGPRX4	MTNR1A
OPN3	OPN4	OPRD1	OPRK1	OPRL1	OPRM1	SENP3	OR2A4	OR2C3	PTPN22	MELL1	PHGDH
PCDH15	TAAR5	PPYR1	PTAFR	PTGDR	PTGER1	PTGER2	PTGER3	PTGER4	PTGFR	PTGIR	PTHR1
SSTR4	SSTR5	TAAR6	TACR1	TACR2	TACR3	TAAR1	TAAR9	TAS1R1	LONP2	TBXA2R	OXER1
RPLP0	ACTB	PPIA	PGK1	B2M	GUSB	HPRT1	TBP	TFRC	HMBS	IPO8	POLR2A

= Endogenous Controls   = GPCR $C_T \leq 32$ = GPCR $32 < C_T \leq 35$

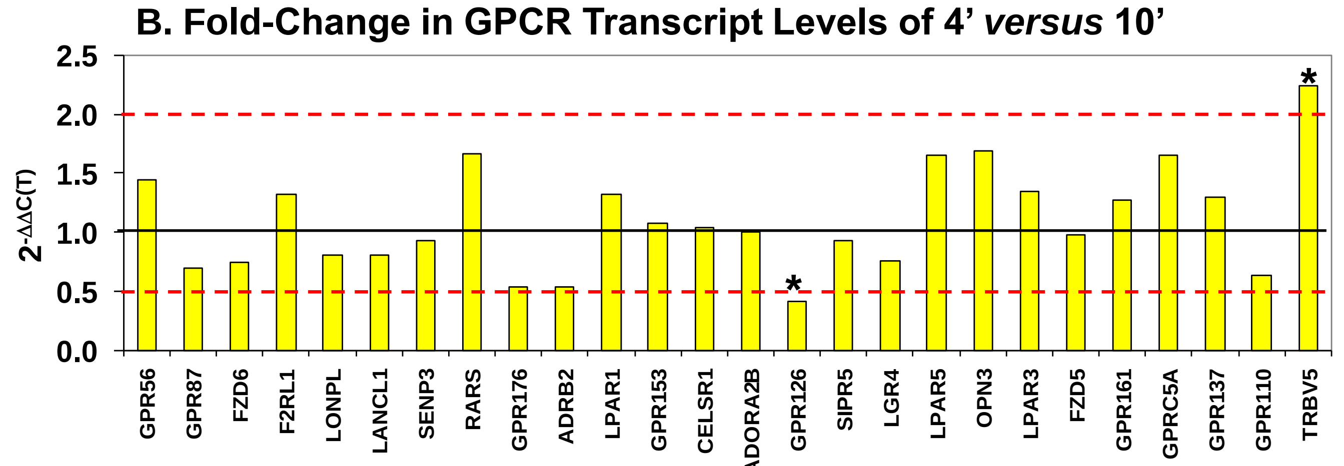
- GPCR transcript levels were assessed for 367 GPCRs from 50 subfamilies
- 30 GPCRs (~10% of profiled GPCRs) were shown to have cycle thresholds (C_T) ≤ 32
- Among the subfamilies represented were LPA receptors, adrenoreceptors, retinoic acid receptors, and proteinase-activated receptors
- Many orphan receptors were also identified including receptors that have been implicated in tumorigenesis (GPR56 and GPR110)

Figure 2. Keratinocytes Can Be Passaged Multiple Times with Few Significant Changes in GPCR Transcript Levels

