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Epistatic interactions between modifier genes confer strain-specific redundancy for *Tgfb1* in developmental angiogenesis

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Abstract

Tgfbm1 (chromosome 5, $P = 8 \times 10^{-5}$) and *Tgfbm3* (chromosome 12, $P = 6 \times 10^{-11}$) were identified as loci that modify developmental angiogenesis of *Tgfb1*−/− mice. Congenic mice validated these loci and demonstrated epistatic interaction between them. The novel locus, *Tgfbm3*, encompasses ~22 genes, colocalizes with both tumor susceptibility and atherosclerosis susceptibility loci, and is enriched in genes regulating cell growth and morphogenesis. The use of gene knockout and/or transgenic mice that predispose to a complex trait, such as vascular development/angiogenesis, facilitates the identification of modifiers by simplifying genetic analysis. Identification of genes that modify response to lack of transforming growth factor β1 (TGFβ1) will enhance the understanding of TGFβ1 action in vivo and may help predict which patients would respond well to anti-TGFβ therapy. Identification of angiogenesis-modifying genes may provide new targets for angiogenesis therapies and analysis of polymorphisms therein may contribute to assessment of risk for diseases involving angiogenesis.

Keywords: Angiogenesis; Epistatic interaction; Genetic modifier; Mouse embryogenesis; Prenatal lethal; TGFβ; Yolk sac

Introduction

Deviations in normal embryonic and fetal development of the vascular system can contribute to adult pathologies with late age of onset, such as atherosclerosis and hypertension. Differential angiogenic capacity can also influence the ability to heal wounds and influence the progression of fibrotic diseases and cancer. The development of the vascular bed is regulated multifactorially with both strong environmental [1–4] and genetic influences [5–7]. Inbred mouse strains show innate variation in endogenous vascularity and in angiogenic capacity both during embryogenesis and postnatally [6–11], making it feasible to dissect the genetic components regulating angiogenesis [5]. However, because the genetics of regulation of angiogenesis is highly complex, it can be difficult to map those genes that act together to modify this process since each individual

Abbreviations: BC, backcross; HHT, hereditary hemorrhagic telangiectasia; QTL, quantitative trait locus; STB, survival to birth; TGFβ, transforming growth factor β.

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modifier gene contributes only a small fraction of the genetic variance to the overall effect on angiogenesis, leading to weak linkage for any individual gene [6].

We have taken an approach that we believe simplifies the genetics of such modifier screens by potentiating mice toward defects in prenatal vascular development, in our case using the *Tgfb1*−/− mouse [10–12]. Utilizing this approach, we expect that fewer loci would be identified, but that each locus would be more amenable to mapping at high resolution. The loci identified will certainly be important in developmental angiogenesis and may well also be important, either directly or indirectly, in the transforming growth factor β (TGFβ)[7] signaling pathway.

TGFβ1 is a key regulator of normal angiogenesis and vascular integrity as demonstrated by the phenotypes of mice [13] and humans [14] with null mutations in components of the TGFβ1 signaling pathway. Homozygosity for null mutations in *Tgfb1, Tbr1, Tbr2, Eng*, or *Acvrl1* results in lethal embryonic vascular defects in mice [13]. Hemizygous mutation of *TGFBR2* results in Marfan syndrome [15], and hemizygosity for *ENG* or *ACVRL1* results in hereditary hemorrhagic telangiectasia (HHT), a vascular dysplasia with late age of onset [14]. Genes encoding components of the TGFβ1 signaling pathway, including *TGFB1* [16] and *TBRI* [17], have been shown to be functionally polymorphic in humans and confer susceptibility to atherosclerosis [16,18], hypertension [16,19], and other vascular diseases [20], as well as breast and prostate cancers [21,22]. TGFβ1 is also important in pathological angiogenesis, such as tumor angiogenesis [13,23] and during wound healing [24].

