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The roles and regulation of TBX3 in development and disease

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Abstract

TBX3, a member of the ancient and evolutionary conserved T-box transcription factor family, is a critical developmental regulator of several structures including the heart, mammary glands, limbs and lungs. Indeed, mutations in the human *TBX3* lead to ulnar mammary syndrome which is characterized by several clinical malformations including hypoplasia of the mammary and apocrine glands, defects of the upper limb, areola, dental structures, heart and genitalia. In contrast, TBX3 has no known function in adult tissues but is frequently overexpressed in a wide range of epithelial and mesenchymal derived cancers. This overexpression greatly impacts several hallmarks of cancer including bypass of senescence, apoptosis and anoikis, promotion of proliferation, tumour formation, angiogenesis, invasion and metastatic capabilities as well as cancer stem cell expansion. The debilitating consequences of having too little or too much TBX3 suggest that its expression levels need to be tightly regulated. While we have a reasonable understanding of the mutations that result in low levels of functional TBX3 during development, very little is known about the factors responsible for the overexpression of TBX3 in cancer. Furthermore, given the plethora of oncogenic processes that TBX3 impacts, it must be regulating several target genes but to date only a few have been identified and characterised. Interestingly, while there is compelling evidence to support oncogenic roles for TBX3, a few studies have indicated that it may also have tumour suppressor functions in certain contexts. Together, the diverse functional elasticity of TBX3 in development and cancer is thought to involve, in part, the protein partners that it interacts with and this area of research has recently received some attention. This review provides an insight into the significance of TBX3 in development and cancer and identifies research gaps that need to be explored to shed more light on this transcription factor.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Keywords

Transcription factor; T-box factors; TBX3; Heart development; Limb development; Mammary gland development; Lung development; Ulnar mammary syndrome; Obesity; Rheumatoid arthritis; Cancer; Signalling; Target genes; Co-factors; Stem cells

1. Introduction

The T-box 3 gene (*TBX3*) is a member of the ancient T-box gene family which is conserved across a wide spectrum of species. A mouse mutation that results in a short tail identified in 1927 led to the discovery and ultimate cloning of *Brachyury*, the prototype of the family, in 1990 and, as shown in Table 1, there are currently 17 paralogues in human, mouse and rat that are grouped into five subfamilies, namely Brachyury (T), T-brain (Tbr1), TBX1, TBX2, and TBX6 (Papaioannou, 2014). Tbx3 is a member of the Tbx2 subfamily which includes Tbx2, Tbx4 and Tbx5. Members of this subfamily originated from the duplication of a primordial gene by an unequal crossing over event which initially gave rise to the *Tbx2/Tbx3* and *Tbx4/Tbx5* cognate gene pairs and their subsequent duplication led to the four independent genes. Functional studies have shown that T-box family members are transcription factors with a highly conserved DNA binding domain known as the T-box. They can activate and/or repress their target genes through binding a partially palindromic sequence (T(G/C)ACACCT AGGTGTGAAATT) known as the T-element, or half sites within this sequence as well as protein co-factor binding sites (Wilson and Conlon, 2002). Their importance has been well established in the field of developmental biology where they play essential roles from as early as cell-fate determination all the way through to organogenesis (Packham and Brook, 2003; Papaioannou, 2001). Not surprisingly, numerous human congenital developmental syndromes are associated with mutated T-box genes and there is significant evidence implicating T-box factors as major contributors of cancer processes as either oncoproteins and/or tumour suppressors (Wansleben et al., 2014).

1.1. TBX3 gene location and structure

The human *TBX3* gene maps to the reverse strand of chromosome 12 at position 12q23–24.1 and consists of 7 exons within a 4.7 kb region which spans from 114670255 bp to 114684175 bp (ENSEMBL assembly release GRCh38.p12) (Fig. 1). It encodes a 723 amino acid protein with part of exon 1, exons 2 and 3, and part of exon 4, encoding the conserved T-box domain, exon 1 and 2 encode one of two repression domains (R2), exon 6 encodes the activation domain and part of exon 6 and 7 encode the second repression domain (R1) (Bamshad et al., 1997; Carlson et al., 2001; He et al., 1999).

1.2. TBX3 mRNA structure and splicing

Alternative processing and splicing gives rise to at least 4 distinct TBX3 isoforms with *TBX3* and *TBX3+2a* being the predominant isoforms. *TBX3+2a* results from alternative splicing of the second intron which leads to the addition of the +2a exon and consequently this isoform has an additional 20 amino acids within the T-box DNA binding domain (Fig. 1) (Bamshad et al., 1997; Fan et al., 2004). Considering the location of the extra 20 amino acids in the TBX3+2a isoform, it is tempting to speculate that it may regulate a different set

of downstream targets to TBX3. Indeed, an initial study by Fan et al. (2004) indicated that whereas Tbx3 inhibits senescence in mouse embryonic fibroblasts (MEFs), Tbx3+2a accelerated the process. Subsequent studies have, however, shown that the two TBX3 isoforms have similar roles, at least functionally if not always mechanistically. For example, as will be seen later, they can both bind and repress several common target genes during embryonic development and cancer and they can both inhibit the process of mRNA splicing by directly binding RNAs containing the core motif of a T-element (Hoogaars et al., 2008; Krstic et al., 2016, 2019; Rodriguez et al., 2008; Zhao et al., 2014). It therefore seems likely that the function of the TBX3 and Tbx3+2a isoforms may vary slightly across different cell types.

2. TBX3 protein

2.1. TBX3 functional domains

TBX3 is a transcription factor characterised by a DNA-binding domain (DBD) also called the T-box, a nuclear localization signal (NLS), two repression domains (R2 and R1) and an activation domain (A) (Fig. 1). The NLS, T-box and R2 domains are 100% conserved between human and mouse and their R1 and activation domains share 98.4% and 77.5% homology respectively. The T-box is situated in the amino terminus (position 105–287; REFSEQ: accession NM 005996.3) and consists of 182 amino acids. It recognizes highly related DNA sequences, called T-elements, although it can also recognise variations within the consensus T-element sequences. The NLS is a 6 basic amino acid cluster ‘RREKRK’, which spans amino acid residues 292–297. While the R2 consists of 77 amino acids and is located within the T-box (123–200), the dominant repressor domain R1 consists of 56 amino acids and is in the C terminus (567–623). The activation domain consists of 77 amino acids and is located between amino acids 423–500 of the TBX3 protein (Carlson et al., 2001).

2.2. DNA binding properties of TBX3

Coll et al. (2002) resolved the 3-dimensional structure of the TBX3 DBD interacting with its palindromic consensus target DNA (5′TAATT TCACACCTAGGTGTGAAAT3′). The crystallographic data showed that TBX3 recognises the core 10 base pair sequence (5′TTTCACACCT3′) referred to as the half T-element. Furthermore, the authors show that the TBX3 DBD interacts with the GC base pairs 3 and 5 through direct hydrogen bonds, with TA base pairs 8 and 9 through hydrophobic interactions, and with base pairs 1, 2 and 4 through an indirect mechanism. Hoogaars et al. (2008), confirmed that both TBX3 isoforms bind the half T-element efficiently and that the +2a region does not alter the DNA binding ability of the TBX3 DBD. Coll et al. (2002) further showed that Tbx3 binds its consensus sequence as two monomers, with each one recognising one of the half T-elements in the palindromic target sequence. It was however predicted that TBX3, like all other T-box members identified to date, will bind its biological downstream effectors as a single monomer through the half T-element of the palindrome target sequence (Coll et al., 2002; Wilson and Conlon, 2002).

To date, only TBX3 target sites with sequences closely related to half-sites of the original palindrome have been identified. Indeed, while there is still a paucity of information

available on TBX3 target genes, studies have shown that TBX3 can regulate diverse cellular processes through its ability to transcriptionally repress or activate biologically relevant factors through mechanisms involving single half T-elements. For example, TBX3 directly represses transcription of the tumour suppressors, $p19^{ARF}$ ($p14^{ARF}$ in humans) through CACCTCTGGTGCCA in primary breast tumours (Lingbeek et al., 2002), $p21^{WAF1/CIP1}$ through a GTGTGA close to the initiator in chondrosarcoma (Willmer et al., 2016a, 2016b), *E-cadherin* through CAGGTGT in melanoma (Rodriguez et al., 2008) and *TBX2* through GACACCT in breast cancer and melanoma cells (Li et al., 2014). Furthermore, Weidgang et al. (2013) showed that in mouse embryonic stem cells (mESCs), Tbx3 directly bound highly conserved T-elements to activate the promoters of *Eomes*, *T* and *Sox17* which are essential for mesoderm differentiation. Lu et al. (2011) also reported that TBX3 directly binds a conserved T-element at −700 bp of the *Gata6* promoter to activate it in mESCs in order to promote extra embryonic endodermal differentiation. Interestingly, TBX3 represses *PTEN* through a region of its promoter which lacks putative T-elements, but which forms an important regulatory unit for *PTEN* transcriptional activators. This raises the possibility that TBX3 may also repress some of its target genes through interfering with transcriptional activators (Burgucu et al., 2012).

2.3. Phosphorylation of TBX3

While there are several predicted post-translational modification sites for TBX3 including 10 ubiquitination, 1 acetylation, 2 methylation and 29 phosphorylation sites only the SP190, SP692 and S720 phosphorylation sites have been fully characterised. The kinases involved are cyclin A-CDK2 at either SP190 or SP354, p38 MAP kinase at SP692 and AKT Serine/Threonine Kinase 3 (AKT3) at S720 (Peres et al., 2015; Willmer et al., 2016; Yano et al., 2011). The SP190 motif within the DBD is highly conserved across T-box factors and species suggesting that it must have an important regulatory role. Indeed, while the kinase responsible for phosphorylating TBX3 at this site has not been identified, a SP190 pseudo phosphorylated TBX3 protein has reduced ability to bind and transcriptionally repress $p21^{WAF1/CIP1}$ and consequently to promote proliferation (Willmer et al., 2016a, 2016b). Phosphorylation within the N-terminal (1–371) half of the TBX3 protein by the cyclin ACDK2 complex is important for stabilizing TBX3 during the S phase of the cell cycle and allowing for its functional role in driving S phase progression (Willmer et al., 2015). SP motifs are the minimum consensus sequences for cyclin A-CDK2 and since SP190 and SP354 are the only SP motifs within this region either one or both must be involved in this phosphorylation. Furthermore, phosphorylation of TBX3 by the p38 MAP kinase at SP692 in embryonic kidney cells enhances its ability to transcriptionally repress its well-known target, *E-cadherin*, to promote migration (Yano et al., 2011). Importantly, in melanoma, phosphorylation of TBX3 at S720 by AKT3 promotes its protein stability, nuclear localisation, transcriptional repression of *E-cadherin*, and its role in cell migration and invasion (Peres et al., 2015).

