*Commentary*

# Non-redundant tumour suppressor functions of transforming growth factor beta in breast cancer

Transforming growth factor betas (TGF*b*s) are multifunctional peptides thought to be involved in growth regulation, development, differentiation and immune modulation of virtually all tissues in organisms, from fruitflies to humans. Together, these ligands account for a large number of intercellular signals governing cell fate. There are three mammalian isoforms (TGF*b* 1-3) which are the product of different genes. Appropriate levels of TGF*b* activity are essential for the well being of the organism. Lack of sufficient TGF*b* can result in severe inflammatory disease, deficient wound healing, congenital malformations including that of heart and palate and increased tumourigenesis. Conversely, excessive TGF*b* activity leads to scarring, development of fibrotic diseases in multiple organ systems, immune suppression, and possibly, enhancement of later stages of tumour progression. Over the last few years it has become clear that TGF*b* pathways are significantly involved in many processes related to carcinogenesis. Mutations in multiple TGF*b* signal transduction pathway components have been implicated in human colon and breast cancer.

The TGF*b*s 1, 2 and 3 exhibit both distinct and overlapping spatial and temporal patterns of expression and localization in tissues throughout mouse embryonic development and in the adult, with pronounced embryonic expression in areas undergoing morphogenesis (Pelton *et al* 1991). Most cells secrete TGF*b*s as large latent polypeptides that require activation – mostly proteolytic activation – before becoming biologically functional. These secreted latent polypeptides are always bound to alpha 2-macroglobulin (alpha 2M), which scavenges excess TGF*b*s and limits its local action (O'Connor-McCourt and Wakefield 1987). TGF*b* ligands signal through serine/threonine kinase receptors TGF*b*R1 and 2 to the intracellular mediators known as SMAD proteins and they differentially regulate gene expression (Massague 1998) (figure 1). TGF*b*R3 can also associate with the ligand and present it to TGF*b*R2. The functional specificities of the various receptor and SMAD protein combinations are not yet clear. The complexity found at all levels of TGF*b* function makes TGF*b* 1, 2 and 3 ideal molecules for playing significant roles in morphogenetic processes during development and in homeostatic processes in the adult. *In vivo* studies in mouse models with targeted null mutations in the three *Tgfb* genes are consistent with TGF*b* involvement in all of these areas (Shull *et al* 1992; Sanford *et al* 1997; Proetzel *et al* 1995).

Between the three strains of TGF*b* gene knockout (KO) mice, over three dozen different phenotypes have been characterized (table 1). All but those marked with an asterisk have been characterized by Doetschman's group at the University of Cincinnati Medical Center, Ohio, or through its collaborative efforts. There is clearly an overlap in the developmental localization patterns of the three TGF*b*s (Pelton *et al* 1991), and they all signal through one common receptor, TGF*b*R2, and one SMAD4 signalling molecule (Massague 1998). The three TGF*b*s usually, though not always, have similar functions *in vitro* (Roberts and Sporn 1990). However, the fact that the 3 *Tgfb* knockout mice have between them over 30 knockout phenotypes, none of which are redundant or overlapping, indicates that *in vivo* each TGF*b* ligand has unique functions.

The role of TGF*b* ligands and the corresponding receptor (TGF*b*R2) in breast cancer is very complex. A biphasic role for TGF*b* has been proposed in breast cancer. In normal breast and in early stages of breast cancer development TGF*b* acts as a suppressor, but later it facilitates tumour progression. A decreased expression of TGF*b*R2 in human breast tissue contributes to breast cancer progression and is related to a more aggressive phenotype in both *in situ* and invasive carcinomas (Walker 2000). Also, dominant negative interference of TGF*b*R2 shows defective alveolar epithelial differentiation and

