Evidence for Altered Peroxisome Proliferator Activated Receptor (PPAR) Pathway Activity in a Transgenic Mouse Model of Scleroderma (TβRIIΔk-fib): Analysis of Mouse Skin, Lung and Explanted Cells.

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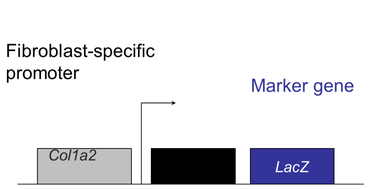
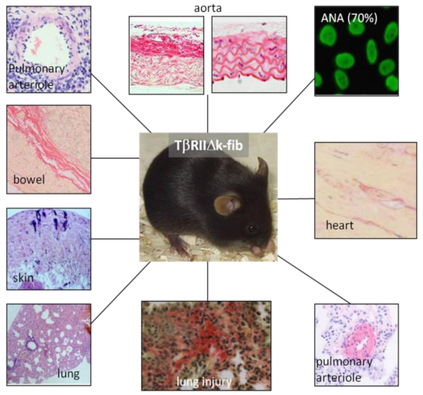
Background/purpose:

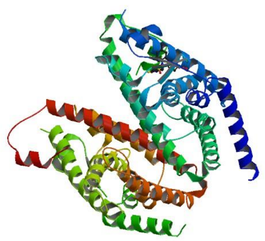
The TβRIIΔk-fib transgenic (TG) mouse model of scleroderma carries a fibroblast-specific TGFβ receptor II mutation resulting in balanced up-regulation of TGFβ signalling and replicates key fibrotic and vasculopathic features of scleroderma, including susceptibility to lung fibrosis and pulmonary hypertension.





This study examines evidence of PPAR pathway perturbation in whole tissue or explanted cells from adult or neonatal TG mice compared with wildtype (WT) littermates.

Lanifibranor (formerly known as IVA337) is a new chemical entity that activates all PPAR isoforms and is undergoing clinical trials in scleroderma at present.



Methods:

Gene expression differences between whole skin biopsies, fibroblasts or aortic smooth muscle cells (aSMCs) from TβRIIΔk-fib and WT sex-matched littermate mice (n=3 each group) were quantified using MouseRef-8v1.1 expression BeadChips (Illumina, USA). In total, 42 illumina microarray gene expression profiles were analysed. After normalisation (Bioconductor Lumi), the genes were ranked according to differential expression. Data were expressed as pairwise analysis comparing the mean expression of two groups (t test), with FDR correction for multiple testing (number of tests/rank of p value). The PPAR pathway gene list obtained from http://software.broadinstitute.org/gsea/msigdb/cards/KEGG_PPAR_SIGNALING_PATHWAY was cross-referenced with each microarray. Within the GSEA cohort of genes the number with significant differential expression or a trend to difference (P< 0.10) in analysis was determined. A total list of 12800 genes were tested.

Results:

For the individual substrate RNAs analysed the number of genes were whole lung (n=3), adult skin fibroblasts (n=2), neonatal skin fibroblasts (n=4), neonatal lung fibroblasts (n=3) and adult vascular smooth muscle cells (n=11). Overall, for the pathway genes within the GSEA cohort 42 of 69 annotated genes showed expression and were included in the illumina microarray for these samples. Other genes were not present or did not show significant signal for evaluation.

The genes within the PPAR pathway showing either significant difference ($p<0.05$) or trend of difference are summarised in **Table 1** below. Overall, 11 genes showed significant difference between WT and TG explant cells and 82% (n=9) were reduced in the TG cells. 25 genes showed a trend of difference in ≥1 of the test substrates and the majority, 18 (72%), were down-regulated in TG mice. This suggested down-regulation of the PPAR pathway in the TβRIIΔk-fib mouse model. As shown in Table 1, there were most differences for the aortic smooth muscle cells and this is notable as the altered phenotype of these in culture is likely to reflect an altered in vivo environment, with elevated TGFβ activity in the vessel wall rather than intrinsic SMC abnormalities since the smooth muscle cells are not predicted to express the kinase-deficient transgene.

Table 1. Individual genes within the PPAR GSEA that show trend or differential expression between transgenic and wildtype littermate mice.

Adult whole lung	Adult skin FB	Adult lung FB	Neonatal skin FB	Neonatal lung FB	Vascular aSMC	
ACADM	ACADL	*CPT1C*	ACAA1	ANGPTL4	*ACOX3*	*PDPK1*
ACSL4	*RXRA*	*SCD*	*ACSL1*	*APOA1*	ANGPTL4	*PPARG*
PDPK1			CPT1C	*PDPK1*	*CPT2*	NR1H3
			FABP5		*FABP5*	LPL
					SCP2	FABP3
					FADS2	

Italicised trend only, $p<0.10$), others $p<0.05$
FB = fibroblast explant culture; aSMC = aortic smooth muscle cell explant culture

Conclusions:

- This mouse model provides a potential platform for *in vivo* experiments to provide mechanistic support for trials of IVA337 in scleroderma.
- Whole tissue is less informative that explanted cells with whole skin showing no significantly differentially expressed genes.
- These results are not indicative of highly dysregulated PPAR pathway expression, although overall there is a signal that the PPAR pathway, assessed by GSEA, may be reduced in TG mice.
- The trend is seen most often in aSMC explant culture.