EGR-1 AND EGR-2 IN MYOCARDIAL FIBROSIS OF TGF-ß TRANSGENIC MICE AND THEIR ASSOCIATION WITH TIMP-1

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BACKGROUND

Recently we found strong association between cardiac TIMP-1 expression and the severity of myocardial fibrosis in TGF-ß1 transgenic mice on different genetic backgrounds (B6 and CBAxB6 F1)[1]. Fibrosis is usually charaterized by increased expression of the tissue inhibitors of matrix metalloproteinases (TIMPs).

Early growth response factors (EGRs), a family of transcription factors have been associated with TGF-ß induced activation of fibroblasts and regulation of collagen synthesis[2,3]. However, their role in cardiac fibrosis or their association with TIMP-1 is still unkown.

In the present study, we aimed to investigate the strain dependent molecular regulation of cardiac TIMP-1 and its association with profibrotic transcription factors EGR1 and EGR2.

METHODS

Animal model:

Male B6-TGFß and CBAxB6-TGFß transgenic mice were generated as previously described [4,5].

Experimental groups:

TGF-ß1 transgenic mouse strains and wild type control strains were used as follows:

1) B6-TGFß (n=8)
2) CBAxB6-TGFß F1 (n=9)
3) B6 (n=6)
4) CBAxB6 F1 (n=6)

Cardiac samples of 14-days old male mice were analyzed for mRNA and protein expression. Circulating TGF-ß1 levels were analyzed in plasma samples.

Ingenuity Pathway Analysis (IPA) Molecule Activity Predictor module was used on expression data to construct the proposed pathway.

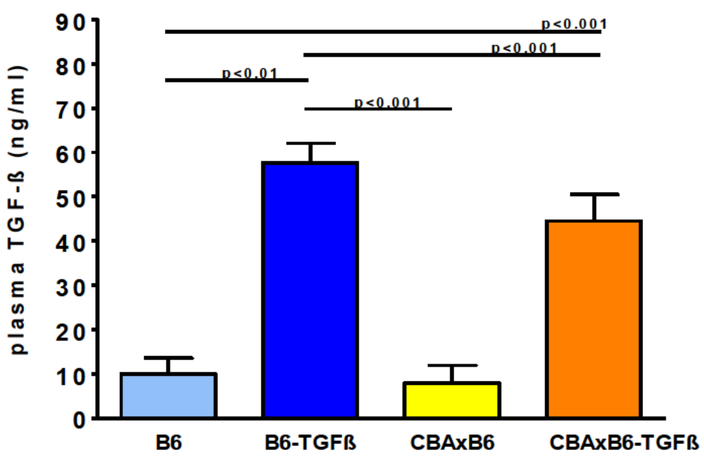
Statistical analysis:

Data are presented as mean+SD. ANOVA and and Kruskal-Wallis test were performed.