**Table 1.** Non-overlapping phenotypes of *Tgfb* KO mice.

| *Tgfb1* | *Tgfb2* | | *Tgfb3* |
|---|---|---|---|
| Pre-morula lethality | Perinatal lethality | Skeletal defects | Prenatal scarring* |
| Yolk sac lethality* | Heart defects | Occipital bone | Postnatal lethality: cleft palate |
| Reproductive defects | Ventricular septum defects | Parietal bone | Dilated conducting airways* |
| Multifocal autoimmunity | Dual outlet right ventricle | Squamous bone | Collapsed bronchioles* |
| Platelet defect | Dual inlet left ventricle | Palatine bone (cleft palate) | Adult: Skin homeostasis |
| Colon cancer | Inner ear defect – missing limbus | Alisphenoid bone | |
| Skin cancer* | Urogenital defects | Mandibular defects | |
| Failing heart | Dilated renal pelvis | Shortened radius and ulna | |
| Reduced FABP activity | Agenesis (females only) | Missing deltoid tuberosity | |
| Delayed wound healing | Uterine hornectopia | Missing third trochanter | |
| | Testicular ectopia | Sternum malformations | |
| | Testis hypoplasia and vas deferens dysgenesis | Rib barreling | |
| | Lung-prenatal | Rib fusions | |
| | Dilated conducting airways | Spina bifida | |
| | Collapsed bronchioles | Ocular defects | |
| | Hair follicle development | Post. chamber hypercellularity | |
| | | Partial fusion of cornea to lens reduced corneal stroma | |

branching morphogenesis of the mammary gland (Joseph et al 1999). Many investigators now believe that development of resistance to TGF*b* by tumour cells represents a key event in the progression of malignancy. For these reasons there has been considerable interest in the signal transduction pathways utilized by these different TGF*b* family members. Genes that cause growth abnormalities related to differentiating alveolar epithelium in pregnant mice are most involved in breast cancer development. In pregnant mice, *Tgfb1* is moderately expressed. And, *Tgfb2* has highly specific and significant expression levels in differentiating alveolar epithelium; *Tgfb3* is found in ductal stroma and epithelium (Robinson *et al* 1991).

Many studies that describe the roles of TGF*b* in breast cancer development, whether they involve breast cancer cell lines or transgenic mouse models, suggest that TGF*b*1 is the breast cancer tumour suppressor (Walker 2000). *Tgfb1* knockout (KO) mice develop an autoimmune-like inflammatory disease (Shull *et al* 1992) and colon cancer (Engle *et al* 1999), but not breast cancer (Boivin *et al* 1996). KO mice for *Smad3*, through which all TGF*b*s can signal, develop metastatic colon cancer (Zhu *et al* 1998); whereas, KO mice for *Tgfb1* develop non-metastatic colon cancer, suggesting that either TGF*b*2 or 3 can inhibit metastasis in the colon. However, no other cancers spontaneously develop in *Tgfb1* KO mice. These data strongly suggest that TGF*b*1 is not a tumour suppressor gene in mouse breast tissue. Consequently, one of the other TGF*b*s is likely to be a tumour suppressor in breast. The major phenotype of *Tgfb3* KO mice is a cell adhesion defect resulting in cleft palate, which in turn leads to a perinatal lethality (Proetzel *et al* 1995). A recent study confirms the role of TGF*b*3 as a local factor that causes cell death of alveolar epithelium postpartum in post-lactational involution and remodelling of mammary gland to the virgin-like state (Nguyen and Pollard 2000). A spectrum of developmental defects in different tissues undergoing remodelling and epithelial-mesenchymal interactions are seen in *Tgfb2* KO mice, and they die within 2 h of birth from cardiopulmonary abnormalities (Sanford *et al* 1997).

Most of the available data on TGF*b*2 and its functions in breast cancer research is highly correlative, and most research on TGF*b* ligands in breast cancer has involved addition of TGF*b*1 or inhibition of all 3 TGF*b*s through inhibition of a common receptor, TGF*b*R2. Since growth factors and their signalling pathways are major targets for therapeutic approaches, and since TGF*b* signalling pathways are very likely involved in breast cancer development, it is imperative to determine which TGF*b* could be a therapeutic target. It would be unwise to assume that targeting any one of the three TGF*b*s will give the same result. At the time of lobular cell differentiation during pregnancy, the predominant isoform that expressed in the differentiating alveolar epithelium is TGF*b*2 (Robinson *et al* 1991). Furthermore, women who are at lower risk for breast cancer have higher levels of TGF*b*2 in the breast