2.4. Interacting proteins

Increasing evidence suggests that the function of TBX3 as either a transcriptional repressor or transcriptional activator is, in part, modulated by protein co-factors. For example, during embryogenesis it can interact with other transcription factors such as Nkx2–5, Msx and

Sox4 to assist it binding to its target genes to regulate heart development (Bakker et al., 2008; Boogerd et al., 2008, 2011, Christoffels et al., 2000, 2004; Hoogaars et al., 2008; Stennard and Harvey, 2005). In the cancer context, TBX3 can interact with histone deacetylases (HDACs) to repress target genes. Indeed, it interacts with HDACs 1, 2, 3 and 5 to repress the tumour suppressor $p14^{ARF}$ in breast cancer and with HDAC5 to repress *E-cadherin* to promote metastasis in hepatocellular carcinoma (Dong et al., 2018a, 2018b; Yarosh et al., 2008). Lastly, Kumar et al. (2014a, 2014b) showed that TBX3 interacts with CAPERα to repress the long non-coding RNA, *UCA1*, resulting in the bypass of senescence through loss of UCA1-mediated stabilisation of *p16INK4A* mRNA.

3. Expression and function of TBX3 during development

TBX3 plays multiple roles during embryonic development as evidenced by the abnormalities reported for homozygous and heterozygous mice as well as the phenotype of individuals with the ulnar mammary syndrome (UMS) which results from mutations in human *TBX3*.

During mouse embryonic development, Tbx3 is expressed in the inner cell mass of the blastocyst, in the extraembryonic mesoderm during gastrulation, and in the developing heart, limbs, musculoskeletal, mammary glands, nervous system, skin, eye, liver, pancreas, lungs, pituitary glands and genitalia (Chapman et al., 1996; Tümpel et al., 2002; Davenport et al., 2003; Moorman et al., 2004; Cho et al., 2006; Lin et al., 2007; Bakker et al., 2008; Mesbah et al., 2008; Pontecorvi et al., 2008; Lüdtke et al., 2009, 2016; Begum and Papaioannou, 2011; Colasanto et al., 2016; Emechebe et al., 2016; Ichijo et al., 2017; López et al., 2018; Quarta et al., 2019; Karolak et al., 2019). Importantly, *Tbx3* null embryos show defects in, among other structures, the heart, mammary glands and limbs and they die *in utero* by embryonic day E16.5, most likely due to yolk sac and heart defects (Davenport et al., 2003). These observations, underscored by studies described below, have illustrated that Tbx3 plays crucial roles in the development of the heart, mammary glands, limbs and lungs.

3.1. Heart development

During the onset of cardiogenesis, the linear heart tube undergoes looping and forms a chamber myocardium, which consists of ventricular and atrial chambers, and a non-chamber myocardium, which consists of the inflow and outflow tract (IFT and OFT), the atrioventricular canal (AVC) and the inner curvatures (Christoffels et al., 2004). During the process of looping, a chamber myocardiumspecific gene program, which includes expression of *Nppa, Cx40, Cx43*, and *Chisel*, initiates proliferation and differentiation in specific regions of the heart tube to form the chamber myocardium (Christoffels et al., 2000; Delorme et al., 1997; Van Kempen et al., 1996). In contrast, regions that form the non-chamber myocardium do not express this specific gene program and largely retain the phenotype of the early myocardium. Cells from the non-chamber myocardium form the cardiac conduction system (CCS), which controls the co-ordinated contraction of the heart (Christoffels et al., 2004; Greulich et al., 2011).

During early heart development, *Tbx3* is exclusively expressed in the non-chamber myocardium of the AVC and the OFT (Christoffels et al., 2004). At E10.5, *Tbx3* expression is found in the AVC, atrioventricular bundle (AVB), sinoatrial node (SAN) and OFT. In the

formed heart at E16.5, Tbx3 fully delineates the CCS and is expressed in the SAN, AVB, atrioventricular node (AVN) and proximal bundle branches (BBs) (Fig. 2A). *Tbx3* expression is particularly important for the formation of the CCS, AVB, and the ventricular septum of the heart and Tbx3-deficient embryos develop ventricular septal defects, delay in heart looping and outflow tract malformations (Bakker et al., 2008; Mesbah et al., 2008; Ribeiro et al., 2007; Washkowitz et al., 2012). It is believed that Tbx3 contributes to the developing CCS by firstly modulating cell division which results in constrictions between chambers, and secondly by directly repressing chamber myocardium genes by cooperatively binding their promoters along with other transcription factors (Christoffels et al., 2004; Hoogaars et al., 2004; Washkowitz et al., 2012). Indeed, Tbx3 binds cooperatively with Msx1 and Msx2 in the repression of *Cx43* and with Nkx2.5 to repress *Nppa* in the non-chamber myocardium to block chamber formation (Fig. 2A) (Boogerd et al., 2008; Hoogaars et al., 2004). Furthermore, *Tbx3* mutant hearts show elevated expression of *Cx40*, *Cx43*, and *Nppa* in the non-chamber AVC and ectopic expression of Tbx3 leads to the upregulation of CCS genes, such as *Lbh* and *Hcn4*, and the development of functional conduction tissue (Fig. 2A) (Hoogaars et al., 2007). These results show that Tbx3 exerts an important function by repressing the chamber-specific genetic program in regions from which functional tissues of the non-chamber myocardium are formed.

Tbx3, alongside with Tbx18 and Shox2, is also important for the development of the functional SAN (Espinoza-Lewis et al., 2009; Hoogaars et al., 2007; Wiese et al., 2009). The SAN is the pacemaker of the heart and initiates the heartbeat and controls the rate and the rhythm of contraction throughout life (Hoogaars et al., 2007; Protze et al., 2017). Lineage tracing showed that the SAN originates from Tbx3 positive cells in the early heart tube (Mohan et al., 2018). Importantly, Tbx3 was shown to regulate the pacemaker gene program and phenotype by suppressing the atrial differentiation gene program in the SAN. Ectopic expression of Tbx3 in the atria of mouse models resulted in the development of functional ectopic pacemakers and induced Tbx3 expression reprogrammed terminally differentiated cardiomyocytes into pacemaker cells (Bakker et al., 2012; Hoogaars et al., 2007). Recently, SAN-like pacemaker cells were generated from human pluripotent stem cells without genetic manipulation and these cells were positive for Tbx3, Tbx18 and Shox2 and were shown to be able to function as a biological pacemaker in vivo (Protze et al., 2017). Taken together, Tbx3 plays an important role in the pacemaker function of the heart.

To date, very little is known about signalling pathways that regulate *Tbx3* expression during heart formation. However, there is increasing evidence that the Bone Morphogenetic Protein (BMP) pathway is an important upstream modulator of *Tbx3* expression (Fig. 2A) (Yamada et al., 2000; Yang et al., 2006). BMPs are members of the transforming growth factor β (TGF-β) gene family and play a critical role in the formation of the non-chamber myocardium (Shi et al., 2000). Yamada et al. (2000) showed that *Tbx3* expression patterns overlap with those of Bmp2 during chick embryonic heart development and that ectopic expression of Bmp2 induces *Tbx3* expression in non-cardiogenic tissue capable of developing into cardiac tissue. Moreover, Yang et al. (2006) showed that Tbx3 expression is downregulated when the Type I Bmp receptor is ablated and that *Tbx3* is a direct target of Bmp Smads in vivo.

3.2. Mammary gland development

In mice, the development of the mammary glands (Fig. 2B) begins at E10.5 with the formation of the milk line between the forelimb and hindlimb of each flank, which forms an ectodermal ridge characterized by *Wnt10b* expression (Veltmaat et al., 2004). At E11.5 the mammary placodes begin to form and by E13.5, five pairs of mammary placodes have developed along the milk line and start to expand into mammary buds and by E18.5 the branching ductal system has formed. Studies have shown that Fibroblast growth factor (Fgf) signalling is critical for the induction and maintenance of mammary placodes 1, 2, 3 and 5 and for the expression of the earliest known breast differentiation marker, Lymphoid enhancer factor 1 (Lef1), a downstream mediator of Wnt signalling (Mailleux et al., 2002). Wnt signalling plays a critical role in mammary bud formation as illustrated by *Lef1* null mice developing a reduced number of mammary buds and when the pathway is inhibited by Dickkopf-1, bud formation is completely lost at E11.5 (Andl et al., 2002; van Genderen et al., 1994).

Tbx3 first appears at E10.5 in the mesenchymal milk line and at E11.5 it is one of the earliest markers of mammary gland epithelium in the placodes. *Tbx3* continues to be expressed at E13.5 in the mammary buds and by E18.5 *Tbx3* is expressed in the mesenchyme surrounding the nipples (Chapman et al., 1996; Davenport et al., 2003) (Fig. 2B). The functional significance of Tbx3 expression during mammary gland development has been demonstrated in several studies. Loss of *Tbx3* in homozygous mutant mice results in failure of placode induction and heterozygous mutant mice have decreased ductal tree development and failed nipple formation (Davenport et al., 2003; Jerome-Majewska et al., 2005; Rowley et al., 2004). Interestingly, failure of placode induction in homozygous mutant mice, places Tbx3 activity upstream of both Fgf and Wnt signalling (Fig. 2B) (Rowley, et al., 2004). This is evidenced by the loss of *Wnt10b* and *Lef1* expression, as well as Fgf signalling when *Tbx3* is absent (Davenport, et al., 2003). Interestingly, both Wnt and Fgf signalling, have also been described to feed into the regulatory network of Tbx3 during mammary gland development (Fig. 2B). Indeed, when Wnt or Fgf signalling is inhibited by CK1–7 or SU5402 respectively in early bud formation, *Tbx3* expression is completely abolished. Taken together, these results indicate that Wnt, Fgf and Tbx3 are involved in feedforward and feedback loops to regulate the expression of each other (Eblaghie et al., 2004). Cho et al. (2006) also provided evidence that a reciprocal negative regulation between Bmp4 and Tbx3 expression is crucial for mammary gland positioning (Fig. 2B). Furthermore, Nrg3 transmits signals downstream of Tbx3 and Fgf signalling from somite to the overlying ectoderm to promote their local aggregation in the mammary placode and subsequent placode formation (Howard et al., 2005; Howard and Ashworth, 2006).

3.3. Limb and digit development

Limb development (Fig. 2C) is initiated by the emergence of small buds from the lateral body wall, consisting of a lateral plate mesoderm (LPM) and an overlying ectodermal layer. The outgrowth and patterning of the limb buds is dependent on three key signalling centres: the apical ectodermal ridge (AER), the dorsal ectoderm (DE) and the zone of polarizing activity (ZPA). These induce and co-ordinate specific outgrowth of the limb bud along the dorsal-ventral, anterior-posterior, and proximal-distal axes (Capdevila and Belmonte, 2001).

