RNA-seq based transcriptomic profiling of human and mouse dorsal root ganglion reveals a potential role for Protease Activated Receptor 3 (PAR3) in pain processing

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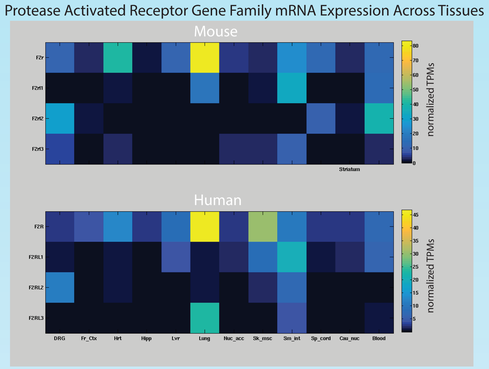
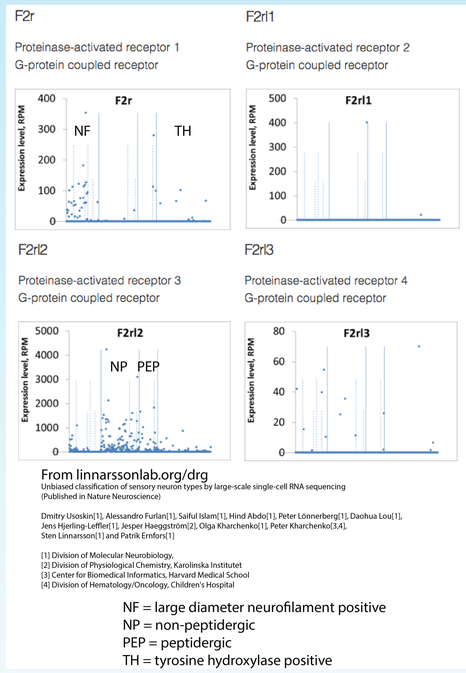
The dorsal root ganglion (DRG) contains sensory neurons that innervate the surface of the body and many visceral organs. Included amongst these neurons are nociceptors, specialized neurons that detect damaging or potentially damagind stimuli, which are required for the detection of actue pain and play a key role in the development and maintenance of chronic pain states. RNA-seq has recently been used to elucidate the transcriptome of this tissue in mouse and rat but the transcriptome of human DRG has not been explored. We obtained fresh, lumbar DRG tissue from female human donors and performed 75bp paired-end polyA+ RNA-sequencing on the Illumina platform. The sequenced fragments were mapped to the Gencode v14 reference transcriptome / hg19 reference genome to yield 80M mapped fragments, and relative transcript abundance in TPMs (Transcripts per Million reads) was quantified using the Tophat-Cufflinks toolkit. We compared our RNA-seq dataset to publicly available mouse DRG RNA-seq data and performed integrative analysis with RNA-seq data from several tissues associated with drug side effects (e.g. heart, small intestines, whole brain) to perform an unbiased search for conserved gene expression in DRG across both species. We find there is broad conservation of known DRG and/or nociceptor enriched genes (e.g. P2XR3, SCN10A, SCN11A, NTRK1, MRGPRD) across mouse and human DRGs. Information theory approaches were used to identify tissue-specific genes in human and mouse DRG compared to 13 other tissues in human and mouse. We find strong correlation of expression across tissues between species for a few hundred DRG-enriched transcripts, including known genes enriched in the DRG and previously unidentified ones. A conspicuous DRG enriched gene is F2RL2 which encodes PAR3. PAR3 expression in DRG is amongst the highest for all G protein coupled receptors (GPCRs) in human and mouse and mapping of existing cellular expression databases suggests neuronal expression in a population of nociceptors. To test the potential role of PAR3 in pain, we developed a novel ligand for this receptor using our synthetic tethered ligand discovery platform for PARs and show that it robustly activates calcium signaling in trigeminal ganglion neurons and causes mechanical hypersensitivity after hindpaw injection in mice but is devoid of activity at PAR2. Ongoing experiments are further evaluating the specificity of this compound. Our unbiased, human transcriptome approach to target discovery reveals PAR3 as a novel pain target.