A. Normalized C_T Values of 4' *versus* 10' Subculture Levels



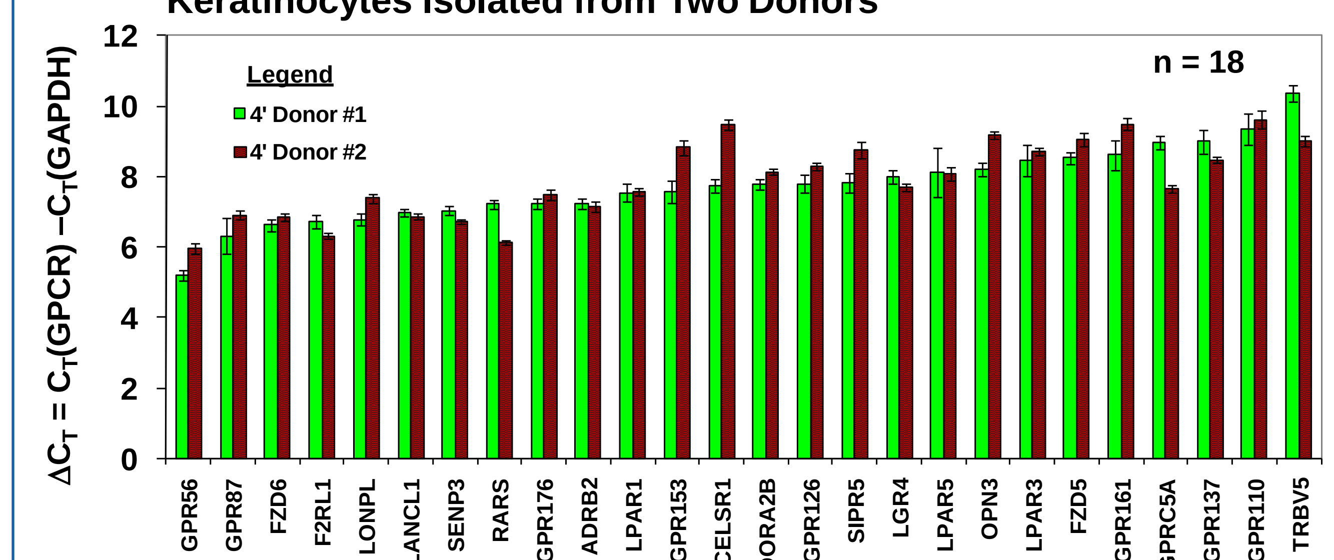
B. Fold-Change in GPCR Transcript Levels of 4' *versus* 10'



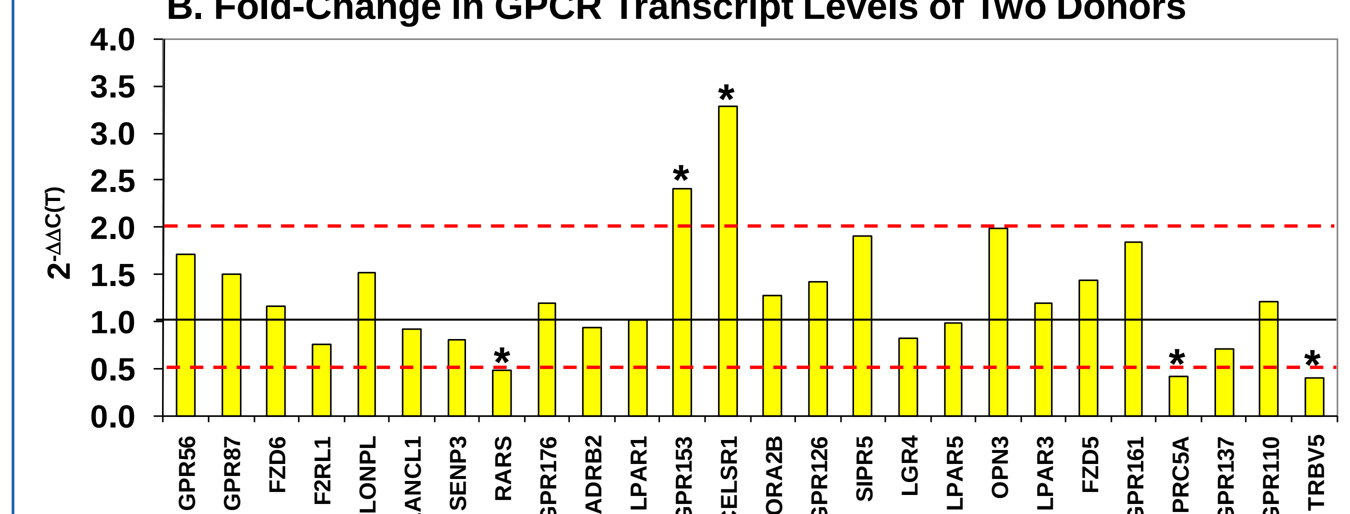
- Only 2 GPCRs were shown to have > 2-fold changes in transcript levels, allowing large expansion of primary banks of cells for use in primary HTS screens.

