Published in final edited form as:
J Mammary Gland Biol Neoplasia. 2013 June ; 18(2): 143–147. doi:10.1007/s10911-013-9282-8.

The T-box transcription factors TBX2 and TBX3 in mammary gland development and breast cancer

Nataki C. Douglas[1] and **Virginia E. Papaioannou**[2]

[1]Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, Columbia University Medical Center, New York, New York, USA 10032

[2]Department of Genetics and Development, College of Physicians and Surgeons, Columbia University Medical Center, New York, New York, USA 10032

Abstract

TBX2 and *TBX3*, closely related members of the T-box family of transcription factor genes, are expressed in mammary tissue in both humans and mice. Ulnar mammary syndrome (UMS), an autosomal dominant disorder caused by mutations in *TBX3*, underscores the importance of *TBX3* in human breast development, while abnormal mammary gland development in *Tbx2* or *Tbx3* mutant mice provides models for experimental investigation. In addition to their roles in mammary development, aberrant expression of *TBX2* and *TBX3* is associated with breast cancer. *TBX2* is preferentially amplified in *BRCA1/2*-associated breast cancers and *TBX3* overexpression has been associated with advanced stage disease and estrogen-receptor-positive breast tumors. The regulation of *Tbx2* and *Tbx3* and the downstream targets of these genes in development and disease are not as yet fully elucidated. However, it is clear that the two genes play unique, context-dependent roles both in mammary gland development and in mammary tumorigenesis.

Keywords

Tbx2; Tbx3; mammary development; breast cancer; transcription factor; T-box

Introduction

The first connection of the T-box gene family with mammary gland development came with the discovery that ulnar mammary syndrome (UMS, MIM 181450) is caused by mutations in *TBX3*. UMS is a rare autosomal dominant disorder so named for defects in the ulna and the mammary glands. Mammary gland abnormalities affect both sexes and include hypoplastic or aplastic breasts and supernumerary, inverted, or absent nipples with an inability to lactate [1, 2]. Axillary apocrine gland, dental, cardiac and reproductive system abnormalities are additional clinical features of UMS [1–5]. Both *TBX3* and the closely related *TBX2* are overexpressed in mammary gland neoplasia and breast cancer cell lines, suggesting a role for these genes in tumorigenesis and cancer progression. Studies in mice highlight the distinct roles that *Tbx2* and *Tbx3* play in mammary gland development and have characterized their roles in mammary gland induction and maintenance [6, 7]. However, the involvement of these genes in postnatal, pregnant, lactating or involuting mammary glands has yet to be explored.

TBX2 and *TBX3* are members of the T-box family of transcription factor genes, which are defined by the evolutionarily conserved DNA binding domain, the T-box. TBX2 and TBX3

Tel. 212 305-4753, FAX 212 923-2090, vep1@columbia.edu.

share 95% identical amino acid residues in their T-box domains and contain a highly conserved, C-terminal transcription-repression domain [2, 8, 9]. Although *TBX2* is expressed in a wide variety of tissues including the adult breast [10], there are no human developmental syndromes as yet associated with *TBX2.* The *TBX3* expression profile includes the adult breasts, heart, and uterus [1, 11]. UMS is caused by mutations throughout the *TBX3* gene [1, 2, 5]. Characterization of the crystal structure of TBX3 bound to DNA supported the supposition that point mutations affecting the T-box domain impair DNA binding [8]. C-terminal TBX3 mutants associated with UMS have decreased ability to repress transcription and accelerated rates of protein decay [9, 12]. Although some mutations disrupting the T-box domain have been associated with more severe mammary gland defects in males [3], mutations usually result in a spectrum of clinical features with no clear correlation between the location of a mutation and the UMS phenotype.