Herein, we have used classical F_1 intercross mapping to validate the existence of a previously identified TGFβ1 modifier locus on chromosome 5, in an independent cross between C57 and NIH mice ($P = 8 \times 10^{-5}$). This locus has now been named *Tgfbm1*. We have also identified a second strong modifier locus, *Tgfbm3*, on chromosome 12 ($P = 6 \times 10^{-11}$) that interacts with *Tgfbm1* in a multiplicative fashion to determine the developmental response to *Tgfb1* nullizygosity. The validity of these loci was confirmed using mice congenic for the respective modifier regions.

The identification of genes that modify the response to lack of TGFβ1 should enhance our understanding of the mode of action of TGFβ1 in vivo. This is particularly important now that small-molecule inhibitors that act on the TGFβ signaling pathway are being developed to treat fibrosis and cancer [25]. Knowledge of the genetic basis for variation in response to attenuation of TGFβ may eventually be useful in identifying patients that may or may not respond well to anti-TGFβ therapy. Identification of angiogenesis modifying genes may provide new targets for angiogenesis therapies and analysis of polymorphisms therein may contribute to assessment of risk for diseases involving angiogenesis.

Results

Multifactorial nature of Tgfb1−/− genetic modifiers

Tgfb1−/− mice die at either 10.5 days *postcoitum* (dpc) from defects in development of the yolk sac [10–12] or 3 weeks *postpartum* from multifocal inflammation [26]. Yolk sac defects include both abnormal vascular development and anemia [10], but it has been suggested that the primitive hematopoietic defects are secondary to defects in vascular development [27,28]. On mixed genetic backgrounds, there have been reports of *Tgfb1*−/− prenatal loss before 8.5 dpc [11,29,30]; however, on relatively pure C57, NIH, or 129 genetic backgrounds, the fraction of 9.5 dpc *Tgfb1*−/− with yolk sac defects is consistent with this being the major cause of prenatal loss in each of these strains [11,12,31].

We previously identified a polymorphic genetic modifier locus, *Tgfbm1*, on chromosome 5 that accounts for a significant fraction of the genetic variance responsible for determining embryonic fate of *Tgfb1*−/− mice in a NIHC57F1 intercross [11]. The phenotype scored was survival to birth (STB) of *Tgfb1*−/− mice compared to wildtype littermates in a *Tgfb1*+/− F_1 intercross. Since then, the C57 and NIH strains harboring the *Tgfb1* null allele were bred through further backcross generations onto the relevant strain, in order to purify the genetic backgrounds. At backcross generation 5 (BC5) mice were moved from a conventional facility in the United Kingdom to a barrier facility in the United States and re-derived by superovulation and embryo transfer. For each strain, between one and five *Tgfb1*+/− male mice were utilized for this re-derivation step. All breeding in the United States (post-BC6) was performed in a barrier facility.

The STB rate of *Tgfb1*−/− mice on the C57 background was zero in both the Glasgow- and U.S.-bred mice. However, it was notable that in all the crosses, the NIH STB rate in the U.S.-bred mice was roughly half that of the Glasgow-bred mice (Fig. 1), suggesting that the penetrance of the NIH modifier allele(s) was reduced ~40% following transfer to the United States. Nevertheless, within genetically similar populations bred within the same animal house the variance in STB was small. The incidence of NIH *Tgfb1*−/− neonates per litter was evenly distributed between the >30 litters of Glasgow-bred mice and >25 litters of U.S.-bred mice. Moreover, the different batches of Glasgow-bred F_1 intercross mice showed little variance in STB rates (189 *Tgfb1*+/+: 373 *Tgfb1*+/−: 70 *Tgfb1*−/− versus 49 *Tgfb1*+/+: 97 *Tgfb1*+/−: 24 *Tgfb1*−/−; $P = 0.55$). All prenatal death of both the NIH and the C57 *Tgfb1*−/− embryos was due to yolk sac insufficiency [12], since STB rates corresponded directly to the incidence of vascular/hematopoietic abnormalities. The reduced STB rate in U.S.- versus Glasgow-bred *Tgfb1*−/− mice may be explained by genetic and/or environmental differences (see Discussion), but clearly illustrates the multifactorial nature of the *Tgfb1* genetic modifiers.