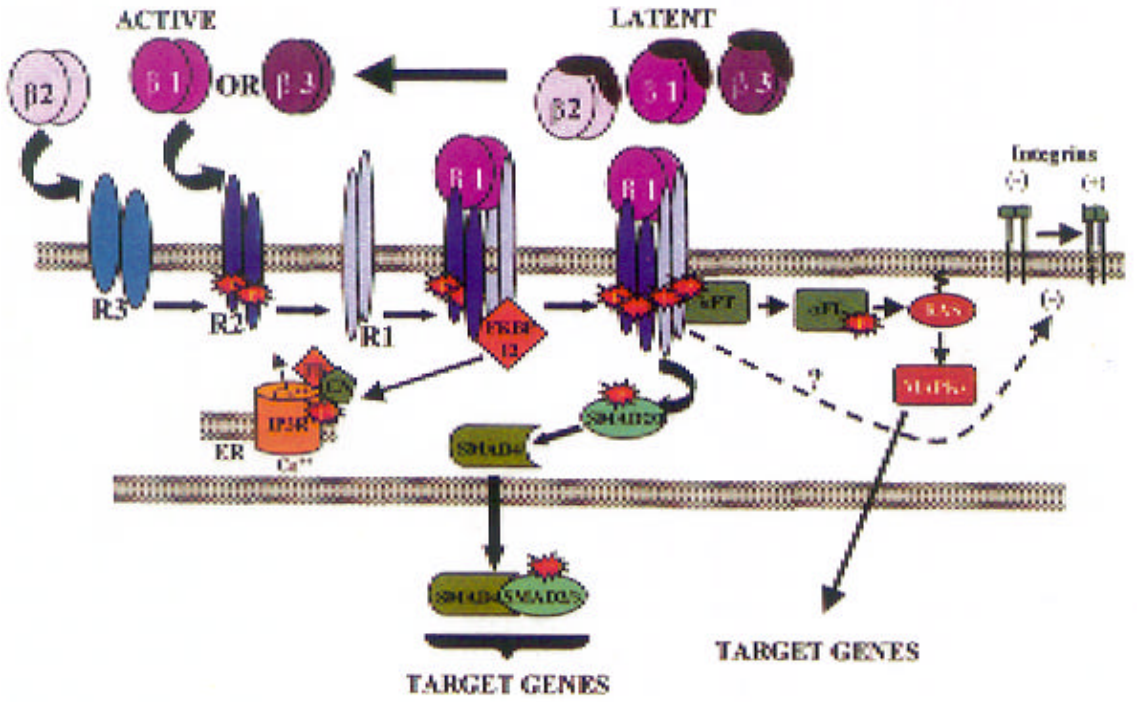


**Figure 1.** TGF*b* signalling via Smads: Upon local activation of TGF*b* ligands, all three TGF*b*s assemble a heteromeric receptor complex with one common receptor, TGF*b*R2. Curiously, TGF*b*2 associates with TGF*b*R3 before interacting with TGF*b*R2 and TGF*b*R1 receptors. The receptor-ligand complex signals to the nucleus through threonine/serine phosphorylation of a series of Smad proteins which then in conjunction with other transcriptional activators and repressors regulate transcription of several target genes. The TGF*b* ligand-receptor complex also modulates several non-genomic signalling pathways such as calcium signalling via FKBP-12.

cystic fluid (Erbas *et al* 1999). Even in culture, TGF*b*1 and TGF*b*2 have been found to have opposite effects on estrogen metabolism in MCF-7 cells, and this is the case even though they both signal through the same receptor, TGF*b*R2 (Ee *et al* 1999; Erbas and Lai 2000). Since TGF*b*2 inhibits (Erbas and Lai 2000) and TGF*b*1 stimulates (Ee *et al* 1999) estrogen production, it is likely that TGF*b*2 is playing the tumour suppressor role. Therefore, targeting the wrong TGF*b* for therapy could lead to either ineffective treatment; worse, it could have the opposite effect of inducing cancer or other undesirable effects. Consequently, it is imperative that it be determined which TGF*b* might be the important target for therapy, and whether that therapy should be designed to stimulate or inhibit TGF*b* function.

Ablation of tumour suppressor genes in the mouse has led to many mouse models for human cancers. However, there are also cases in which a traditional gene knockout results in a lethality that precludes one's ability to investigate the tumour suppressor activities in the desired tissue. To circumvent this and other similar problems, a Cre/LoxP-based conditional KO system has been developed and successfully used (Torres and Kühn 1997). It is relevant to note that TGF*b*2 is hardly detectable in plasma or serum in systemic circulation and that it acts locally in specific tissues in an autocrine/paracrine fashion (Wakefield *et al* 1995). Since *Tgfb2* KO mice die within 2 h of birth, it is necessary to utilize this powerful technology for conditionally ablating the gene only in differentiating alveolar epithelial cells of mammary gland in the adult pregnant mouse. Such an innovative approach will provide a definitive answer to the question whether *Tgfb2* is a tumour suppressor gene for breast cancer in mice. If this turns out to be the case, the new strain of mouse will be a useful animal model for breast cancer and can be used for testing therapeutic approaches.

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