Figure 3. GPCR Transcript Levels Within Keratinocytes Vary Little from Donor to Donor

A. Normalized C_T Values of GPCR Transcript Levels within Keratinocytes Isolated from Two Donors

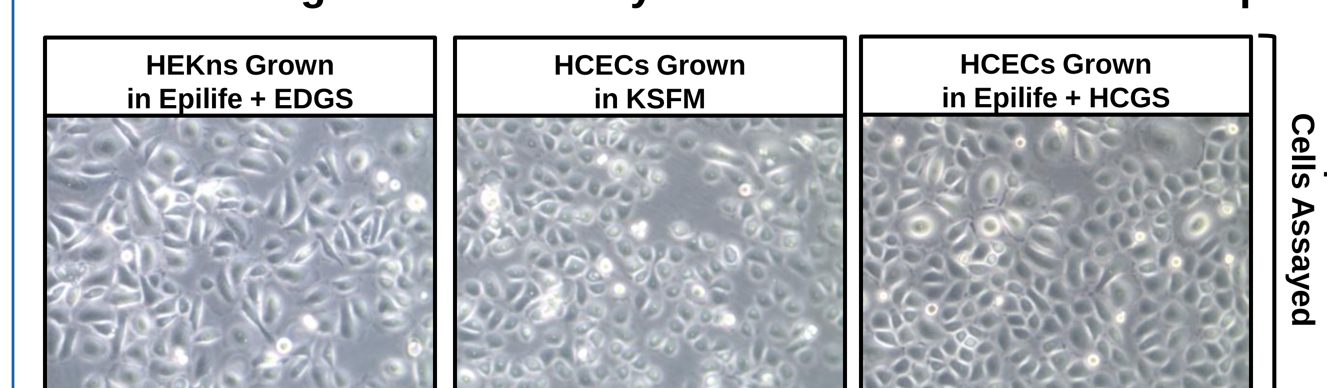


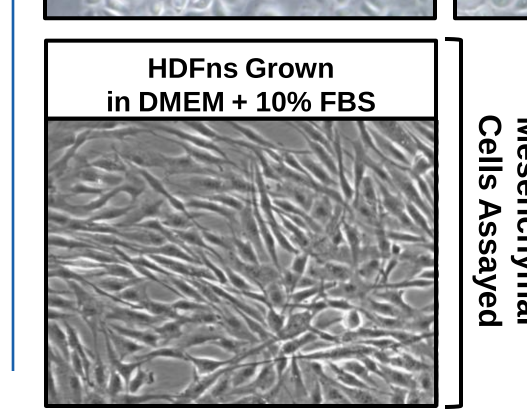
B. Fold-Change in GPCR Transcript Levels of Two Donors



- No additional GPCRs were identified in the subsequent screening of an additional lot of keratinocytes using the Taqman® Gene Signature Array for Human GPCRs.
- Only 5 GPCRs were shown to have > 2-fold changes in transcript levels.

Figure 4. Other Primary Cell Offerings Available From Life Technologies Preliminarily Screened for GPCR Transcripts





- Human Corneal Epithelial Cells (HCECs) were assayed to identify differences in GPCR transcripts between epithelial cell types.
- Neonatal Human Dermal Fibroblasts (HDFns) were assayed to identify differences in GPCR transcripts between epithelial & mesenchymal cells.

Figure 5. Preliminary GPCR Transcript Analysis of Epithelial (HEKn & HCEC) and Mesenchymal (HDFn) Cell Types Available From Life Technologies