Activities of the AER, ZPA and DE depend on complex signalling pathways, with the major contributors being the Fgf and Sonic Hedgehog (Shh) pathways (Fig. 2C). In the limb mesenchyme, Fgf10 induces Fgf8 in the overlying ectoderm and the formation of the AER and Fgf8 induces Shh expression to establish the ZPA. Together, Fgf and Shh signalling promote digit development and control digit number and patterning (Martin, 1998; Ohuchi et al., 2000).

Tbx3 is first expressed at the posterior margin and thereafter in the mesenchyme of the anterior and posterior margins of the early limb buds and at the AER (Fig. 2C). By E13.5, expression of *Tbx3* in the AER is restricted to the tips of the digits (Chapman et al., 1996; Gibson-Brown et al., 1996). Importantly, Tbx3 homozygous mutant embryos display forelimb abnormalities, severe reduction in hindlimb bud development and reduced AER formation (Davenport, et al., 2003). Furthermore, a recent study by Emechebe et al. (2016) revealed that Tbx3 positively regulates Shh signalling to control digit number (Fig. 2C). The authors generated *Tbx3 fl/fl;Cre* mutant mice in which *Tbx3* expression was stopped at different stages of mouse limb development and observed different abnormalities depending on when *Tbx3* expression was halted. Whereas loss of *Tbx3* expression in early development disrupted Shh signalling and resulted in failure of limb initiation and limb abnormalities, later deletion of *Tbx3* in the posterior limb mesenchyme resulted in digit loss. It is important to note that Tbx3 also controls digit number via a Shh-independent, cilium-based Hedgehog pathway and loss of *Tbx3* in the anterior limb results in preaxial polydactyly. In addition, Tbx3 expression has been reported to be downstream of the retinoic acid (RA) signalling pathway which plays a critical role in early limb development. Indeed, loss of components of the RA pathway in mutant mice leads to various forelimb abnormalities ranging from small limbs with digit anomalies to absent limbs (Lohnes et al., 1994; Sandell et al., 2007). Furthermore, an RA–receptor complex directly activates the *Tbx3* promoter and RA deficient embryos show decreased expression of *Tbx3* in the limb (Ballim et al., 2012). *Hand2* and *Tbx3* also form an important regulatory network in limb development. For example, anterior and posterior polarization of the limb bud mesenchyme requires the expression of *Tbx3 (*and *Gli3)* which is regulated by Hand2 and *Hand2* is downregulated in the limbs of *Tbx3* mutant mice (Davenport, et al., 2003; Osterwalder et al., 2014; Sheeba and Logan, 2017). Furthermore, *Tbx3* and *Hand2* are both regulated by the microRNA-processing enzyme Dicer to ensure proper limb bud positioning (Zhang et al., 2011a, 2011b). In addition, experiments in the chick have shown that Tbx3 plays an important role in posterior digit specification, acting together with Tbx2 and the interdigital BMP signalling cascade (Suzuki et al., 2004).

3.4. Lung development

The formation of the lungs is initiated in the ventral wall of the foregut endoderm at E9.0. Primary lung buds and tracheal primordium start to develop at E9.5 and at E10.5 secondary lung buds develop as outgrowths from the primary buds. From E11.5 onwards, the epithelium undergoes branching morphogenesis and eventually forms a respiratory (bronchial) tree (Cardoso and Lu, 2006). Lung development is mediated by members of the Bmp, Wnt, Fgf, and Shh signalling families and in the lung mesenchyme during E10.5 and E14.5 they converge on Tbx3 and Tbx2 to maintain mesenchymal proliferation and lung

branching morphogenesis (Fig. 2D) (Herriges and Morrisey, 2014; Li et al., 2004; Lüdtke et al., 2016). For example, Wnt signalling depends on active Bmp signalling and the loss of *Wnt2/2b* leads to failure of trachea and branching lung formation and inactivation of the Bmp receptors Bmpr1a and Bmpr1b leads to tracheal agenesis and ectopic primary bronchi (Domyan et al., 2011; Goss et al., 2009). Fgf signalling is regulated by, among other pathways, Bmp4 and Shh and when Fgf signalling is disrupted, branching is abrogated (Ohuchi et al., 2000; Pepicelli et al., 1998; Sekine et al., 1999; Weaver et al., 2000). Moreover, *Shh* null mice have hypoplastic lungs due to incorrect branching morphogenesis (Litingtung et al., 1998; Pepicelli et al., 1998). Finally, Tbx2 and Tbx3 regulate mesenchymal proliferation by maintaining pro-proliferative Wnt signalling through direct repression of the Wnt antagonists *Frzb* and *Shisa3* (Lüdtke et al., 2016).

4. TBX3 in stem cell biology

Embryonic stem cells (ESCs) and adult stem cells, are undifferentiated cells which when they divide have the potential to either remain a stem cell or to differentiate into other specialised cells (Mo, et al., 2014; Yin and Zhang, 2015). ESCs are pluripotent cells derived from the inner cell mass (ICM) of the blastocyst and give rise to a plethora of mature cell types that make up the body. Adult stem cells are multipotent progenitor cells found in numerous adult tissues and, as part of the body repair system, they can develop into more than one cell type but they are more limited than ESCs (Barbosa et al., 2012; Becker et al., 1963; Gilbert et al., 2012; Kim and Hirth, 2009). BMP/TGF-β, Notch, Wnt/β-catenin, FGF, LIF/STAT, Hedgehog and Hippo are some of the signalling pathways which function in combination with transcription factors/co-factors, including Tbx3, octamer-binding transcription factor 4 (Oct4), SRY box2 (Sox2), kruppel-like factor 5 (KLF5) and homeobox protein Nanog, to regulate pluripotency and self-renewal of ESCs (Andersson et al., 2011; Huang et al., 2015; Ng and Surani, 2011; Niwa et al., 2009; Zhao et al., 2011). Importantly, several lines of evidence suggest that TBX3 enhances and maintains stem cell pluripotency in vitro by preventing differentiation and enhancing self-renewal (Niwa et al., 2009; Russell et al., 2015; Saunders et al., 2013). For example, LIF maintains the pluripotency of mESCs through regulating the Jak/Stat3 and PI(3)K/Akt signalling pathways which activate Klf4/Sox2 and Tbx3/Nanog respectively to maintain expression of Oct3/4 (Niwa et al., 2009). In the absence of LIF, the upregulation of Tbx3 in mouse pluripotent stem cells is sufficient to maintain adequate expression levels of Oct3/4 to keep pluripotency and low levels of Tbx3 results in reduced pluripotency in mESCs (Niwa et al., 2009; Russell et al., 2015). Furthermore, while *Tbx3* levels are high in undifferentiated mESCs, its levels are downregulated in mESCs undergoing retinoic acid induced differentiation (Ivanova et al., 2006). Recently Tbx3 was also shown to be highly expressed in the interfollicular epidermal stem cells and it was shown to promote proliferation of these cells and to be required for abdominal skin expansion in mice during pregnancy and regeneration during wound repair (Ichijo et al., 2017).

It is important to note that in mESCs, Tbx3 appears to play a dual role in self-renewal and differentiation. Indeed, Tbx3 was demonstrated to be important for self-renewal and extraembryonic endoderm specification in mESCs (Lu et al., 2011; Semrau et al., 2017). In addition, the chromatin remodelling Baf45 complex maintains the pluripotency and

differentiation potential of mESCs and its subunit Dpf2 was recently found to directly activate *Tbx3* expression (Zhang et al., 2019). Importantly, the deletion of *Dpf2* led to the repression of Tbx3 and a reduction of mesodermal differentiation and when Tbx3 was restored, mesodermal differentiation was recovered. The authors further show that Eed, a subunit of PRC2, can bind an intragenic *Tbx3* enhancer to prevent Dpf2 dependent *Tbx3* expression in mesodermal differentiation. During differentiation of mESCs into neural cells, miR-137 is upregulated and it was found to bind the 3′ UTR of *Tbx3* which resulted in the repression of *Tbx3* levels, the inhibition of self-renewal and increased differentiation of mESCs in vitro (Jiang et al., 2013). In early mouse adipocyte precursor cells, miRNA-93 also exhibited the ability to repress *Tbx3* to prevent self-renewal (Cioffi et al., 2015).

Induced pluripotent stem cells (iPSCs) are ESC-like cells that can generate scalable quantities of relevant tissue and are of major interest for their application in personalized regenerative medicine, drug screening, and for our understanding of the cell signalling networks that regulate embryonic development and disease. In vitro studies have shown that expressing Tbx3, KLF4, SOX2, OCT4, Nanog, LIN-28A and C-MYC in somatic cells can reprogram them to form iPSCs (Okita and Yamanaka, 2011; Lee, et al., 2013). Importantly, Han et al., (2010) showed that iPS cells generated with Oct4, Sox2, Klf4 and Tbx3 are superior in both germ-cell contribution to the gonads and germline transmission frequency. They further showed using genome-wide chromatin immunoprecipitation sequencing analysis of Tbx3-binding sites in ESCs that Tbx3 regulates pluripotency-associated and reprogramming factors. In addition, co-expression of Tbx3 and Nr5α2 with Oct4, Sox2, Klf4 and c-Myc enhanced the generation of porcine iPSCs which resembled mESCs (Wang et al., 2013). Ke et al. (2018) also showed that LIF enhanced the levels of p-AKT as well as Tbx3 in marmoset iPSCs and an inhibitor of PI3K drastically reduced this regulation. Consistent with this data, naïve cynomolgus monkey (Cm) iPSCs was shown to express Oct3/4, DPPA5, SOX2, TBX3, KLF4, and KLF5 and expression of these genes in Cm ESCs was LIF-dependent (Honda et al., 2017). Interestingly, two studies showed that Tbx3/TBX3 is not entirely critical for the tenacity or generation of iPSCs (Klingenstein et al., 2016; Russell et al., 2015). Indeed, these studies compared the pluripotency potential of MEFs isolated from *Tbx3*$^{+/+}$ and *Tbx3* null (*Tbx3*$^{-/-}$) mice as well as human foreskin fibroblasts and keratinocytes in which TBX3 was inducibly knocked down. They showed that *Tbx3*$^{-/-}$ MEFs and TBX3 knockdown cells could still be reprogrammed to iPSCs. Together these results indicate that while TBX3 is able to promote the efficacy of iPSC reprogramming, it is not essential for the reprogramming kinetics and maintenance of the pluripotency phenotype. This may be due to alternative pluripotency networks such as DPPA3 being able to substitute for TBX3.

5. TBX3 in human disease

TBX3 has been implicated in human diseases including ulnar mammary syndrome, rheumatoid arthritis, obesity and cancer (Frank et al., 2013; Quarta et al., 2019; Sardar et al., 2019; Willmer et al., 2017).