Tbx2 and *Tbx3* in mouse mammary development

Tbx2 and *Tbx3* are the only members of the T-box family known to be expressed in developing mammary glands. Like their human counterparts, *Tbx2* and *Tbx3* share over 90% sequence identity in their T-box domains and both contain a functional C-terminal transcription-repression domain [6, 9, 12]. At embryonic day (E) 9.5, neither gene is expressed in the mesenchyme at the ventro-lateral body wall between the forelimb and hindlimb where the mammary line will form within 1–2 days. Expression is first detected at E10.5 as a continuous stripe in the mesenchyme underlying the mammary line, with *Tbx3* exhibiting a broader domain of expression than *Tbx2* (Fig. 1A, B). While *Tbx2* is expressed only in mesenchyme at E10.5 and E11.5, *Tbx3* is also expressed in the epithelium of mammary placode (MP) 1 and MP3 at E10.5 and in all 5 pairs of placodes at E11.5 (Fig. 1A–D, **arrowheads**). At E12.5 and E13.5, all mammary buds express *Tbx3* as well as *Lef1,* a downstream target of Wnt signaling and an early marker of mammary differentiation [6, 7, 13, 14]. At E18.5, both *Tbx2* and *Tbx3* are expressed in mesenchyme underlying the nipple sheath [7], but only *Tbx3* is expressed in the epithelium of the branching mammary ducts. The overlapping, yet distinct expression patterns of *Tbx2* and *Tbx3* are maintained throughout gestation. *Tbx2* expression in adults has not as yet been described, but *Tbx3* is expressed in virgin, pregnant, lactating and involuting mammary glands [6, 15].

Effects of *Tbx2* and *Tbx3* mutations on mammary gland development

Homozygosity for a null mutation of *Tbx2* results in embryonic lethality at midgestation, presumably due to heart abnormalities [16], while homozygosity for a null mutation of *Tbx3* results in embryonic lethality from mid to late gestation due to yolk sac and heart abnormalities [6]. Because of UMS, mammary gland development has been studied extensively in *Tbx3* heterozygous and homozygous null mutants. At E13.5, expression of *Lef1* is similar in *Tbx3* heterozygous and wild type embryos, indicating normal mammary bud formation [7]. At E18.5, *Tbx3* heterozygotes have a significantly higher incidence of failed nipple and ductal tree development of mammary gland (MG) 1 and MG2 (Fig. 1E, F). When present, ductal trees in all mammary glands exhibit a trend towards fewer branches, with significantly fewer branches in MG1 and MG2 [7]. In *Tbx3* null homozygotes between E11.5 and E13.5, neither *Wnt10b* nor *Lef1* is expressed and at E12.5 there was no histological evidence of mammary placode formation in five mutant embryos. By E13.5, however, histological examination revealed two rudimentary mammary buds in one mutant embryo and a single bud in a second embryo, both in the position of MG2 [6]. Adult virgin *Tbx3* heterozygous mice have a higher incidence of aplasia of MG1, MG2, and MG3 and reduced branching in ductal trees of all 5 pairs of mammary glands (Fig. 1G, H). Taken together, these data indicate that *Tbx3* is essential for normal mammary placode induction

and that haploinsufficiency of Tbx3 impairs mammary bud maintenance as well as the extent of ductal tree development.

Tbx2 homozygous mutants have no discernible defects in mammary placode induction [7]. Although all mammary glands are present in adult *Tbx2* heterozygous virgin females, the extent of ductal tree development is reduced, with a significant reduction in MG1. In *Tbx2; Tbx3* double heterozygous mice, there is a higher incidence of aplasia of MG1, MG2, and MG3 at E18.5 compared with *Tbx3* heterozygotes. These data reveal a genetic interaction between *Tbx2* and *Tbx3* in maintenance of mammary placodes [7].