	C_T			
GPCR	HEKn (Epilife + EDGS)	HCEC (Epilife + HCGS)	HCEC (KSFM)	HDFn (DMEM + 10% FBS)
ADORA2B	30.7	31.7	32.4	
ADRB2	30.2	31.3	32.2	33.8
BAI2				34.7
BDKRB1	32.5	34.6		32.4
GPR137	31.4	32.3	33.1	31.0
CALCRL	34.8			
CCRL1				28.9
CD97	32.6	32.6	32.5	30.8
CELSR1	30.6	32.8	32.7	
CELSR2	33.0			
CHRM2				33.4
DRD5				33.9
SIPR1	34.4			32.5
LPAR1	30.6	31.5	32.2	29.6
SIPR3	33.4			30.4
LPAR2	32.5			
S1PR2	32.9			31.7
LPAR3	30.9	32.5	32.6	
S1PR5	31.3	33.6		
EDNRA	34.8		34.9	34.8
EMR2	34.9			
ELTD1				33.9
F2RL1	29.5	32.0	32.6	
F2RL2				33.4
FZD1	34.0			33.0
FZD10	34.2			
FZD2	32.9	35.0		31.8
FZD3	33.2	33.2	33.2	
FZD4				33.6
FZD5	31.8	32.9	33.9	34.8
FZD6	29.4	30.4	30.6	31.2
FZD7	33.0	34.7		29.7
GABBR1	31.1	33.9	33.4	32.9
GPR176	30.5	31.5	32.0	28.7
GPR1				33.1
GPR110	31.7	32.8	33.1	
GPR115	32.6	34.1	34.9	
GPR124				29.6
GPR125	31.0	33.3	33.8	34.8
GPR126	31.3	33.9	34.2	
GPR135	34.8			
GPR153	29.2	30.6	30.8	31.4
GPR161	32.0	33.9	33.9	32.5
GPER				32.9
TRBV5	32.3			

	C_T			
GPCR	HEKn (Epilife + EDGS)	HCEC (Epilife + HCGS)	HCEC (KSFM)	HDFn (DMEM + 10% FBS)
GPR37	34.4			34.8
GPR39	32.2	34.5		
LGR4	31.3	32.9	33.4	31.5
GPR56	28.1	30.3	30.9	33.3
GPR63	34.2	34.2		
GPR68	32.7			34.3
GPR85	34.6			
GPR87	29.7	31.4	31.7	
LPAR5	30.5	33.0	33.3	
GPRC5B				33.7
HDAC3	29.9	31.1	31.4	31.0
HTR1B	32.9			
HTR7	34.0			
LANCL1	29.9	31.6	31.9	31.4
LANCL2	33.1	33.8	34.3	32.9
LPHN2	34.9	32.2	32.7	30.6
LPHN3	34.9			
LTB4R	33.7			
LTB4R2	32.7			
MC1R				33.3
MRGPRF				32.0
MRGPRX3	32.9			
RARS	29.4	29.6	30.1	29.2
OPN3	31.3	33.6	33.3	33.9
SENP3	30.3	30.9	31.9	31.1
OR2A4	32.6			34.2
PHGDH	28.2	30.1	30.6	31.9
OXTR				32.1
P2RY11	33.5			
P2RY2	32.6	33.8	34.1	
PTAFR	33.4	31.9	30.9	
PTGER2				32.4
PTGER3				34.9
PTGER4	33.8			
PTGFR				31.5
PTGIR				31.8
GPRC5A	31.6	32.2	31.2	33.8
SMO	32.0	34.2	34.8	
GPR173				34.8
SSTR1				34.0
LONP2	29.8	31.5	31.7	31.9
TBXA2R				33.4
GPR137B	32.1	33.4	33.0	31.8
VIPR1	32.6	33.8		
VN1R1	33.7			34.3

= GPCR $C_T \leq 32$ = GPCR $32 < C_T \leq 35$ = GPCR $C_T > 35$

- Analysis of transcript levels from epithelial cell types indicate significant changes (i.e., >> 2-fold) in GPCR transcripts between HEKns and HCECs.
- Analysis of transcript levels from HCECs grown in varying medium conditions indicate little to no change in GPCR transcript levels.
- Analysis of transcript levels within epithelial vs. mesenchymal cell types indicates significant differences in endogenous transcript levels.

CONCLUSIONS

- Many of the endogenous receptors identified in this study have been, and continue to be, targets of pharmaceutical development to treat tumor metastasis, pain, neurological and inflammatory disorders.
- The transcript levels of HEKn endogenous receptors are shown to be surprisingly consistent upon increasing passage of the cells, as well as from donor-to-donor, demonstrating the ability to expand primary cell banks to accommodate the scale required for primary HTS screens.
- Preliminary assessment of the GPCR transcripts of other primary cell offerings available from Life Technologies demonstrates significant differences in GPCR expression between epithelial and mesenchymal cell types.

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