Protease Activated Receptor Gene Family mRNA Expression Across Tissues



RNA-seq-based single cell expression for PAR family receptors in mouse DRG



From linnarssonlab.org/drg
Unbiased classification of sensory neuron types by large-scale single-cell RNA sequencing
(Published in Nature Neuroscience)

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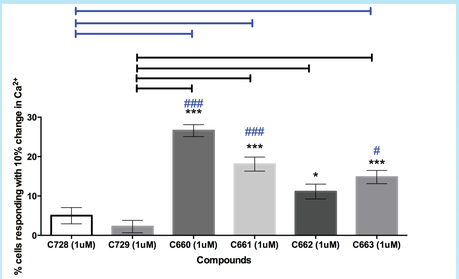
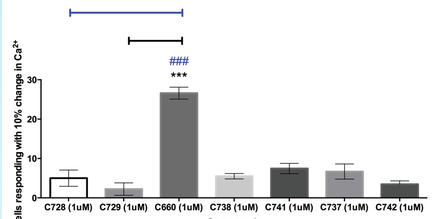
NF = large diameter neurofilament positive
NP = non-peptidergic
PEP = peptidergic
TH = tyrosine hydroxylase positive

Calcium imaging in Trigeminal Ganglion neurons from mice reveals that a PAR3-directed synthetic tethered ligand induces Calcium influx

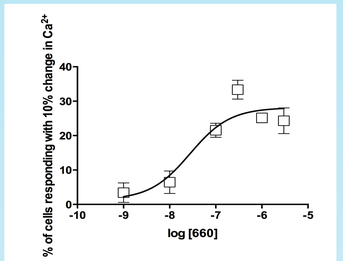


728 = Peg_3 - Hdc
729 = PGTEFNFARESFP - Peg_3 - Hdc
660 = TFRGAPPNSFEEF - Peg_3 - Hdc
661 = GAPPNSFEEF - Peg_3 - Hdc
662 = TFRGAP - Peg_3 - Hdc
663 = TFR - Peg_3 - Hdc

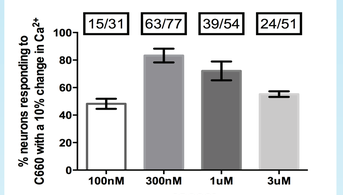


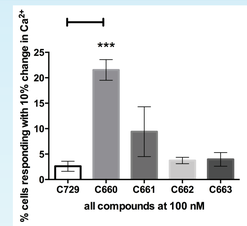
728 = Peg_3 - Hdc
729 = PGTEFNFARESFP - Peg_3 - Hdc
660 = TFRGAPPNSFEEF - Peg_3 - Hdc
738 = TFRGAPPNSFEEF
741 = RTFRGAPPNSFEEF - Peg_3 - Hdc
737 = FEEF - Peg_3 - Hdc
742 = NSFEEF - Peg_3 - Hdc

Compound 660 is a putative PAR3 agonist with an effect largely on neurons

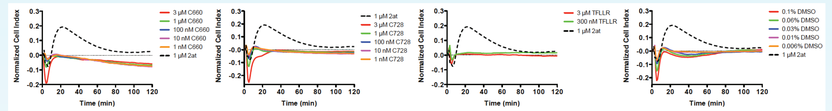


EC_{50} = 27.6 nM (95% CI = 6.7 - 114 nM)