RESULTS

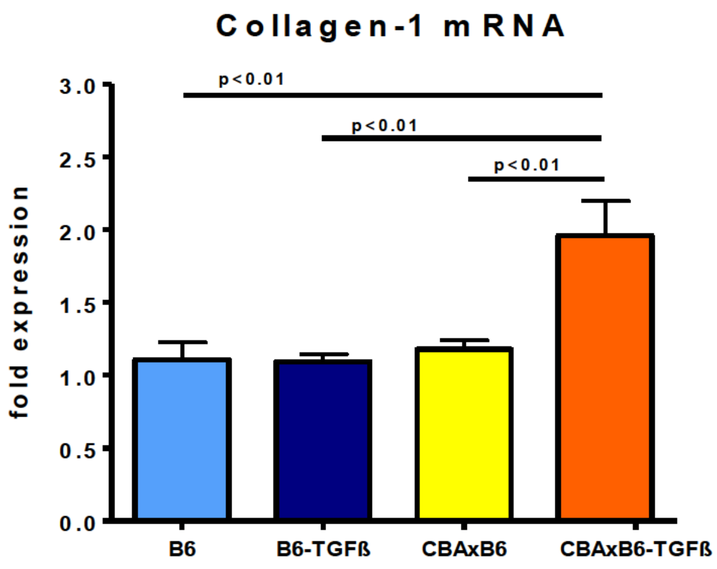
Plasma TGF-ß1 levels

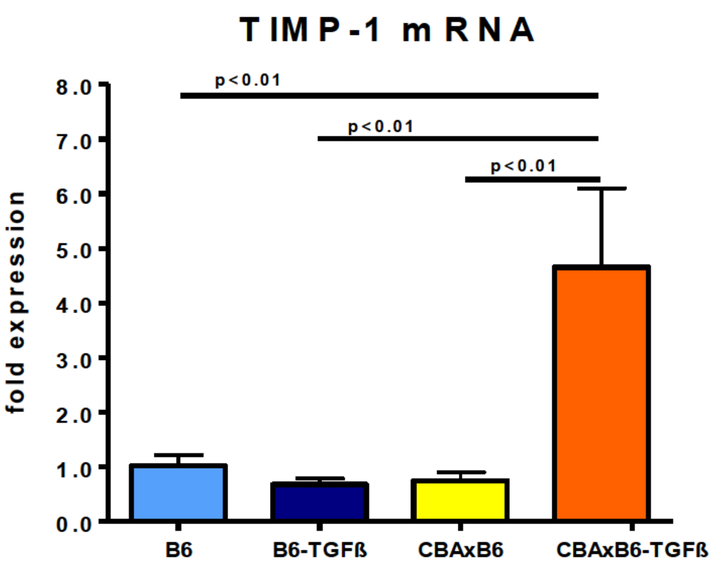
Both transgenic strains had elevated circulating TGF-ß1 levels, as compared to wild type controls.

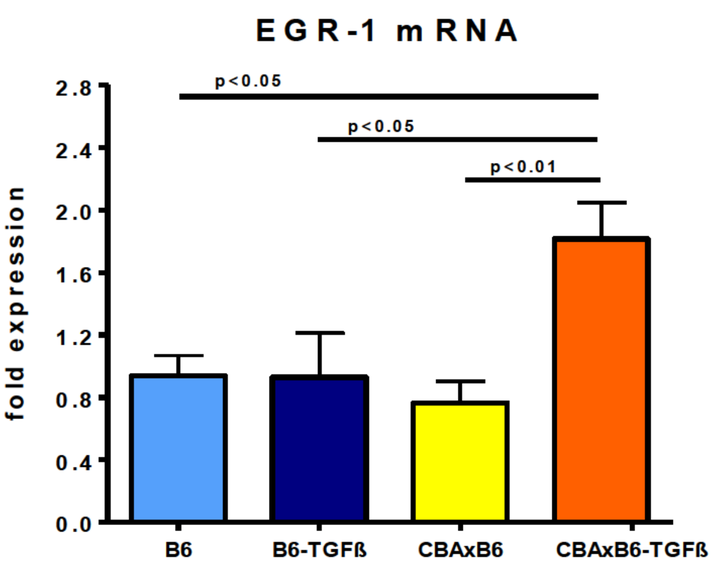


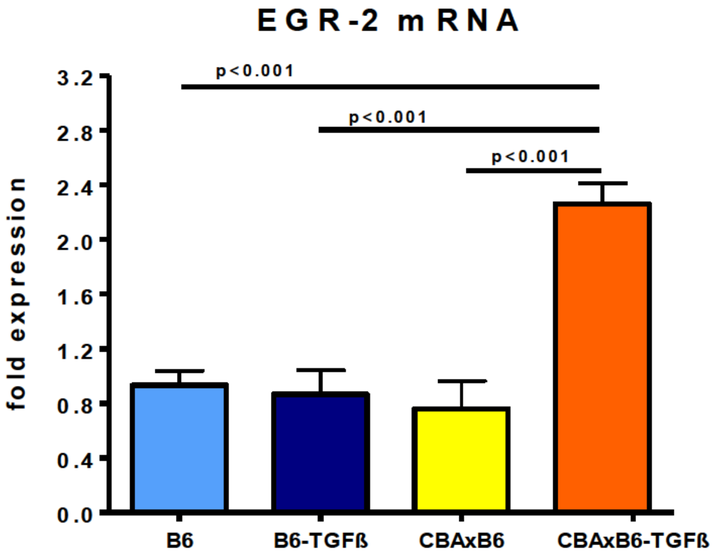
Cardiac gene expression

Despite the comparable elevated plasma TGF-ß1 levels in both transgenic strains, the cardiac mRNA expression of collagen-1, TIMP-1, EGR-1 and EGR-2 were elevated only in CBAxB6-TGFß transgenic mice.