Association with human mammary neoplasia

TBX2 and *TBX3* are overexpressed in a number of cancers, including pancreatic, melanoma, and breast [10]. Amplification of the chromosomal region 17q22-q24, a region containing *TBX2, RPS6KB1* and several other candidate oncogenes, is common in breast cancers [17]. *TBX2* is preferentially amplified in *BRCA1/2*-associated tumors compared with sporadic tumors, whereas *RPS6KB1* is not [18, 19]. Furthermore, *TBX2* was amplified equivalently in ductal carcinoma *in situ* and invasive tumors, suggesting that *TBX2* amplification occurs early in the development of *BRCA1/2*-associated tumors [18]. *In situ* hybridization and Northern blot analyses confirmed that *TBX2* is overexpressed in primary tumors and breast cancer cell lines in which *TBX2* is amplified [20, 17]. *TBX3* is also overexpressed in a subset of breast cancer cell lines, with higher levels of *TBX3* in those with estrogen-receptor-positive status, such as MCF7, an epithelial adenocarcinoma cell line [11]. Treatment of MCF7 cells with estrogen induces TBX3 expression, suggesting that estrogen increases the number of cancer stem-like cells in *in vivo* breast tumors via TBX3 signaling [21]. In a cohort of 79 breast cancer patients, TBX3 expression was increased in plasma samples from all patients with advanced disease and in the majority of samples from the entire cohort [22]. TBX3 protein is overexpressed in malignant breast epithelial cells compared with nonmalignant breast tissue [23]. Recent characterization of TBX3 mutations in primary breast tumors strengthens the link between TBX3 and mammary tumorigenesis [24, 25].

Molecular mechanisms in development and neoplasia

Regulation of *Tbx2* and *Tbx3* in the developing mammary gland

While more is known about the molecular function of *Tbx3* than *Tbx2* in mammary gland development, very little is known about how either gene is regulated in this tissue. In the MCF7 cell line, *TBX3* is upregulated in response to the phorbol ester PMA in a protein kinase C-dependent manner via the AP-1 factors c-Jun and JunB, which bind to an AP-1-response element in the *TBX3* promoter [26]. In early mammary gland development *in vivo*, induction of *Tbx3* expression depends on WNT signaling, retinoic acid (RA) signaling, and FGF signaling through Fgfr2. In the absence of *Tbx3*, *Wnt10b* and *Lef1*, RA signaling and FGF signaling are all lost [6, 14, 27], indicating feedback mechanisms in these three signaling pathways for induction and/or maintenance of *Tbx3* expression. In addition, overexpression studies have implicated BMP signaling in a reciprocal negative feedback loop with *Tbx3*, an interaction that may play a role in positioning the mammary placodes during development [28].

Downstream targets and mode of action

Both Tbx2 and Tbx3 are transcriptional repressors and, by virtue of their highly similar DNA binding domains, can bind similar promoter sequences. However, their distinctive mutant phenotypes indicate that they do not function redundantly. In the mouse, overexpression of human TBX3 in mammary epithelium causes hyperplasia by increasing

proliferation and is associated with an increase in mammary stem-like cells. TBX3 was shown to directly bind and repress NFκBIB, an inhibitor of the NFκB pathway known to play a role in regulating cell proliferation [29]. Combined with the hypoplastic phenotype seen in *Tbx3* mutants [6, 7], this indicates a proliferation-promoting role.

An important discovery was the direct transcriptional repression by both TBX2 and TBX3 of the cyclin dependent kinase inhibitors, Cdkn2a ($p19^{ARF}$ in mouse; $p14^{ARF}$ in humans), $p16^{INK4a}$, and $p21^{CIP}$, which are involved in proliferation control. *TBX2* was identified as a potent immortalizing gene in primary fibroblasts and *TBX3* was found to inhibit senescence in mouse immortalized neuronal cells [20, 30]. The senescence pathway is a protective mechanism against cancer and is mainly mediated by $p21^{CIP}$, $p16^{INK4a}$ and $p19^{ARF}$, which stabilizes p53 levels. TBX2 and TBX3 regulate p19/p14 through a variant T-box binding element (TBE) [31] and bind a consensus TBE in the $p21^{CIP}$ promoter [32, 33]. There is evidence that Tbx3 can promote the growth of mammary epithelial cells *in vitro*, with no effect on differentiation or apoptosis, through its ability to repress $p19^{ARF}$ and $p21^{CIP}$, independently of the p53/Mdm2 pathway [15]. However, there is evidence that the hypoplastic mammary gland phenotype in heterozygous *Tbx3* mutants is independent of both p53 and $p19^{ARF}$ [7].