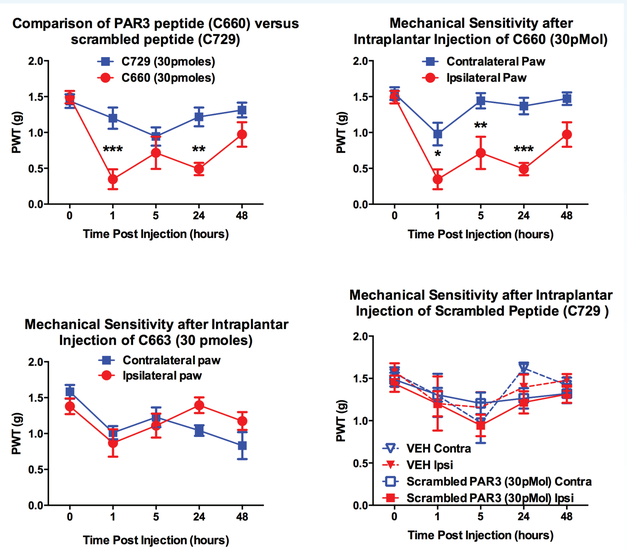


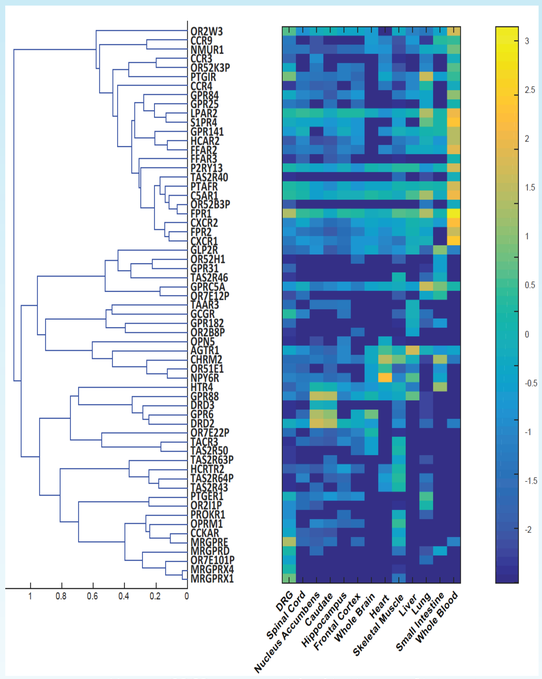


Compound 660 is devoid of action on PAR2 expressing 16-HBE cells

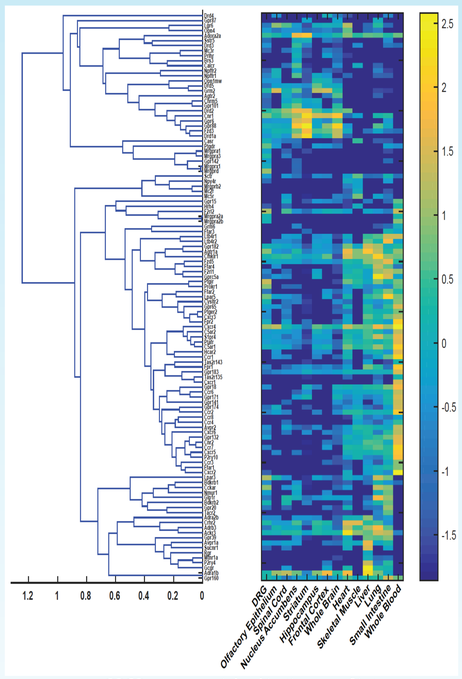


Compound 660 induces a pain response in mice





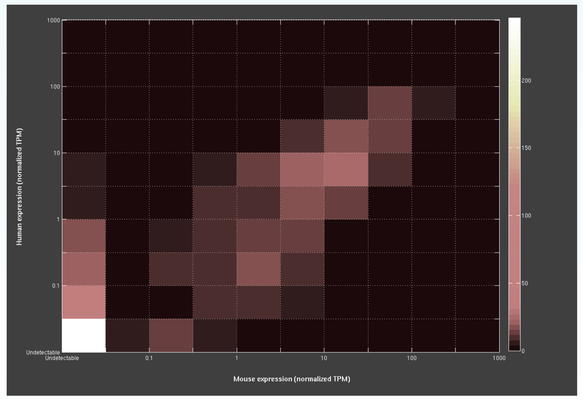
Human GPCR genes enriched in tissues of interest



