Functional Prostaglandin-Endoperoxide Synthase 2 Polymorphism Predicts Poor Outcome in Sarcoidosis

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Online Data Supplement

Methods

Functional studies

Lung fibroblast lines were established from uninvolved lung tissue taken from patients undergoing lung tumour resection. These methods have been described in detail elsewhere[E1]. Fibroblasts were grown to confluence and PGE_2 levels were measured in the tissue culture medium basally and 24 hours following addition of $TGF\beta_1$ (1ng/ml) using a specific enyzme immunoassay (Amersham, Bucks, UK) as previously described [E1].

For the EMSA studies nuclear extracts from human lung fibroblasts (HFL-1) were prepared as described[E2]. EMSAs, using radiolabelled probes **GAATTTACCTTTCCCGCCTCTCTTTCCAAG** (probe G) and **GAATTTACCTTTCCCCCCTCTCTTTCCAAG** (probe C), were performed as previously described[E3] with 5 ug of nuclear extract per binding reaction using standard conditions of a gel-shift kit from Promega. Antibody supershift and blocking assays were performed using antibodies specific for Sp1, Sp2, Sp3, Sp4 and Egr-1 (Santa Cruz Biotechnology) or isotype control antibodies, added to the nuclear extracts and incubated for 60 min at room temperature prior to the addition of the labelled probes. For experiments with human recombinant (hrec) Sp1 protein, 150ng of hrecSp1 (Promega) was incubated for 20 min at room temperature prior to addition of labelled probe. Protein-DNA complexes were separated in 6% polyacrylamide gels in 0.25x TBE at ambient temperature.

Reference List

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