The association of both *TBX2* and *TBX3* with mammary and other types of neoplasia has been a driver of discovery and in some cases mechanisms relevant to normal development have been uncovered in breast cancer cell lines. For example, interactions between *Fgf* and *Tbx3* signaling are involved in mammary gland initiation [14] and FGF9 expands the stem cell population in breast cancer cells and cell lines through FGFR signaling and *TBX3* [21]. However, whether other mechanisms identified in breast cancer cells apply to normal mammary gland development has yet to be determined. For example, TBX3 physically interacts with histone deacetylases (HDACs) in MCF-7 cells and its repressive function on $p14^{ARF}$ is dependent on HDACs [23]. In the same cell line, it was found that in response to stress induced by UV irradiation, Tbx2 protein is phosphorylated by p38 mitogen-activated protein (MAP) kinase, which leads to increased Tbx2 protein levels, nuclear localization and an increase in the ability of Tbx2 to repress the *p21* promoter [34]. Whether TBX3 functions by recruiting HDACs to the TBE in the p14ARF promoter and whether Tbx2 repression of *p21* is enhanced in response to a stress-induced senescence pathway in development needs to be further explored.

Same or different functions?

A distinction between TBX2 and TBX3 function was highlighted using shRNA to knock down either TBX2 or TBX3 in MCF-7 cells, which overexpress both genes. Knockdown of TBX2 decreased proliferation without affecting migratory ability whereas knockdown of TBX3 slightly increased proliferation and reduced migratory ability. At least in this context, TBX2 acts as a powerful growth promoter, whereas TBX3 is mainly required for cell migration [35]. The effect of TBX3 on migratory behavior may underlie the increased invasiveness observed in response to the phorbol ester PMA since knockdown of TBX3 abrogates PMA-induced cell motility in MCF-7 cells [26]. The demonstration that E-cadherin is a downstream target of TBX3 in invasive melanoma cells provides a possible mechanism for the effect on migratory behavior [36].

On the other hand, conflicting results were found with immortalized but otherwise normal mammary epithelial cell lines not expressing *TBX2* (mouse HC11 and human MCF10A). Expression of *TBX2* was sufficient to induce changes characteristic of epithelial-mesenchymal transition (EMT) with increased cell motility and invasion. *TBX2* was also upregulated upon induction of EMT in primary human mammary epithelial cells. Conversely, silencing endogenous *TBX2* in malignant human breast carcinoma cell lines

MDA-MB-435 and MDA-MB-157 led to gain of epithelial characteristics, the loss of mesenchymal markers and the abolition of tumor cell invasion and metastatic potential. Like TBX3, TBX2 was found to directly repress E-cadherin [37]. These conflicting results on whether or not TBX2 promotes migratory cell behavior could be the result of cell type-dependent differences and highlight the need to consider the functions of these genes in specific contexts.

Conclusions

UMS highlights the role of *TBX3* in human mammary gland development, while studies in the mouse have shown that both *Tbx2* and *Tbx3* play important roles in the induction and maintenance of mammary placodes and in the growth of the mammary ductal tree. An important unexplored area is the role of these genes in the adult mammary gland during pregnancy, lactation and involution. *TBX2* and *TBX3* are overexpressed in primary breast cancers and breast cancer cell lines. The association of aberrant expression of both genes with human breast cancer has led to the idea that TBX2 and/or TBX3 could be used as prognostic biomarkers for breast cancer treatment [22].