5.1. TBX3 in ulnar mammary syndrome

In humans, heterozygous mutations of *TBX3* that result in haploinsufficiency lead to ulnar mammary syndrome (UMS, OMIM 181450) (Bamshad et al., 1997). UMS is an autosomal dominant developmental disorder, characterized by a number of clinical features including mammary and apocrine gland hypoplasia, upper limb defects, malformations of areola, dental structures, heart and genitalia (Chen and Chen, 2017). Interestingly, not all tissues and organs that express TBX3 are affected in UMS patients. This suggests that specific expression levels of TBX3 may be crucial for its functions in various tissues and/or that other T-box transcription factors, such as TBX2, could substitute for TBX3 in tissues and organs unaffected by UMS. Eighteen UMS causing mutations in the *TBX3* gene have been reported which include 5 nonsense, 8 frameshift (due to deletion, duplication and insertion), 3 missense and 2 splice site mutations (Table 2). While these mutations can occur throughout *TBX3*, those which occur within or upstream of the T-domain (DNA binding) are associated with the most severe phenotype (Meneghini et al., 2006). Missense mutations within the T-domain that alter its structure are responsible for abolishing the DNA binding and transcription activity of TBX3 (Lingbeek, et al., 2002). Furthermore, in vitro studies in which the RD1 was deleted resulted in decreased transcriptional activity of Tbx3 (Carlson et al., 2001). More recent observations suggest that aberrant transcripts and truncated proteins resulting from mutations in *TBX3* contribute to UMS through functions unrelated to its transcriptional activity. Indeed, Kumar et al. (2014) found that TBX3 proteins that model different UMS mutations were unable to perform its pre-mRNA splicing regulatory functions and were capable of interfering with the splicing inhibition function of endogenous wild type TBX3. It is interesting to note that numerous UMS patients have been reported to be obese which is consistent with the recent study by Quarta et al. (2019) that linked haploinsufficiency of Tbx3 in mice to obesity. Taken together, clinical phenotypes arising from mutations in *TBX3* reveal the importance of this gene during the development of multiple tissues and organs.

5.2. TBX3 in obesity

Heterogeneous populations of hypothalamic arcuate nucleus (ARC) neurons, such as the agouti-related protein (Agrp)-expressing and proopiomelanocortin/cocaine- and amphetamine-regulated transcript (Pomc/Cart)-neurons, release specific neuropeptides that control energy homeostasis by controlling appetite and energy expenditure. Energy imbalances and obesity have been associated with the deregulation of these hypothalamic neurons. Interestingly, Tbx3 is expressed in these hypothalamic neurons and has been implicated in the differentiation of human embryonic stem cells into hypothalamic Pomc neurons (Eriksson and Mignot, 2009; Linden et al., 2009; Quarta et al., 2019). Importantly, patients with UMS have shown symptoms consistent with ARC neuron dysfunction, including deficiency in growth hormone production leading to impaired puberty and obesity (Linden et al., 2009). The ablation of Tbx3 function in Agrp and Pomc neurons was recently shown to cause obesity in mice by interfering with the identity, differentiation and plasticity of these hypothalamic neural networks (Quarta et al., 2019). The Drosophila melanogaster *Tbx3* homologue, *omb*, is expressed in the central nervous system of the adult fly and it was also reported to prevent obesity because depleting it by RNAi led to the induction and consequent increase in body fat content (Quarta et al., 2019). Tbx3 thus appears to be a key

player in driving the functional heterogeneity of hypothalamic neurons responsible for governing body weight and energy metabolism and this role is conserved in mice, drosophila and humans.

5.3. TBX3 in rheumatoid arthritis

Rheumatoid arthritis (RA) is characterized by chronic inflammation, which primarily affects the synovial joints leading to tissue damage and physical disability and genome wide association studies have casually linked TBX3 to RA susceptibility (Freudenberg et al., 2011; Julià et al., 2008; Plenge et al., 2007). Furthermore, *Tbx3* was identified as a candidate gene for RA in collagen-induced arthritis (CIA) mouse models (Sardar et al., 2019). Compared to control mice, mice with allelic variants in the *Eae39r* locus (which harbours the *Tbx3* and *Tbx5* genes) developed more severe CIA which correlated with increased Tbx3 serum levels but decreased TBX3 intracellular levels. Tbx3 was shown to repress B lymphocyte proliferation and it was thus proposed that decrease intracellular levels of Tbx3 results in their increased proliferation and activation. This is likely to cause an activated humoral immune response which is associated with chronic inflammation of the synovium leading to RA. Tbx3 may thus be an important player in regulating the immune system and a candidate biomarker for the diagnosis of RA severity. This is consistent with the limb defects seen in UMS which suggests the involvement of TBX3 in bone development pathways which are closely associated with immune pathways (D'Amelio and Sassi, 2016; Frank et al., 2013).

5.4. TBX3 in cancer

TBX3 is overexpressed in a wide range of carcinomas (breast, pancreatic, melanoma, liver, lung, gastric, ovarian, bladder and head and neck cancers) and sarcomas (chondrosarcoma, fibrosarcoma, liposarcoma, rhabdomyosarcoma and synovial sarcoma) and there is compelling evidence that it contributes to several hallmarks of cancer (Fig. 3). Indeed, uncontrolled cell proliferation and the bypass of senescence and apoptosis are early events in oncogenesis, and TBX3 has been shown to impact these processes as well as to promote tumour formation, angiogenesis and metastasis (Dong et al., 2018b, 2018a; Feng et al., 2018; Krstic et al., 2019; Wang, 2018; Willmer et al., 2017).

5.4.1. The role of TBX3 in promoting proliferation and bypassing senescence, apoptosis and anoikis—

A fundamental trait of cancer cells is uncontrolled proliferation and normal cells have several checkpoints that serve as barriers to prevent this from happening. For example, cell cycle arrests, senescence (irreversible cell cycle arrest), and programmed cell death pathways, including apoptosis and anoikis, prevent inappropriate cell division and/ or survival and the bypass of these processes can result in cancer (Hanahan and Weinberg, 2011). At a molecular level, these checkpoints are triggered and maintained by negative regulators of the cell cycle such as $p14^{ARF}$/$p19^{ARF}$, p53, $p21^{WAF1/CIP1}$, $p16^{INK4a}$, the retinoblastoma protein (Rb) and PTEN (Barnum and O'Connell, 2014). For example, in response to diverse oncogenic stresses, $p14^{ARF}$ and $p16^{INK4a}$ are upregulated. This results in $p14^{ARF}$ sequestering the p53 antagonist, MDM2, which leads to the upregulation of p53 and consequently activation of p53 target genes including $p21^{WAF1/CIP1}$, an important inhibitor of cell cycle progression and an inducer of

senescence and apoptosis (Berkovich et al., 2003; Brugarolas et al., 1995; Inoue et al., 1999; Pomerantz et al., 1998). $p16^{INK4a}$, like other members of the Ink4 family, functions by blocking the kinase active sites of cyclin-dependent kinases (CDKs) 4 and 6 thereby preventing their interaction with their cognate cyclins and thus preventing CDK-cyclin mediated phosphorylation of Rb (DeGregori, 2004). Hypo-phosphorylated Rb interacts with and sequesters the E2F family of transcription factors which results in a G1 cell cycle arrest and the maintenance of the senescence phenotype (DeGregori, 2004). TBX3 contributes to tumour progression, in part, by inhibiting the $p14^{ARF}$/p53/$p21^{WAF1/CIP1}$ and $p16^{INK4a}$/pRb tumour suppressor pathways to bypass key cell cycle checkpoints, cellular senescence, apoptosis and anoikis.

Several groups have reported that TBX3 can promote cell proliferation by directly repressing *$p14^{ARF}$/$p19^{ARF}$*, *$p21^{WAF1/CIP1}$*, *$p57^{KIP2}$* or *PTEN*. Indeed, Tbx3 expression promoted the proliferative ability of normal and tumorigenic mammary epithelial cells (MECs) by transcriptionally repressing *$p19^{ARF}$* which was accompanied by the downregulation of $p21^{WAF1/CIP1}$ (Platonova et al., 2007). It is important to note that *p53-null* MECs exhibited a similar growth response to TBX3 suggesting that the negative impact of TBX3 on *$p21^{WAF1/CIP1}$*, occurs independently of p53. Similarly, Suzuki et al., (2008) demonstrated that Tbx3 expression in hepatic progenitor cells negatively impacts *$p19^{ARF}$* levels resulting in significantly increased proliferative potential. In chondrosarcoma cells, *TBX3* is upregulated transcriptionally by c-Myc and post-translationally by cyclin A/CDK2 and it is required for transition through S-phase (Willmer et al., 2015). Furthermore, TBX3 promotes chondrosarcoma cell proliferation by directly binding to and repressing the *$p21^{WAF1/CIP1}$* promoter at a T-element at −121 bp (Willmer et al., 2016a, 2016b). More recently, TBX3 was shown to promote proliferation of papillary thyroid carcinoma cells through repressing *$p57^{KIP2}$* (Li et al., 2018a, 2018b). This resulted from TBX3 binding and recruiting the PRC2 and HDACs 1 and 2 to the region of the *CDKN1C* promoter that regulates *$p57^{KIP2}$* expression. TBX3 may also promote proliferation by repressing *PTEN*, an inhibitor of PI3K/AKT-mediated cell growth, proliferation and survival (Leslie and Downes, 2004). Indeed, TBX3 levels were significantly upregulated in 33 head and neck squamous cell carcinoma (HNSCC) patients and this correlated with reduced expression of PTEN. Furthermore, in the same study the authors show that TBX3 represses both basal and induced PTEN levels in HeLa and HEK cells (Burgucu et al., 2012).

Carlson et al. (2001) demonstrated that MEFs stably overexpressing wild type Tbx3, but not a Tbx3 protein in which the dominant RD1 is mutated, were able to form colonies and proliferate for more than 50 passages. This suggests that Tbx3 can promote unlimited cell division and bypass senescence and that the RD1 plays an important role in these abilities. Furthermore, Fan et al. (2004) showed that Tbx3, and not its isoform Tbx3+2a, could immortalise MEFs and Brummelkamp et al. (2002) identified Tbx3 as a key anti-senescence factor in a genetic screen of conditionally immortalised mouse striatal cells. The mechanism responsible was shown to involve the ability of Tbx3 to directly repress *$p19^{ARF}$* and mutations within the Tbx3 DBD dramatically abrogated this ability. Yarosh et al. (2008) subsequently showed that TBX3 interacts with HDACs 1, 2, 3 and 5 to repress *$p14^{ARF}$* through a T-box binding site in its initiator. TBX3 can also promote proliferation and prevent senescence by co-operating with CAPERα to repress *UCA1* and consequently the

p16^{INK4a}/Rb pathway. *UCA1*, a long non-coding RNA, stabilises *p16^{INK4a}* mRNA by sequestering the p16^{INK4a} antagonist HnRNP A1 and in this way promotes senescence. Importantly, knockdown of either *CAPERα* or *TBX3* increased senescence-associated β-galactosidase activity in human foreskin fibroblasts which was accompanied by an increase in p21$^{WAF1/CIP1}$, p16^{INK4a} and pRb (Kumar et al., 2014b).