mouse GPCR genes enriched in tissues of interest

HUMAN gene	TPMs	mouse gene	TPMs
GABBR1	60.899182	Gabbr1	272.70117
GPR137	45.40635	Tpra1	87.312274
GPR108	34.459632	Ackr1	80.123407
ADORA3	33.474923	Gabbr2	76.063797
ADIPOR2	30.663467	Adipor1	67.925437
FPR1	30.523826	Gpr137	54.040483
GPRC5C	29.77098	Gprc5b	54.027669
ADIPOR1	29.498253	Adora1	50.097483
MRGPRE	28.604894	Gprc5c	45.553466
LPAR1	27.818753	Gpr137l	40.690596
EDNRB	20.830163	Adipor2	37.486532
LPAR3	18.100564	Celsr2	35.903037
PTH1R	17.634788	Celsr3	35.511932
CHRM4	17.552687	Gpr158	33.226844
GPR37L1	17.380704	Grm4	30.201461
TPRA1	17.230207	Lpar1	28.420717
LPAR6	14.873211	Grm7	27.673721
GPR107	14.581799	S1pr3	25.96008
GPR35	13.112902	Pigr	25.039054
GPR34	13.066746	Gpr153	23.280654
GABBR2	12.856543	Gpr108	21.565523
P2RY12	12.620165	Oprl1	21.265752
PTGIR	11.799004	Htr1d	17.739822
C3AR1	11.602172	Gpr162	17.697283
LGR5	11.445026	Ednrb	16.453364
CX3CR1	11.21423	Pth1r	14.228776
HRH1	10.955698	Gpr107	13.783334
MRGPRX1	10.630897	Lpar3	13.320037
FZD8	10.184205	Adra2c	13.163794
GPR161	9.4839037	F2rl2	12.819316
ADORA1	9.2329088	Smo	12.744353
P2RY11	8.5807304	P2ry2	12.640432
EDNRA	8.2870689	P2ry1	12.579224
GRM4	8.0843596	Grm8	11.993404
FZD2	7.9543229	Gpr149	11.506304
GPR162	7.7317711	Mrgprd	10.793742
FZD7	7.329072	Cxcr4	10.640899
CXCR4	7.2845549	Fzd1	9.8000288
CELSR2	7.101535	Htr7	8.7280826
MRGPRC3	6.1406414	Lpr4	8.6045538
ADRA2C	6.1355086	Cckar	8.5683879
LTB4R	6.0719573	Gpr27	8.3130657
GPR158	5.868561	Npy1r	7.747148
S1PR3	5.6935389	Gpr135	7.7400505
SMO	5.4914923	Gpr45	7.6151506
F2RL2	5.2705813	Pgrr3	7.3505657
MRGPRF	5.123891	Agtr1a	7.2657532
GNRHR2	4.7330393	Gpr19	7.2522276
GPR173	4.7102926	Pgrr1	7.2245399
QRFPR	4.6750642	Gpr173	7.1780346
S1PR1	4.6725413	Prokr1	7.0731603
GPR158	4.6593499	Gpr146	6.9637478
CCR10	4.6093951	Mrgpre	6.6439718
GPR183	4.5655685	Gpr35	6.48045
LPAR5	4.5568767	Lgr5	6.0431707
OPN3	4.320984	Cnr1	6.0146247
GRM7	4.3083156	Opn3	5.7995653
GPR65	4.2836659	Chrm4	5.7783818
GPR27	4.1682371	Gpr161	5.5418923
HTR1D	4.1233478	Gpr26	5.4743855
PTGER4	4.0598358	Gpr157	5.4586954
C5AR1	3.9920254	Lpar5	5.3612286
S1PR2	3.9323787	Ntsr2	5.2077203
FZD1	3.8745316	Fzd7	5.0587923
PTGER3	3.8391747	F2r	4.7770253
P2RY14	3.8349216	Pygb	4.6079647
GCGR	3.7662475	Gpr37	4.55118
PTGDR	3.6251516	Pgfr	4.4648307
CCR1	3.5764689	Adcyap1r1	4.4062781
GPR146	3.5540423	Htr1b	4.2186334
NTSR2	3.3652911	Kiss1r	4.1746573
CYSLTR2	3.3228899	S1pr1	4.0758456
PTAFR	3.2768431	Gpr63	3.9448704
LGR4	3.2262258	Gpr85	3.8589331
GPR83	3.0946264	Pgrr4	3.8046944
LPAR2	3.0566798	Hrh3	3.7092351
GPR153	2.9845253	Oxtr	3.6022591
P2RY13	2.9587157	Hrtr2	3.4221518
GPR4	2.924482	Gpr3	3.369035
OPRL1	2.8633313	Ptpsm3	3.3671101
CMKLR1	2.8569411	Mchr1	3.3650117
CALCRL	2.851978	Npy2r	3.348556
MRGPRD	2.7785642	Gpr68	3.0932683
BDKRB2	2.7254395	Adora2a	3.0898857
FZD4	2.6998107	Oprd1	3.0165251
PTGFR	2.6934388	Gpr61	2.979284
MRGPRX4	2.5587054	Gpr139	2.9614292
HTR7	2.5403253	Prok2	2.8573667
GPR61	2.4884806	Cnr1	2.8159758
GPR85	2.467626	Fzd8	2.7820871
LTB4R2	2.4528457	Fzd2	2.6571422
P2RY6	2.4445371	Gpr62	2.5715121
GPR160	2.4400938	S1pr2	2.5527864
GPR68	2.4301072	Adra2a	2.5298841
PTGER2	2.2718333	Fzd4	2.4509398
FPR3	2.2669277	Fzr3	2.3112049
PITPNM3	2.024078	Ccr10	2.2614063
CELSR3	2.0102391	Oprm1	2.2273799
GRM8	1.9648761	Celsr1	2.198999
GPR17	1.9445879	Mrgpra3	2.1612777

Top 100 expressed GPCR genes in DRG



Broad conservation of GPCR gene expression in mouse and human DRG
R = 0.79 p < 0.01 (colormap = number of genes in cluster with increasing heat)

Conclusions

1) GPCR expression in human and mouse DRG is similar with some notable differences mostly in terms of magnitude of expression
2) Of the PAR receptor family, PAR3 is most highly expressed in human and mouse DRG and is high in DRG compared to almost all other tissues
3) A PAR3-derived synthetic tethered ligand robustly activates Calcium signaling in mouse TG neurons and induces mechanical hypersensitivity in vivo consistent with a role for PAR3 (f2rl2 gene) in nociceptive plasticity
4) This work demonstrates an unexpected role for PAR3 in sensory neurobiology and creates a strong rationale for further work on PAR3