SUPPLEMENTARY INFORMATION

SUPPLEMENTARY DISCUSSION

The compatibility of the donor and recipient immune systems plays an essential role in the success of haematopoietic stem and progenitor cell (HSPC) transplantation. Among all of the factors influencing the immune systems, genes from the human major histocompatibility complex (MHC) locus have been shown to be the most important genes determining tissue histocompatibility. When the adult zebrafish whole kidney marrow (WKM) competitive transplantation assay was developed and the chemical screening was performed, knowledge of zebrafish MHC was very preliminary. In addition, although wild-type zebrafish have been inbred for generations, unlike the BL6 mice, they still harbor diverse sets of MHC genotypes. This fact might have a substantial impact on the average survival and chimerism of transplanted zebrafish recipients in the adult zebrafish transplantation experiments performed in this study. In fact, on average we had about 50-70% of recipients surviving up to 4 wpt (week post transplant).

Even though the donors and recipients were not completely immune-matched, this does not compromise the reliability of evaluating the chemicals' effects on enhancing WKM engraftment for the following reasons: (1) The donor WKM cells were dissected from multiple donors and pooled together before being split into different chemical treatment groups. Therefore, each treatment group should receive cells with the same mixture of MHC genotypes; (2) In each chemical screen, more than 150 adult *casper* recipients were mixed, 10 of which were randomly assigned to each group. Therefore, the recipients' MHC genotypes were also randomly distributed among all the treatment groups; (3) All screen hits were repeated in at least two more experiments with different groups of donors and recipients, the MHC genotypes of which should be independent of the previous experiments.

The next step in improving the overall efficiency and reproducibility of the zebrafish WKM transplantation assay is to more comprehensively understand the zebrafish MHC system and try to match the donor and recipient immune systems. We have already started this initiative recently in our lab[1]. We think this will improve the survival rate and make the assay more suitable for evaluating long-term engraftment.

SUPPLEMENTARY VIDEOS

Video S1 & S2. Confocal time-lapse imaging of HSPC trafficking to and engrafting CHT (caudal haematopoietic tissue) in zebrafish embryos.

Tg(Runx1+23:GPF;flk1:DsRed2) embryos were treated with chemicals between 24-46 hpf. Chemicals were washed off at 46 hpf. Embryos were immediately mounted in 1% low-melting-point agarose and imaged between 54-63 hpf. Time-lapse movies were taken on a spinning disk confocal microscope with a 28°C incubation chamber. Images were taken every 2 minutes and focused on the CHT region.

Video S1: DMSO-treated embryo

Video S2: 5 μM 11,12-EET-treated embryo

SUPPLEMENTARY TABLES

Table S1: Chemicals used in the zebrafish embryo suppressor screen.

Chemical Names	Sources	Highest Doses (μM)	Target Pathways
SB 203580	Tocris	100	p38
PD98059	Calbiochem	50	MEK1
U0126	Tocris	30	MEK1/2
GW5074	Tocris	50	C-RAF
LY294002	Sigma	10	PI3K
wortmannin	Sigma	1	PI3K AKT
PI-103	Cayman	10	PI3K
rapamycin	Enzo	400	mTOR
H89	Sigma	5	PKA
SQ22536	Sigma	200	Adenylate Cyclase
GW9662	Cayman	5	PPARγ antagonist
BADGE	Tocris	10	PPARγ antagonist
Ciglitazone	Enzo	10	PPARγ agonist
WY14643	Tocris	10	PPARα agonist
TPCA	Sigma	50	IKK
Bay 11-7082	Cayman	1	NF-κB
PP2	Sigma	10	SRC
AG1478	Sigma	50	EGFR
GM6001	Enzo	25	MMP
SU 5402	Tocris	30	FGF/VEGF
Indomethacin	Sigma	20	COX
Dorsomorphin	Tocris	20	BMP
L-NAME	Sigma	10	NOS
SU1498	EMD	50	VEGF
Cyclopamine	Sigma	20	SHH
NSC 23766	Tocris	50	RAC
Compound W	Tocris	10	NOTCH
AS605240	Sigma	10	PI3Kγ [2]
PIK75	Cayman	3	PI3Kα
TGX221	Gift of Nathaniel Gray (HMS)	20	PI3Kβ
PIK-294	Gift of Nathaniel Gray (HMS)	30	PI3Kδ

Table S2: Sequences of morpholinos (MO) used in zebrafish embryo studies.