Apoptosis is a physiologically ubiquitous cellular program that eliminates damaged or abnormal cells and cancer cells acquire mechanisms to evade apoptosis to confer upon them a survival advantage and resistance to anti-cancer agents (Hanahan and Weinberg, 2011). *Tbx3* is upregulated in rat bladder carcinoma cells and depleting *Tbx3* in these cells dramatically reduced cell growth and cell adhesion while promoting apoptosis (Ito et al., 2005). On the other hand, the ectopic overexpression of TBX3 or TBX3+2a in human mesangial cells inhibited apoptosis (Wensing and Campos, 2014). Furthermore, the co-expression of Tbx3, with Myc or H-RasVal17 can transform MEFs and bypass Myc-induced apoptosis through the repression of *p19^{ARF}/p53/ p21$^{WAF1/CIP1}$* (Carlson et al., 2002). Interestingly, a Tbx3 N-terminal truncated protein had no effect on Myc-induced apoptosis suggesting that the C-terminus of Tbx3 harbours a motif(s), probably RD1 and/or the activation domain, that may be required for inhibiting apoptosis (Carlson et al., 2002). Importantly, Renard et al. (2007) demonstrated that *TBX3* is a direct transcriptional target of β-catenin/Tcf and that β-catenin mediated upregulation of TBX3 confers resistance to doxorubicin-induced apoptosis in U2OS osteosarcoma and HCT116 colorectal carcinoma cells. Similarly, Zhang et al. (2011a, 2011b) showed that human DLD-1 colorectal cancer cells treated with an aqueous extract of the herb, Fructus Ligustri Lucidi, inhibited TBX3 expression which resulted in the upregulation of *p14^{ARF}* and *p53* and subsequently sensitization of the cells to doxorubicin-induced apoptosis. TBX3 can also confer resistance to anoikis, another form of programmed cell death that occurs when cells lose contact with the ECM or neighbouring cells and it serves as a barrier to metastasis (Gilmore, 2005). Indeed, TBX3 overexpression in HNSCC cells increased their resistance to anoikis thus enabling them to survive without appropriate ECM interaction (Humtsoe et al., 2012). Importantly, when TBX3 was depleted in HNSCC cells, they exhibited a significantly reduced ability to adhere to culture plates, had dramatically lower numbers of live cells and they exhibited a two-fold increase in fragmented nuclei and a significant increase in activated caspase 3 suggestive of apoptosis (Humtsoe et al., 2012). It is worth noting that the bypass of anoikis has also been linked to cancer drug resistance (Ko, et al., 2009). It will therefore be interesting to investigate if the ability of TBX3 to confer resistance to anoikis may be another mechanism by which it confers cancer drug resistance.

In contrast to the above findings, it is interesting to note that in pancreatic ductal adenocarcinomas (PDAC), melanoma and breast carcinomas, TBX3 has no effect, or negatively regulates proliferation, in favour of promoting cell migration, a phenotypic trade-off which is common in cancer (Gallaher et al., 2019). Indeed, ectopic overexpression of TBX3 in human PDAC cell lines had no effect on proliferation but enhanced the migratory and invasive ability of the cells (Perkhofer et al., 2016). Furthermore, non-tumourigenic radial growth phase (RGP) melanoma cells genetically engineered to overexpress *TBX3* had significantly reduced proliferative ability but increased migratory ability and the opposite was observed when TBX3 was depleted in advanced melanoma cells (Peres et al., 2010;

Peres and Prince, 2013). Similarly, the upregulation of *TBX3* in breast and melanoma cells stimulated with RA or TGFβ1 led to diminished proliferative rates but increased migration (Ballim et al., 2012; Li et al., 2013). The ability of TBX3 to inhibit proliferation correlated with decreased levels of its homologue, *TBX2*, a powerful pro-proliferative factor in melanoma and breast cancer. The mechanism by which TBX3 mediated the anti-proliferative effect downstream of TGFβ1 was shown to be through it directly repressing a T-element in the *TBX2* promoter (Li et al., 2014). Together this suggests that TBX3 inhibits breast cancer and melanoma proliferation through repressing *TBX2* and while the mechanisms that enable it to promote or inhibit proliferation in different cellular contexts are largely unknown, there is strong evidence that it may be co-factor dependent.

5.4.2. The role of TBX3 in tumour formation, angiogenesis and metastasis—

Malignant cells form tumours, generate a tumour-associated neo vasculature (angiogenesis) which supplies the tumour with nutrients and oxygen and removes metabolic wastes, and they break away from the primary tumour and metastasise and invade distant organs (Hanahan and Weinberg, 2011). Several studies have suggested that TBX3 contributes to these advanced oncogenic processes in colon cancer, hepatocarcinoma, breast cancer, melanoma, PDAC and chondrosarcoma. Indeed, knocking down TBX3 in colon and liver carcinoma cell lines reduced anchorage-independent growth in vitro, and expressing a dominant negative mutant Tbx3-Y149S in these cell lines, diminished their ability to form tumours in mice (Renard et al., 2007). In hepatocarcinoma patient samples, the expression of TBX3 positively correlated with histological grade, tumour size and cancer cell metastasis (Li et al., 2018a, 2018b). Ectopic overexpression of TBX3 enhanced the migratory and invasive ability of human PDAC cell lines and promoted angiogenesis in vitro and in vivo which correlated with increased expression of angiogenesis-associated genes such as *FGF2* and *VEGF-A* (Perkhofer et al., 2016). The ectopic expression of TBX3 in chondrosarcoma cells also enhanced their ability to form tumours in mice and knockdown of TBX3 in liposarcoma, rhabdomyosarcoma and chondrosarcoma, resulted in diminished substrate-dependent and -independent cell proliferation and migration (Willmer et al., 2016a, 2016b).

Five different mutations were identified in *TBX3* in breast tumour samples and there is evidence to suggest that *TBX3* is a potential driver gene in breast cancer. High *TBX3* mRNA levels were found in breast cancer cells and estrogen receptor (ER)-positive breast tumour samples which correlated positively with a metastatic prognosis (Chen, et al., 2009; Fillmore et al., 2010; Stephens et al., 2012). Furthermore, knocking down TBX3 in ER-positive MCF-7 breast cancer cells resulted in the inhibition of anchorage independent growth and migration (Peres et al., 2010). In addition, TBX3 was shown to mediate breast cancer cell migration downstream of the PKC and TGF-β signalling pathways (Li et al., 2014, 2013; Mowla et al., 2011). Moreover, TBX3 was identified as a potential regulator of the transition from ductal carcinoma *in situ* (DCIS) to invasive breast cancer. Transient and stable overexpression of TBX3 or TBX3+2a enhanced the survival, colony forming and invasive abilities of DCIS-like and non-invasive breast cancer cells (Krstic et al., 2016, 2019). The mechanism involved was demonstrated to occur through the two TBX3 isoforms directly upregulating *SNAI2*, which encodes SLUG, and thereby inducing EMT. The authors provide compelling evidence that in breast cancer patient samples, there is a strong

correlation between elevated levels of *TBX3* and *SLUG* and that this is associated with poor prognosis.

TBX3 is also overexpressed in advanced melanoma and can drive the transition from non-invasive RGP melanoma to invasive vertical growth phase (VGP) melanoma (Hoek et al., 2004; Peres et al., 2010; Rodriguez et al., 2008). Ectopic expression of TBX3 alone in RGP cells was sufficient to drive them to assume a VGP phenotype and knockdown of TBX3 in advanced melanoma cells inhibited their tumour forming ability and their aggressive phenotype (Peres et al., 2010; Peres and Prince, 2013). A key mechanism by which TBX3 promotes melanoma migration and metastasis was shown to occur through its ability to directly repress *E-cadherin* (Rodriguez et al., 2008). In the same study, high levels of TBX3 were shown to correlate with low expression of E-cadherin in metastatic melanoma tissue samples and the depletion of TBX3 caused an increase in E-cadherin levels and decreased melanoma invasiveness in vitro. The association between TBX3 and E-cadherin, and its consequences on migration and invasion, were also reported with similar results for squamous cell carcinoma and human hepatocellular carcinoma (Feng et al., 2018; Humtsoe et al., 2012).

The BRAF-MAPK, AKT and PKC pathways have been identified as upstream regulators of the TBX3/E-cadherin axis in melanoma and bladder cancer. $BRAF^{V600E}$ and AKT3 are constitutively activated in approximately 50% and 70% of melanomas respectively and they play critical roles in melanoma formation and invasion (Dhawan et al., 2002; Palmieri et al., 2015; Siroy et al., 2016). The overexpression of TBX3 in a subset of melanomas was shown to result from it being transcriptionally upregulated by $BRAF^{V600E}$ and phosphorylated by AKT3 (Boyd et al., 2013, Peres et al., 2015). AKT3 phosphorylation of TBX3 enhanced its ability to repress *E-cadherin* and promoted migration (Peres et al., 2015). Levels of *miR-137* and *TBX3* mRNA correlate inversely in a panel of melanoma cell lines as well as a cohort of primary melanoma patients and *miR-137* was shown to be an important component of the TBX3/E-cadherin axis in melanomagenesis (Peres et al., 2017). *TBX3* was identified as a direct target of *miR-137* in non-malignant RGP cells and re-expression of *miR-137* in advanced melanoma cells inhibited their migration by repressing *TBX3* and upregulating E-cadherin levels. In human bladder cancer cells, the regulation of *E-cadherin* by TBX3 occurs in a PLCε/PKC-dependent manner (Du et al., 2014). When PLCε was silenced in bladder cancer cells, TBX3 levels decreased while E-cadherin levels increased, and this correlated with a decrease in invasive capability. Furthermore, this situation was partly reversed when the PKC pathway was stimulated, suggesting that TBX3 is downstream of PLCε/PKC in bladder cancer.

5.4.3. TBX3 in cancer stem cells—Cancer stem cells (CSCs) are a small sub-population of tumour cells which exhibit capabilities such as self-renewal, differentiation and tumorigenicity (Clarke et al., 2006). They are resistant to standard chemotherapies and are thought to be one of the main contributors to cancer development, drug resistance and clinical relapse (Yu et al., 2012). Understanding the role of CSCs within the tumour micro-environment has thus sparked much interest and there is evidence that TBX3 contributes to the expansion of these cells within tumours. The treatment of a panel of ER-positive breast cancer cell lines with 17-β-Estradiol resulted in a significant increase in the number of CSCs

and enhanced tumorsphere formation and the downstream effectors were shown to be FGF9 and TBX3 (Fillmore et al., 2010). Additionally, breast tumours that responded best to chemotherapy were shown to express lower levels of TBX3 while tumours that express high levels of TBX3 had the greatest recurrence rates. These results highlight the importance of the FGF/Tbx3 signalling pathway in the expansion of breast CSCs and reveal another mechanism by which TBX3 aids breast cancer progression, recurrence and drug resistance (Fillmore et al., 2010; Dong et al., 2018a, 2018b). CSCs derived from PDACs also express high levels of TBX3 and perpetuate themselves through an autocrine TBX3-ACTIVIN/NODAL signalling loop to sustain stemness (Perkhofer et al., 2016). In these cells, TBX3 co-localized with the pluripotency marker OCT3/4 and was found to be bound to pluripotency genes involved in the ACTIVIN/NODAL pathway. These findings indicate that TBX3 is a key player in regulating pluripotency-related genes in CSCs and that this may be another mechanism by which it contributes to cancer formation and tumour aggressiveness.