Acknowledgments

This work was supported by grant number R37HD033082 (V. E. P.) from the Eunice Kennedy Shriver National Institute of Child Health and Human Development, grant number K12HD000849 (N.C.D.) from the Reproductive Scientist Development Program and a grant from the Amos Medical Faculty Development Program/Robert Wood Johnson Foundation (RWJF) (N.C.D.). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NICHD, NIH or RWJF. We thank Sharon Prince, Daniel Concepcion and Andrew J. Washkowitz for critical reading of the manuscript.

Abbreviations

E	embryonic day
EMT	epithelial-mesenchymal transition
HDAC	histone deacetylase
MAP	mitogen-activated protein
MG	mammary gland
MP	mammary placode
RA	retinoic acid
TBE	T-box binding element
UMS	ulnar mammary syndrome

References

1. Bamshad M, Le T, Watkins WS, Dixon ME, Kramer BE, Roeder AD, et al. The spectrum of mutations in *TBX3*: genotype/phenotype relationship in ulnar-mammary syndrome. American Journal of Human Genetics. 1999; 64:1550–62. [PubMed: 10330342]
2. Bamshad M, Lin RC, Law DJ, Watkins WS, Krakowiak PA, Moore ME, et al. Mutations in human *TBX3* alter limb, apocrine and genital development in ulnar-mammary syndrome. Nature Genetics. 1997; 16:311–5. [PubMed: 9207801]
3. Meneghini V, Odent S, Platonova N, Egeo A, Merlo GR. Novel *TBX3* mutation data in families with Ulnar-Mammary syndrome indicate a genotype-phenotype relationship: mutations that do not disrupt the T-domain are associated with less severe limb defects. Eur J Med Genet. 2006; 49(2): 151–8. [PubMed: 16530712]