MO Names	Target Ref Seq	MO Sequence
* *junb*_ATG	NM_213556	CGGTTGCTCCATTTTTGTTGACATG
*c-jun*_ATG	NM_199987	TTCCATCTTGGTAGACATAGAAGGC
*c-jun*_5'UTR	NM_199987	GCGAAACTTTTAGTTCAGAAGCAGT
** *gna12*_ATG	NM_001013277	CGCACCACGCCAGCCATCCTGTCCA
** *gna13a*_ATG	NM_001012243	AAATCCGCCATCTTTGTAGTAGCGA
** *gna13b*_ATG	NM_001013263	AGGAAATACGCCATCTTTGTGCAAC
*pik3ca1*_ATG	XM_009306175.1	GTCTTGGAGGCATGATTTGTAATCC
*pik3cb*_ATG	XM_005157466.2	GCATGGCAGAGAACACACTGAAGCC
***MO2-*pik3cg*_ATG	NM_213306.1	CATCATCACTGGCTTGCTGTTCCAT
*pik3cd*_ATG	NM_201199.1	GGCATCGTGCAGGAAACTCATCTAC

***** The *junb*_ATG morpholino is a published sequence[3]. But due to the high similarity of the first 23 bp coding sequences between *junb* and *junbl* (only differ by 4 bp, including a consecutive 3 bp), this morpholino might potentially knock down both alleles at the same time.
** Published morpholino sequences[4].
*** Published morpholino sequence[5].

Table S3: Microarray analysis of 36 hpf wild-type zebrafish embryos treated with DMSO or 5 μM 11,12-EET between 24-36 hpf. (See the separate Excel sheet, Supplementary Table S3)

Total RNA was extracted from 36 hpf zebrafish embryos treated with DMSO or 5 μM 11,12-EET between 24-36 hpf, with 3 biological replicates each and n=25 in each group. Microarray hybridization was performed with the Affymetrix GeneChip Zebrafish Genome Array. Genes with q<0.1 by SNR test were considered differentially expressed.

Table S4: RNA-seq analysis of human umbilical cord blood CD34+ HSPCs and the human myeloid cell line U937 with DMSO or 5 μM 11,12-EET for 2 hrs. (See separate Excel sheets, Supplementary Table S4).

- Sheet 1 lists genes upregulated in CD34+ HSPCs with log2fc>0.5 (log2 fold change).
- Sheet 2 lists genes downregulated in CD34+ HSPCs with log2fc<-0.5.
- Sheet 3 lists genes upregulated in U937 with log2fc>0.5.
- Sheet 4 lists genes downregulated in U937 with log2fc<-0.5.
- Sheet 5 lists the overlapping genes upregulated in CD34+ HSPCs and U937.
- Sheet 6 lists all increased or decreased bio-functions, predicted by IPA based on the overlapping gene set. Labeled in orange are bio-functions summarized under the categories “Cell-to-cell interaction” and “Cellular movement” with significant p values and an activation z-score >2, accounting for the top bio-functions on the list.

Table S5: Comparison of major haematopoietic phenotypes and signaling pathways regulated by PGE2 and EETs.

	PGE2	EETs
Zebrafish embryo treatment between 24-36 hpf	Increase HSPCs in AGM Increase HSPCs in CHT	Increase HSPCs in AGM Increase HSPCs in CHT
Zebrafish embryo treatment after 40 hpf	Decrease HSPCs in CHT and increase HSPCs retained in AGM*	Increase c*myb*+ progenitors in CHT, but not *Runx1+23:GFP* HSCs
Adult marrow engraftment 2-6 hr treatment	Increase engraftment	Increase engraftment
Downstream signaling pathway	Sensitive to Gαs inhibition	Insensitive to Gαs inhibition, but sensitive to Gα12/13 inhibition

***** Unpublished data, personal communication with Trista E. North and Owen J. Tamplin.

References

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