5.4.4. The tumour suppressor role of TBX3—During oncogenesis, tumour suppressor genes are frequently silenced by methylation (Jones and Baylin, 2002). Interestingly, *TBX3* is methylated in metastatic cervical cancer, DU-145 prostate cancer cells, bladder cancer, urothelial carcinoma, in the AGS gastric cancer cell line, glioblastoma and glioblastoma stem cells, and the methylation of *TBX3* was associated with a poor overall survival, resistance to cancer therapy and a more invasive phenotype (Beukers et al., 2015; Eriksson et al., 2015; Etcheverry et al., 2010; Kandimalla et al., 2012; Lee et al., 2014; Lyng et al., 2006; White-Al Habeeb et al., 2014; Yamashita et al., 2006). A comparison of genome wide CpG methylation profiles of three primary glioblastoma cell lines and glioblastoma stem cells with normal brain and neuronal stem cell controls revealed that *TBX3* was one of 202 genes that were hypermethylated within their promoters and 5′UTRs in primary glioblastoma cell lines and glioblastoma stem cells (Lee et al., 2014). Gene ontology analyses of this subset of CpG methylated genes implicated them in, amongst other functions, the regulation of metabolism. These findings are interesting because the methylation of *TBX3* in glioblastoma is associated with resistance to standard therapy and metabolic pathways have been implicated as important mediators of resistance to anti-cancer agents (Etcheverry et al., 2010; Zaal and Berkers, 2018). It would therefore be important to follow up on whether re-expressing *TBX3* in glioblastoma cells alters their metabolic pathways and whether it will lead to the sensitivity of these cells to radiation and chemotherapy.

In a gastric cancer cell line, AGS, de-methylation with 5-aza-2′-deoxycytidine (5-aza-dC) followed by an oligonucleotide array revealed that *TBX3* was one of 579 genes which were upregulated 16-fold or more (Yamashita et al., 2006). The authors showed that in this gastric cancer cell line, but not in 5 other gastric cancer cell lines tested, methylation of the CpG island in the 5′ region of the *TBX3* gene effectively silenced its expression (Yamashita et al., 2006). Importantly, the authors reveal that 5-aza-dC treatment negatively impacted growth of these cells which may suggest a tumour suppressor role for TBX3 in a subset of gastric cancer cells. However, given the large pool of methylated genes identified, the significance of *TBX3* methylation in this context requires further investigation.

Interestingly, TBX3 mRNA and protein levels are overexpressed in fibrosarcoma cells and patient derived tissue samples relative to primary fibroblasts and normal adjacent tissue respectively (Willmer et al., 2016a, 2016b). Investigation of the functional significance of this expression revealed that knockdown of TBX3 promoted substrate dependent and independent proliferation, migration and the formation of tumours in mice with significantly increased volume and weight. In the same study the authors genetically engineered a fibrosarcoma cell line to overexpress TBX3 and they showed that TBX3 conferred tumour suppressor properties on these cells which corroborated their knockdown data (Willmer et al., 2016a, 2016b). Recently, Oh et al. (2019) showed that TBX3 is expressed at very low levels in alveolar and embryonal rhabdomyosarcoma cells and the ectopic overexpression of TBX3 resulted in the inhibition of proliferation and migration of these cells. The authors showed that the mechanism for the inhibition of proliferation involved the direct repression of *TBX2* by TBX3. They also demonstrate that PRC2 together with its regulator, JARID2, co-operate with the methyltransferase H3K27me to silence *TBX3* in skeletal muscle cells. It would be interesting to investigate whether this is the mechanism by which TBX3 levels are kept low in rhabdomyosarcoma.

5.4.5. TBX3 and its homologue, TBX2, in cancer—TBX2 and TBX3 are highly related members of the TBX2 sub-family. As shown in Fig. 4, they both have a T-box DNA binding domain, 2 repression domains and an activation domain (Paxton et al., 2002; Sinha et al., 2000). The TBX2 and TBX3 DNA binding domains share 95% homology and their repression domains located in the C-terminus share 66.67% homology. However, their second repression domains and their activation domains are found at different positions and share no homology (He et al., 1999). Based on the high degree of homology between their DNA binding domains it was initially expected that they would regulate common target genes and have redundant functions. However, there is now compelling evidence that they also have distinct functions in development and cancer. For example, *TBX2* and *TBX3* have overlapping expression patterns and co-operate in mammary gland development but there is also evidence that they have distinct spatial expression patterns and functions. Indeed, during the induction of the mammary gland, *TBX2* expression is restricted to the mesodermal cells of the milk line and *TBX3* is only expressed in the epithelial cells of the emerging mammary placodes (Chapman et al., 1996; Davenport et al., 2003). Importantly, while Tbx3 heterozygous mutations result in failed nipple and ductal tree development, Tbx2 heterozygous mutations have no distinct effect on placode formation but leads to a reduction in ductal tree development (Jerome-Majewska et al., 2005).

TBX2 and TBX3 are also overexpressed in numerous cancers and they both can contribute to similar oncogenic processes including bypassing senescence and apoptosis, promoting proliferation and EMT and conferring drug resistance (Wansleben et al., 2014). This suggests that they are both able to regulate the same target genes in certain contexts. However, relatively little is known about the genes that they regulate to impact these processes as well as the molecular mechanisms underlying their target gene specificity. There is some evidence that in different contexts, TBX2 and TBX3 are both capable of binding and repressing a variant half T-site that is present close to the $p19^{ARF}/p14^{ARF}$ and $p21^{WAF1/CIP1}$ transcriptional start sites to bypass senescence and promote proliferation

(Brummelkamp et al., 2002; Jacobs et al., 2000; Lingbeek et al., 2002; Prince et al., 2004; Willmer et al., 2016a, 2016b). This repression of $p19^{ARF}/p14^{ARF}$ and $p21^{WAF1/CIP1}$ appears to specifically require the homologous DNA binding and C-terminal repression domains of TBX2 and TBX3. It would be interesting to determine whether TBX2 and TBX3 have redundant functions in regulating the $p19^{ARF}/p14^{ARF}$ and $p21^{WAF1/CIP1}$ promoter in cancers where they are both expressed or if their ability to regulate these target genes is regulated by other factors. In this regard it is worth noting that in breast cancer and melanoma cell lines where TBX2 and TBX3 are both overexpressed, their individual knock down resulted in different phenotypes. While TBX2 functioned as a powerful pro-proliferative factor, TBX3 impacted the later oncogenic processes of tumour formation and cell migration (Peres et al., 2010). This suggests that they must be regulating different target genes when they are both simultaneously expressed. Indeed, whereas TBX2 was shown to be required to maintain proliferation and suppress senescence in melanomas by repressing expression of $p21^{WAF1/CIP1}$ (Vance et al., 2005), TBX3 was found to enhance melanoma invasiveness by down-regulating expression of *E-cadherin* (Rodriguez et al., 2008). It is worth noting that in the Rodriguez study, both TBX2 and TBX3 were able to bind the same site in the *E-cadherin* promotor in vitro, but only TBX3 was able to bind the promoter in vivo and the depletion of TBX3 but not TBX2 led to an increase in endogenous $p21^{WAF1/CIP1}$ levels. This suggests that while TBX2 and TBX3 can both bind the same sites in vitro, their ability to recognize half T elements in vivo can vary. Interestingly, in two different studies TBX2 and TBX3 were separately implicated in gastric cancer and their levels were shown to correlate inversely with E-cadherin levels (Liu et al., 2019; Miao et al., 2016). This raises the possibility that they are both capable of repressing *E-cadherin* in some contexts and begs the question as to what regulates this ability in other contexts for example in melanoma. It is possible that their target gene specificity may be regulated by posttranslational modifications by differential signalling cascades or by their associated cofactors. Indeed, the phosphorylation of TBX3 by AKT was shown to enhance its ability to repress *E-cadherin* in melanoma (Peres et al., 2015). In addition, EGR1 has been identified as a TBX2 co-factor in breast cancer and rhabdomyosarcoma and their interaction has been shown to drive cell proliferation by inhibiting EGR1 dependent gene expression of $p21^{WAF1/}$*CIP1, PTEN*, *NDRG1* and *CST6* (D'Costa et al., 2014; Mohamad et al., 2018; Redmond et al., 2010). It will be interesting to determine if TBX3 is also able to interact with EGR1 in breast cancer and the impact of this on EGR1 target gene regulation. Furthermore, there is evidence that TBX2 and TBX3 are differentially expressed in some cancers which appears to relate, in part, to their ability to repress one another (Mohamad et al., 2018; Rodriguez et al., 2008). TBX3 also mediates the anti-proliferative role of TGF-β by repressing *TBX2* at a half T-element site. To better understand the modes of action and functions of TBX2 and TBX3, it is essential that their repertoire of target genes, as well as their interacting proteins and the protein domains involved, are identified.

6. Conclusion

The transcription factor TBX3 is a key player in embryonic development, stem cell maintenance and oncogenesis. During development, mutations resulting in decreased levels of TBX3 lead to ulnar mammary syndrome. This condition affects organs where TBX3

plays a functional role, for example, the heart, mammary gland, limbs and lungs. In contrast, when TBX3 levels are upregulated in postnatal tissue, it contributes to a wide array of epithelial derived cancers and a subset of soft tissue and bone sarcomas by impacting several cancer processes. There is also evidence that in certain cellular contexts TBX3 may function as a brake to prevent tumour progression. A serious gap in TBX3 biology is our understanding of the molecular mechanisms that (i) regulate the overexpression of TBX3 in cancer, (ii) mediate the oncogenic functions of TBX3 and (iii) enables TBX3 to switch between a tumour promoter and tumour suppressor. Investigations into these areas have important implications for identifying versatile ways of targeting TBX3 in anti-cancer therapy.

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The corresponding Gene Wiki entry for this review can be found here: https://en.wikipedia.org/wiki/TBX3.