4. Washkowitz AJ, Gavrilov S, Begum S, Papaioannou VE. Diverse functional networks of *Tbx3* in development and disease. WIREs Syst Biol Med. 2012; 4:273–83.
5. Wollnik B, Kayserili H, Uyguner O, Tukel T, Yuksel-Apak M. Haploinsufficiency of TBX3 causes ulnar-mammary syndrome in a large Turkish family. Annales de Genetique. 2002; 45:213–7. [PubMed: 12668170]
6. Davenport TG, Jerome-Majewska LA, Papaioannou VE. Mammary gland, limb, and yolk sac defects in mice lacking *Tbx3*, the gene mutated in human ulnar mammary syndrome. Development. 2003; 130:2263–73. [PubMed: 12668638]
7. Jerome-Majewska LA, Jenkins GP, Ernstoff E, Zindy F, Sherr CJ, Papaioannou VE. *Tbx3*, the ulnar-mammary syndrome gene, and *Tbx2* interact in mammary gland development through a $p19^{Arf}$/p53-independent pathway. Developmental Dynamics. 2005; 234:922–33. [PubMed: 16222716]
8. Coll M, Seidman JG, Muller CW. Structure of the DNA-bound T-box domain of human TBX3, a transcription factor responsible for ulnar-mammary syndrome. Structure. 2002; 10:343–56. [PubMed: 12005433]
9. He M-L, Wen L, Campbell CE, Wu JY, Rao Y. Transcription repression by *Xenopus* ET and its human ortholog TBX3, a gene involved in ulnar-mammary syndrome. Proceedings of the National Academy of Sciences, USA. 1999; 96:10212–7.
10. Abrahams A, Parker MI, Prince S. The T-box transcription factor Tbx2: Its role in development and possible implication in cancer. IUBMB Life. 2010; 62(2):92–102. [PubMed: 19960541]
11. Fan W, Huang X, Chen C, Gray J, Huang T. *TBX3* and its isoform *TBX3+2a* are functionally distinctive in inhibition of senescence and are overexpressed in a subset of breast cancer cell lines. Cancer Research. 2004; 64:5132–9. [PubMed: 15289316]
12. Carlson H, Ota S, Campbell CE, Hurlin PJ. A dominant repression domain in Tbx3 mediates transcriptional repression and cell immortalization: relevance to mutations in Tbx3 that cause ulnar-mammary syndrome. Human Molecular Genetics. 2001; 10:2403–13. [PubMed: 11689487]
13. Chapman DL, Garvey N, Hancock S, Alexiou M, Agulnik S, Gibson Brown JJ, et al. Expression of the T-box family genes, *Tbx1-Tbx5*, during early mouse development. Developmental Dynamics. 1996; 206:379–90. [PubMed: 8853987]
14. Eblaghie MC, Song S-J, Kim J-Y, Akita K, Tickle C, Jung H-S. Interactions between FGF and Wnt signals and *Tbx3* gene expression in mammary gland initiation in mouse embryos. Journal of Anatomy. 2004; 205:1–13. [PubMed: 15255957]
15. Platonova N, Scotti M, Babich P, Bertoli G, Mento E, Meneghini V, et al. *TBX3*, the gene mutated in ulnar-mammary syndrome, promotes growth of mammary epithelial cells via repression of *p19ARF*, independently of *p53*. Cell Tissue Res. 2007; 328:2, 301–16.10.1007/s00441-006-0364-4
16. Harrelson Z, Kelly RG, Goldin SN, Gibson-Brown JJ, Bollag RJ, Silver LM, et al. *Tbx2* is essential for patterning the atrioventricular canal and for morphogenesis of the outflow tract during heart development. Development. 2004; 131:5041–52. [PubMed: 15459098]
17. Bärlund M, Monni O, Kononen J, Cornelison R, Torhorst J, Sauter G, et al. Multiple genes at 17q23 undergo amplification and overexpression in breast cancer. Cancer Res. 2000; 60:5340–4. [PubMed: 11034067]
18. Sinclair CS, Adem C, Naderi A, Soderberg CL, Johnson M, Wu K, et al. *TBX2* is preferentially amplified in *BRCA1*- and *BRCA2*-related breast tumors. Cancer Research. 2002; 62:3587–91. [PubMed: 12097257]
19. Adem C, Soderberg CL, Hafner K, Reynolds C, Slezak JM, Sinclair CS, et al. *ERBB2*, *TBX2*, *RPS6KB1*, and *MYC* alterations in breast tissues of *BRCA1* and *BRCA2* mutation carriers. Genes, Chromosomes & Cancer. 2004; 41:1–11. [PubMed: 15236312]
20. Jacobs JJL, Keblusek P, Robanus-Maandag E, Kristel P, Lingbeek M, Nederlof PM, et al. Senescence bypass screen identifies *TBX2*, which represses *Cdkn2a* ($p19^{ARF}$) and is amplified in a subset of human breast cancers. Nature Genetics. 2000; 26:291–9. [PubMed: 11062467]
21. Fillmore CM, Gupta PB, Rudnick JA, Caballero S, Keller PJ, Lander ES, et al. Estrogen expands breast cancer stem-like cells through paracrine FGF/Tbx3 signaling. Proc Nat Acad Sci. 2010; 107:21737–42. [PubMed: 21098263]