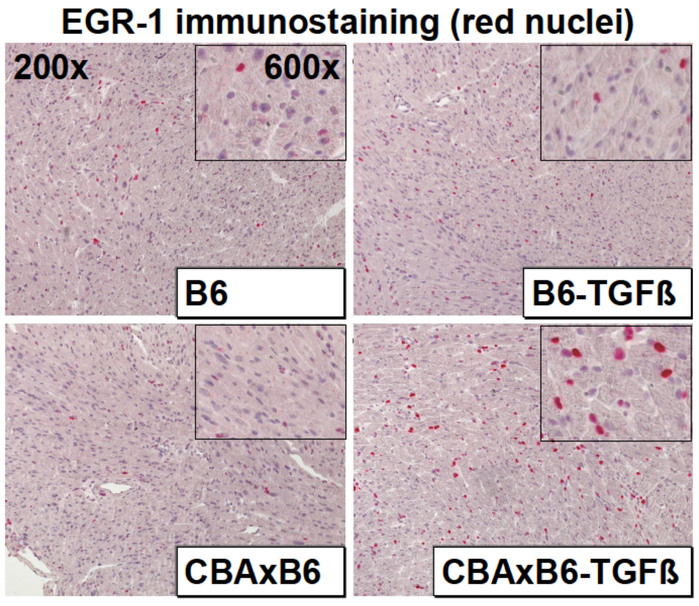




EGR immunostaining

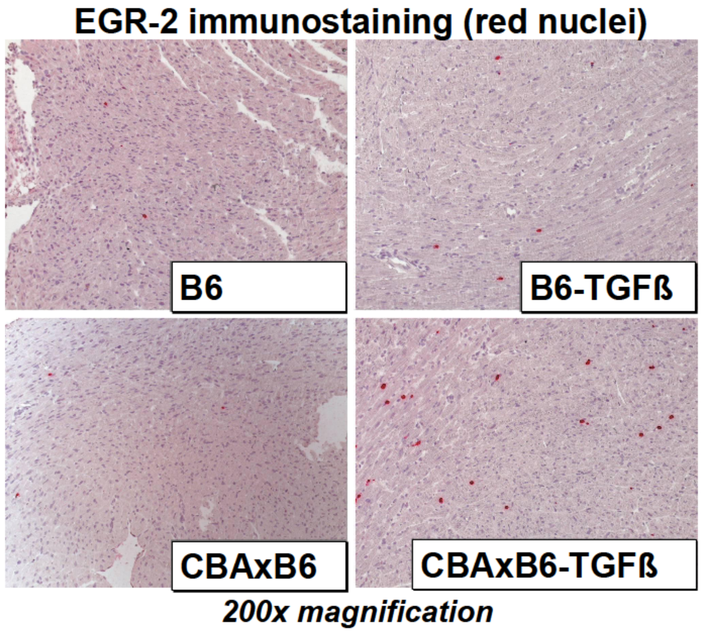
According to gene expression data, CBAxB6-TGFß mice depicted increased number of EGR-1 and EGR-2 positive cardiac cells.

EGR-1 immunostaining (red nuclei)



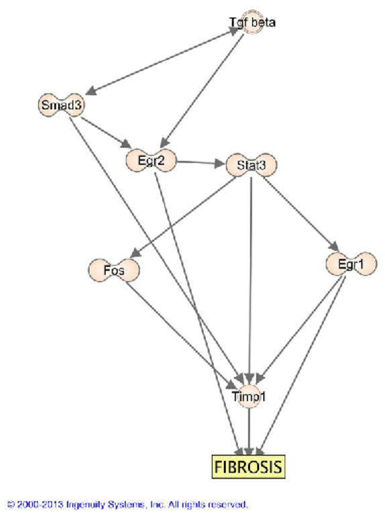
200x and 600x magnification

EGR-2 immunostaining (red nuclei)



200x magnification

Proposed pathway



CONCLUSION

Our results suggest EGR-2 might contribute to the development of TGF-ß induced myocardial fibrosis. We suggest that EGR-1 and EGR-2 might contribute to the strain dependent regulation of TIMP-1 expression in this model .

ACKNOWLEDGEMENTS

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