Abbreviations:

3′ UTR	3′ untranslated region
AER	apical ectodermal ridge
AKT3	AKT Serine/Threonine Kinase 3
AVB	atrioventricular bundle
AVC	atrioventricular canal
AVN	atrioventricular node
Axin2	axis inhibition protein 2
Baf45	BAF complex
BB	bundle branches
BMP	Bone Morphogenetic Protein
CAPERα	Coactivator of AP1 and Estrogen Receptor
CCS	cardiac conduction system
CDKs	cyclin-dependent kinases
Cm	cynomolgus monkey

DBD	DNA-binding domain
DCIS	ductal carcinoma *in situ*
DPPA5	developmental pluripotency-associated 5
ECM	extracellular matrix
ESCs	Embryonic stem cells
Fgf	Fibroblast growth factor
Gata6	GATA-binding protein 6
Gli3	GLI Family Zinc Finger 3
Hand2	*Heart- and neural crest derivatives-expressed protein 2*
Hcn4	Hyperpolarization Activated Cyclic Nucleotide Gated Potassium Channel 4
HDACs	histone deacetylases
HnRNP A1	Heterogeneous Ribonucleoprotein A1
HNSCC	head and neck squamous cell carcinoma
IFT	inflow tract
OFT	outflow tract
iPSCs	Induced pluripotent stem cells
Jak	Janus kinase
KLF5	kruppel-like factor 5
Lbh	Limb bud and heart development
Lef1	Lymphoid enhancer factor 1
LIF	leukemia inhibitory factor
LPM	lateral plate mesoderm
MAP	mitogen-activated protein
MDM2	Mouse double minute 2 homolog
MECs	mammary epithelial cells
MEFs	mouse embryonic fibroblasts
mESC	mouse ESCs
mESCs	mouse embryonic stem cells

NLS	nuclear localization signal
NRE	non-ridge ectoderm
Oct3/4	octamer-binding transcription factor ¾
p-AKT	phosphorylated AKT
PDAC	pancreatic ductal adenocarcinomas
PI(3)K	Phosphatidylinositol-3 kinases
PKC	Protein kinase C
PRC2	polycomb repressive complex 2
PTEN	phosphatase and TENsin Homolog
R2 and R1	repression domains
Rb	retinoblastoma protein
RGP	radial growth phase
SAN	sinoatrial node
Shh	Sonic Hedgehog
Shisa3	*Shisa family member 3*
Sox2	SRY box2
STAT	signal transducer and activator of transcription
TBX2	T-box factor 2
TBX3	T-box factor 3
TGF-β	transforming growth factor β
UCA1	Urothelial Cancer Associated 1
UMS	ulnar mammary syndrome
VEGF-A	Vascular endothelial growth factor A
VGP	vertical growth phase
WNT	Wingless-related integration site
ZIC4	*Zic Family Member 4*
ZPA	zone of polarizing activity
Frzb	*Frizzled related protein*

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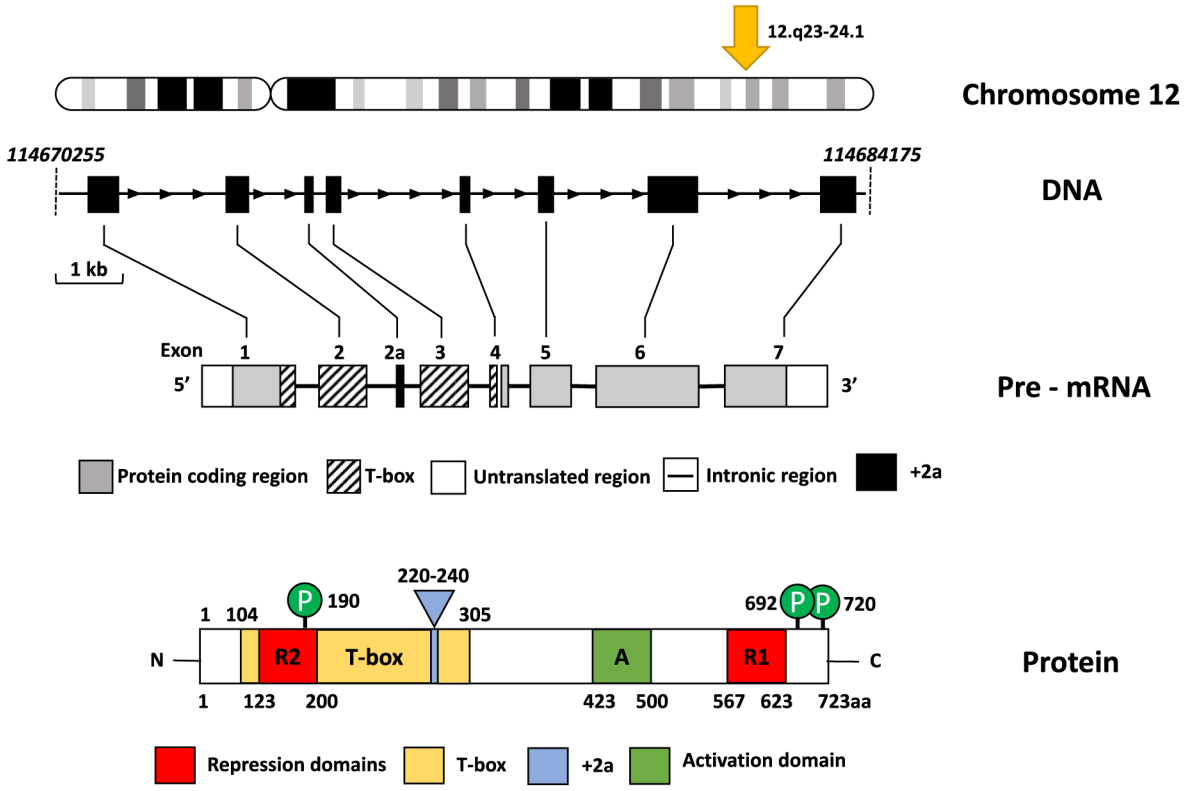

Fig. 1.

Schematic representation of the human *TBX3* gene, pre-*mRNA* and protein structure. The location of *TBX3* on chromosome 12 is depicted with the yellow arrow. The 4.7 kb DNA region is shown with coding regions (exons 1–7) represented by black boxes and the horizontal arrows indicate the direction of transcription. Representative size of region is depicted by thin bracketed horizontal line segment beneath the gene. The exons are linked to the *pre-mRNA* region depicting relative size, position of exons and the +2a splice variant of *TBX3*. The diagram depicting the TBX3 protein shows the DNA binding domain (T-box, yellow boxes), two repression domains (R1 and R2, red boxes), activation domain (A, green box) and the +2a splice variant (blue box). The amino acid residue number is displayed below each box and green circles above the protein diagram correspond to phosphorylation sites (adapted from Willmer et al, 2017).

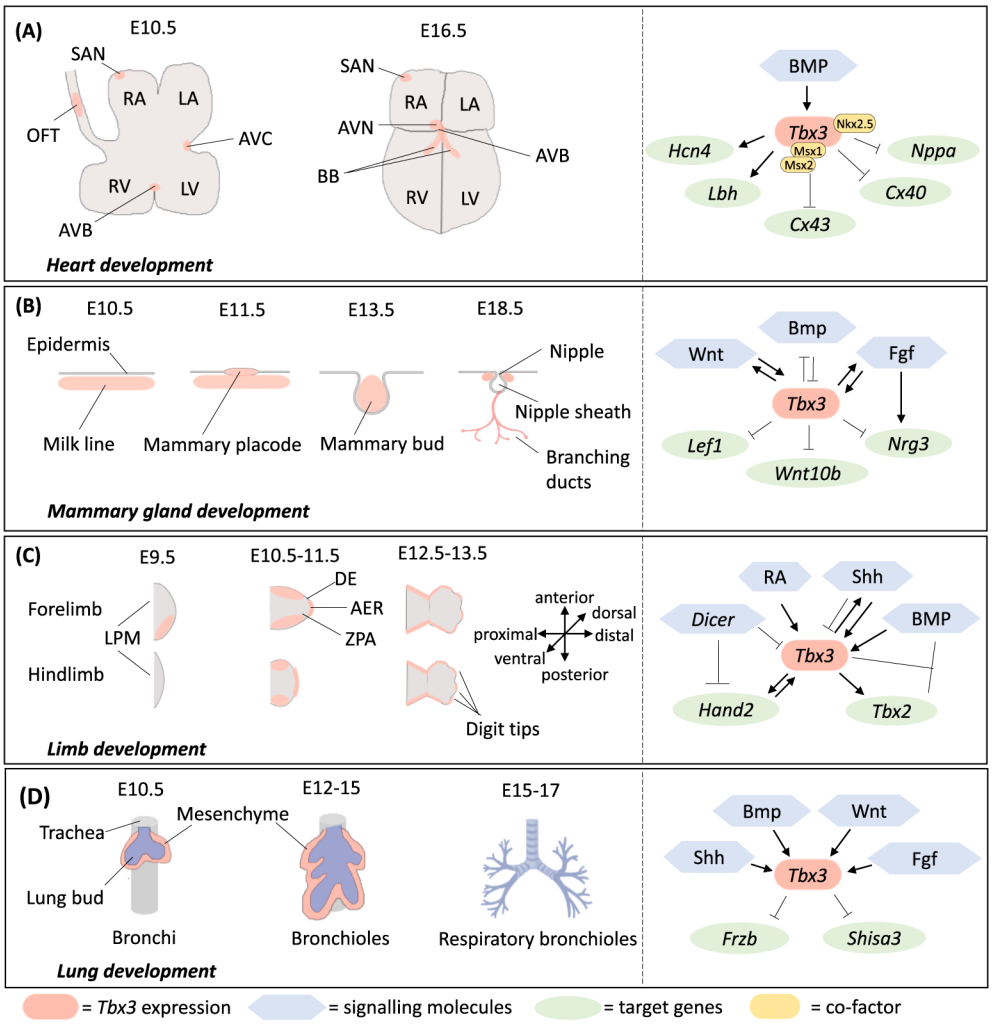

Fig. 2.

Left panels: Expression of *Tbx3* (red) during the development of the mouse (A) heart, (B) mammary gland, (C) limb and (D) lung. (A) At E10.5, *Tbx3* is expressed in the SAN, OFT, AVB and AVC, whereas at E16.5 the topography of *Tbx3* expression delineates the CCS with expression in the SAN, AVN, AVB and BB. (B) *Tbx3* first appears in the mesenchymal milk line at E10.5 and is then expressed in the mammary placodes at E11.5. *Tbx3* expression continues during mammary bud formation at E13.5 and the formation of the branching ductal system at E18.5. Furthermore, *Tbx3* is expressed in the mesenchyme surrounding the nipples.(C) At E10.5, *Tbx3* is expressed in the posterior and anterior margins of the fore and hindlimb buds, as well as the AER. By E12.5, *Tbx3* expression is limited to the tips of the digits. (D) *Tbx3* is expressed in the lung mesenchyme from E10.5 (embryonic stage) to E14.5 (late pseudoglandular stage). Some of the diagrams in this figure are adapted from Washkowitz et al. (2012) and permission was granted by the corresponding author Prof Virginia Papaioannou. Right panels (A)–(D): Signalling molecules and targets that modulate Tbx3 activity during the relevant developmental processes indicated on the left.