22. Lomnytska M, Dubrovska A, Hellman U, Volodko N, Souchelnytskyi S. Increased expression of cSHMT, Tbx3 and utrophin in plasma of ovarian and breast cancer patients. International Journal of Cancer. 2006; 118:412–21.
23. Yarosh W, Barrientos T, Esmailpour T, Lin L, Carpenter PM, Osann K, et al. TBX3 is overexpressed in breast cancer and represses $p14^{ARF}$ by interacting with histone deacetylases. Cancer Res. 2008; 68:693–9. [PubMed: 18245468]
24. Stephens PJ, Tarpey PS, Davies H, Van Loo P, Greenman C, Wedge DC, et al. The landscape of cancer genes and mutational processes in breast cancer. Nature. 2012; 486(7403):400–4. [PubMed: 22722201]
25. Network TCGA. Comprehensive molecular portraits of human breast tumours. Nature. 2012; 490(7418):61–70. [PubMed: 23000897]
26. Mowla S, Pinnock R, Learner VD, Goding CR, Prince S. PMA-induced up-regulation of TBX3 is mediated by AP-1 and contributes to breast cancer cell migration. Biochem J. 2011; 433:145–53. [PubMed: 20942798]
27. Cho K-W, Kwon H-J, Shin J-O, Lee J-M, Cho S-W, Tickle C, et al. Retinoic acid signaling and the initiation of mammary gland development. Dev Biol. 2012; 365:259–66. [PubMed: 22387209]
28. Cho K-W, Kim J-Y, Song S-J, Farrell E, Eblaghie MC, Kim H-J, et al. Molecular interactions between *Tbx3* and *Bmp4* and a model for dorsoventral positioning of mammary gland development. Proc Natl Acad Sci U S A. 2006; 103(45):16788–93. [PubMed: 17071745]
29. Liu J, Esmailpour T, Shang X, Gulsen G, Liu A, Huang T. TBX3 over-expression causes mammary gland hyperplasia and increases mammary stem-like cells in an inducible transgenic mouse model. BMC Developmental Biology. 2011; 11:65. [PubMed: 22039763]
30. Brummelkamp TR, Kortlever RM, Lingbeek M, Trettel F, MacDonald ME, van Lohuizen M, et al. TBX-3, the gene mutated in ulnar-mammary syndrome, is a negative regulator of $p19^{ARF}$ and inhibits senescence. Journal of Biological Chemistry. 2002; 277:6567–72. [PubMed: 11748239]
31. Lingbeek ME, Jacobs JJL, van Lohuizen M. The T-box repressors *TBX2* and *TBX3* specifically regulate the tumor-suppressor $p14^{ARF}$ via a variant T-site in the initiator. Journal of Biological Chemistry. 2002; 277:26120–7. [PubMed: 12000749]
32. Hoogaars WMH, Barnett P, Rodriguez M, Clout DE, Moorman AFM, Goding CR, et al. TBX3 and its splice variant TBX3 + exon 2a are functionally similar. Pigment Cell Melanoma Res. 2008; 21:379–87. [PubMed: 18444963]
33. Prince S, Carreira S, Vance KW, Abrahams A, Goding CR. Tbx2 directly represses the expression of the $p21^{WAF1}$ cyclin-dependent kinase inhibitor. Cancer Research. 2004; 64:1669–74. [PubMed: 14996726]
34. Abrahams A, Mowla S, Parker MI, Goding CR, Prince S. UV-mediated regulation of the anti-senescence factor Tbx2. J Biol Chem. 2007; 283:2223–30. [PubMed: 18025091]
35. Peres J, Davis E, Mowla S, Bennett DC, Li JA, Wansleben S, et al. The highly homologous T-box transcription factors, TBX2 an TBX3, have distinct roles in the oncogenic process. Genes & Cancer. 2010; 1(3):272–82. [PubMed: 21779450]
36. Rodriguez M, Aladowicz E, Lanfrancone L, Goding CR. Tbx3 represses E-cadherin expression and enhances melanoma invasiveness. Cancer Res. 2008; 68(19):7872–81. [PubMed: 18829543]
37. Wang B, Lindley LE, Fernandez-Vega V, Rieger ME, Sims AH, Briegel KJ. The T box transcription factor TBX2 promotes epithelial-mesenchymal transition and invasion of normal and malignant breast epithelial cells. PLoS ONE. 2012; 7(7):e41355. [PubMed: 22844464]