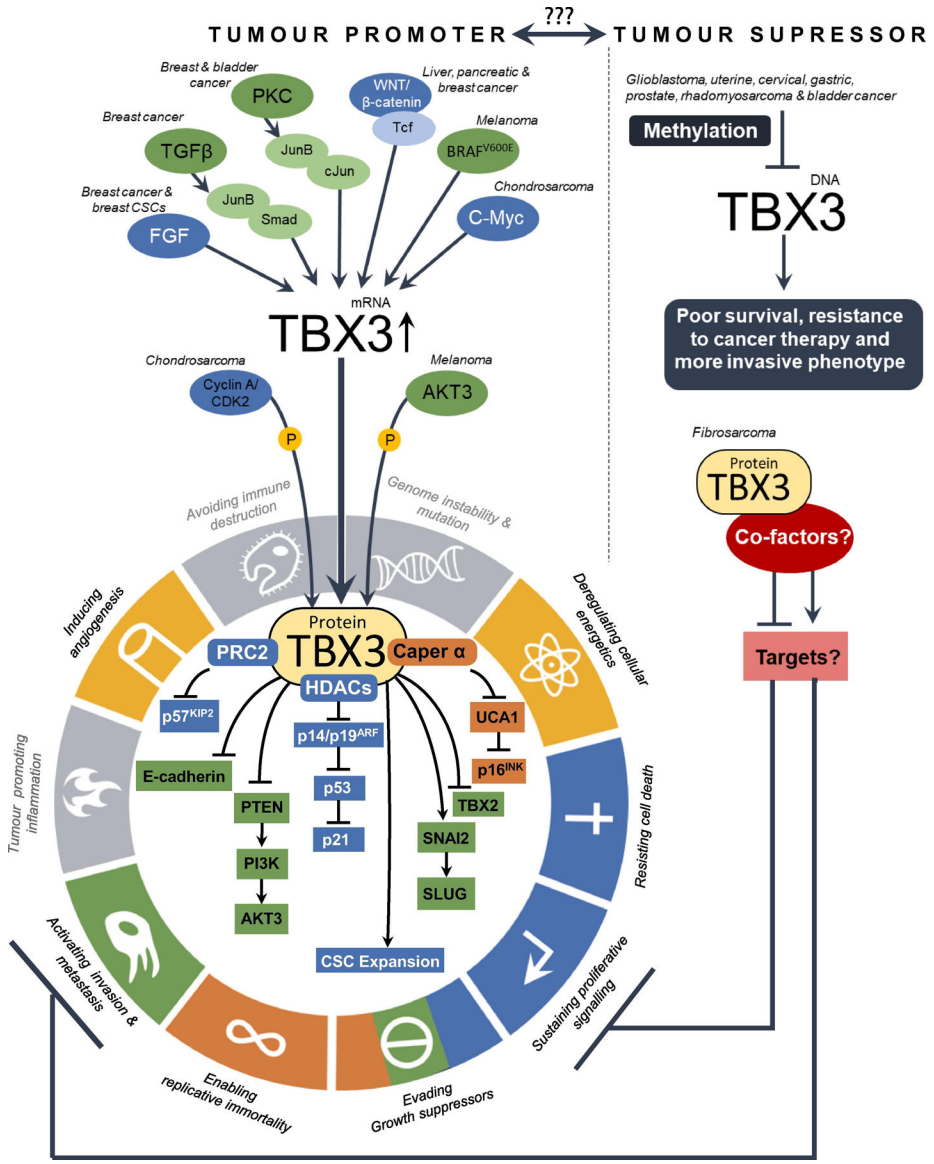

Fig. 3.

Summary of the regulation and roles of TBX3 in cancer. TBX3 is overexpressed in numerous cancers where it promotes several hallmarks of cancer as identified by Hanahan and Weinberg (2011) including (1) sustaining proliferative signalling; (2) evading growth suppressors; (3) resisting cell death; (4) enabling replicative immortality; (5) inducing angiogenesis; (6) activating invasion and metastasis and (7) deregulating cellular energetics. The key signalling molecules responsible for this overexpression and the co-factors and downstream targets that mediate the oncogenic functions of TBX3 are depicted in the figure adapted from Hanahan and Weinberg (2011) with colour coding that matches the appropriate hallmarks of cancer. Right panel: TBX3 also exhibits tumour suppressor activity. As indicated in this panel, it is silenced by methylation in certain cancers and it negatively impacts some hallmarks of cancer in fibrosarcoma and rhabdomyosarcoma. The factors that

upregulate TBX3 in fibrosarcoma as well as the co-factors and target genes that mediate the tumour suppressor functions of TBX3 are yet to be elucidated.

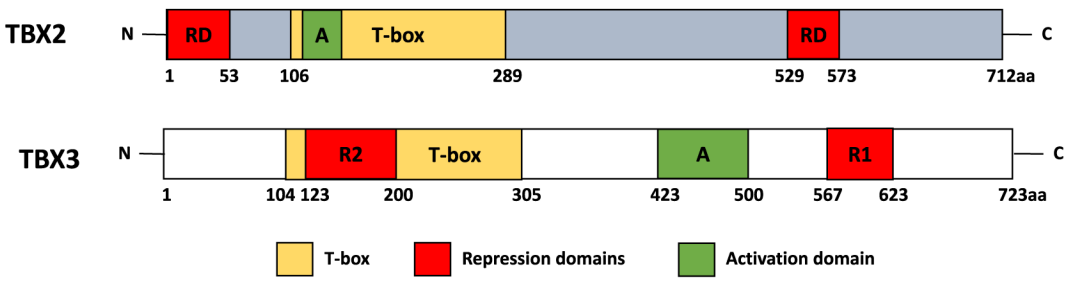

Fig. 4.

Diagrams depict the structural organisation of the human TBX2 and TBX3 proteins. The DNA binding domains (T-box, yellow boxes), repression domain (R1, R2 and RD, red boxes) and activation domains (A, green boxes) are shown and the amino acid residue number is displayed below each box.

Table 1

TBX3 paralogues identified to date in human (green), mouse (blue) and rat (orange) tabulated according to their respective subfamilies (data appropriated from the ensembl genome browser). aa = amino acids, Da = Daltons.

T-box gene subfamily	Gene name	Chromosome	Ensemble transcript ID	UniProt Code	Protein length (aa)	Mass (Da)
T	T	6	ENST00000296946.6	015178	453	47.44
		17	ENSMUST00000074667.8	Q78ZW9	436	47.44
		1	ENSRNOT00000033685.5	F1LXU5	377	43.211
	Tbx19 (Tpit)	1	ENST00000367821.8	O60806	448	48.24
		1	ENSMUST00000027859.il	Q99ME7	446	48.037
		13	ENSRNOT00000063870.1	D3Z977	212	23.695
Tbx1	Tbx1	22	ENST00000649276.1	A0A3B3IS18	504	53.505
		16	ENSMUST00000009241.6	F6ZP09	479	51.677
		11	ENSRNOT00000002597.5	D4A2E9	480	51.774
	Tbx10	11	ENST00000335385.3	075333	385	42.341
		19	ENSMUST00000041871.8	Q810F8	385	42.407
		1	ENSRNOT00000024129.4	D3ZAQ3	344	38.267
	Tbx15	1	ENST00000369429.5	Q96SF7	602	65.757
		3	ENSMUST00000029462.9	070306	602	65.802
		2	ENSRNOT00000067358.2	D3ZJ07	602	65.789
	Tbx18	6	ENST00000369663.10	095935	607	64.753
		9	ENSMUST00000034991.7	G3X919	613	65.434
		8	ENSRNOT00000014657.4	D4A1V6	612	65.595
	Tbx20	7	ENSRNOT00000064783.2	D3ZUF4	298	33.274
		9	ENSMUST00000052946.il	Q9ES03	445	49.096
		8	ENSRNOT00000082744.1	A0A0G2KAH3	446	49.163
	Tbx22	X	ENST00000373294.8	Q9Y458	520	57.910
		X	ENSMUST00000168174.8	E9Q5R8	531	59.869
		X	ENSRNOT00000003190.5	D3ZMK6	518	58.557
Tbx2	Tbx2	17	NST00000240328.4	A0A024QZ86	712	75.066
		11	ENSMUST00000000095.6	Q60707	711	75.081

T-box gene subfamily	Gene name	Chromosome	Ensemble transcript ID	UniProt Code	Protein length (aa)	Mass (Da)
Tbx6	Tbx3	10	ENSRNOT00000004698.7	F1M0C0	364	40.690
		12	ENST00000257566.7	015119	743	79.389
		5	ENSMUST00000018748.8	P70324	741	79.16
		12	ENSRNOT00000084018.1	A0A0G2K8D7	723	77.124
	Tbx4	17	ENST00000240335.1	P57082	545	60.204
		11	ENSMUST00000108047.7	P70325	552	61.101
		10	ENSRNOT00000004736.3	D4A0A2	554	61.387
	Tbx5	12	ENST00000310346.8	Q99593	518	57.711
		5	ENSMUST00000018407.9	Q5CZX7	518	57.832
		12	ENSRNOT00000001893.5	G3V657	517	57.745
Tbx6	Tbx6	16	ENST00000279386.6	095947	436	47.045
		7	ENSMUST00000094037.4	P70327	436	47.006
		1	ENSRNOT00000068543.1	D3ZJK7	436	47.216
	Mga	15	ENST00000219905.il	Q8IWI9	3065	336.159
		2	ENSMUST00000046717.12	A2AWL7	3003	328.802
		3	ENSRNOT00000008528.8	D3ZJB5	3005	329.343
Tbr1	Tbr1	2	ENST00000389554.8	Q16650	682	74.053
		2	ENSMUST00000048934.14	Q64336	681	73.940
		3	ENSRNOT00000065340.3	D4A6N8	680	73.632
	Eomes (Tbr2)	3	ENST00000295743.8	095936	686	72.732
		9	ENSMUST00000035020.14	054839	707	74.801
		8	ENSRNOT00000013530.5	D3ZY52	699	74.203
	Tbx21 (Tbet)	17	ENST00000177694.2	Q9UL17	535	58.328
		11	ENSMUST00000001484.2	Q9JKD8	530	57.852
		10	ENSRNOT00000012538.5	D3ZCM2	528	57.674

Table 2

TBX3 mutations in ulnar mammary syndrome.

Exons	Mutation	Type of mutation
1	*c.88insA*	Ins/Frameshift
	c.227delT	Del/Frameshift
2	*L143P*	Missense
	Y149S	Missense
	c.465_466insTATTGATGGACATT	Ins/Frameshift
	IVS2+1G > C	Splice site mutation
3	*c. 723del*	Frameshift
4	*K273X*	Nonsense
5	*c.991C > T*	missense
	c.992dup	Dup/Frameshift
	Q331X	Nonsense
	S343X	Nonsense
6	*c.1301_1302insGAGGAGCG*	Ins/Frameshift
	Q360X	Nonsense
	Q475X	Nonsense
	c.1586_1587insC	Ins/Frameshift
	IVS6 + 2T > A	Splice site mutation
7	*c.1857delC*	Frameshift