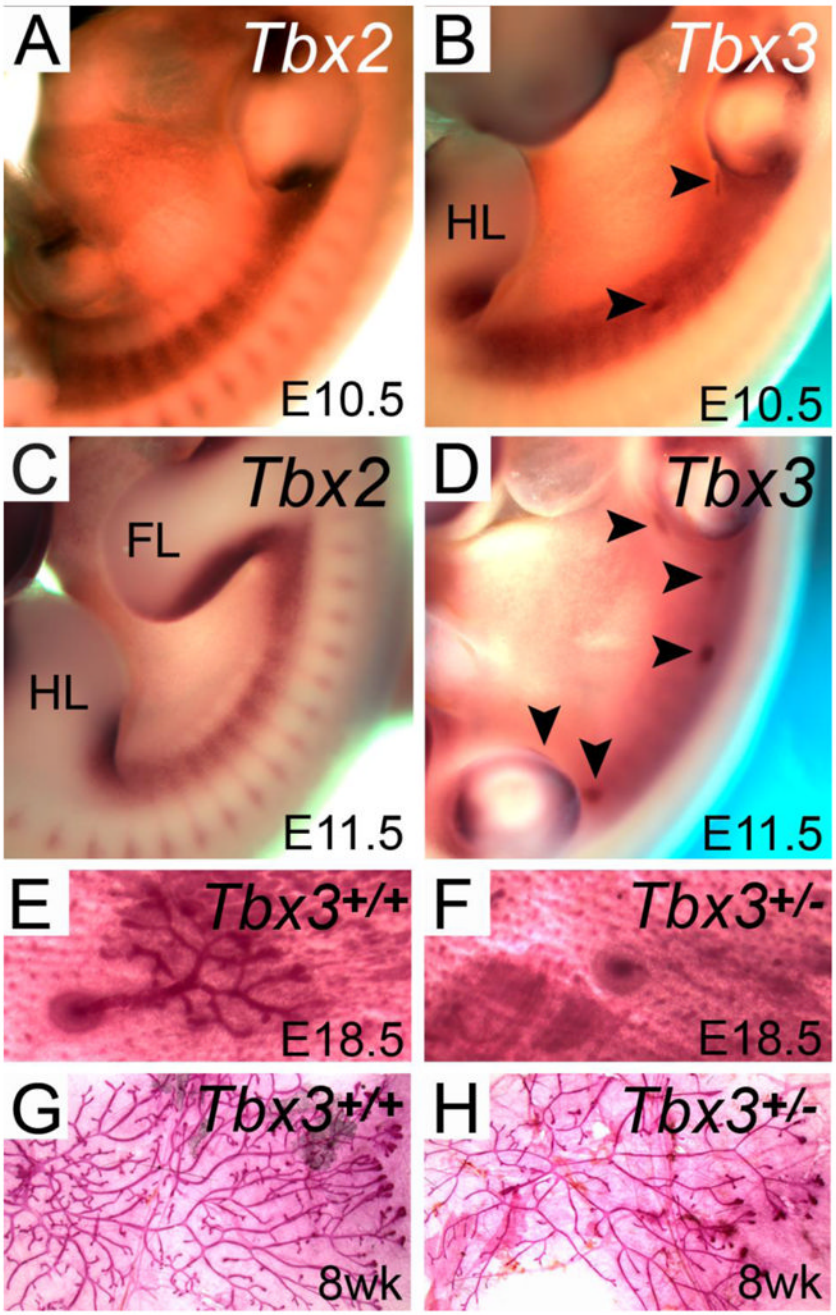

Figure 1.

Expression of *Tbx2* and *Tbx3* in the developing mammary gland and the effect of heterozygosity for a mutation in *Tbx3*. A–D. Whole mount in situ hybridization for *Tbx2* and *Tbx3* at embryonic day (E) 10.5 and E11.5 shows expression of both genes in the mammary line between the forelimb and hindlimb and *Tbx3* expression in the mammary placodes when they first appear (arrowheads). E, F. Skin and mammary gland preparation from late gestation embryos stained with Carmine's Alum to show the nipple and ductal tree, which is reduced in *Tbx3* heterozygotes. G, H. Mammary gland preparations from 8 week old virgin females stained with Carmine's Alum showing a reduction in the branching network of *Tbx3* heterozygotes. (Modified with permission from [7]).