Review

Forkhead transcription factors: key players in health and disease

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Forkhead box (FOX) proteins constitute an evolutionarily conserved family of transcription factors with a central role not only during development, but also in the adult organism. Thus, the misregulation and/or mutation of FOX genes often induce human genetic diseases, promote cancer or deregulate ageing. Indeed, germinal FOX gene mutations cause diseases ranging from infertility to language and/or speech disorders and immunological defects. Moreover, because of their central role in signalling pathways and in the regulation of homeostasis, somatic misregulation and/or mutation of FOX genes are associated with cancer. FOX proteins have undergone diversification in terms of their sequence, regulation and function. In addition to dedicated roles, evidence suggests that Forkhead factors have retained some functional redundancy. Thus, combinations of slightly defective alleles might induce disease phenotypes in humans, acting as quantitative trait loci. Uncovering such variants would be a big step towards understanding the functional interdependencies of different FOX members and their implications in complex pathologies.

Winged helix proteins and Forkhead transcription factors

Transcription factors are proteins required for the initiation and regulation of transcription in all living organisms. They belong to one of two broad classes: (i) 'general' transcription factors, which are part of the basal transcriptional machinery organized around RNA polymerases; and (ii) 'specific' transcription factors that, in response to various biological signals, regulate the expression of relevant target genes by binding to their *cis*-regulatory sequences to activate or repress their transcription. The specific transcription factors share common characteristics, such as the presence of a DNA binding domain (DBD), a transactivation or transrepression effector region.

Such specific transcription factors are grouped according to the structure and degree of homology of their DBD. One such group is the superfamily of 'winged helix' DBDs [Structural Classification of Proteins (SCOP) classification n°46785], whose members are found in Eubacteria, Archaea and Eukaryota. Examples of winged helix DBD-containing proteins include the prokaryotic family of GntR repressors (such as FadR) and the eukaryotic families of 'Forkhead' transcription factors. Interestingly, the linker histones H1 and H5 also contain such domains (Figure 1a). It is not clear whether the similarity of folds among different protein families of the winged helix superfamily results from true homology or convergent evolution. In this review, we focus on the Forkhead transcription factors (SCOP classification n°46832).

The superfamily of Forkhead transcription factors

The evolution of FOX genes and their genomic distribution

The term 'Forkhead' was coined after a mutant of *Drosophila melanogaster* (*fork head*) [1]. At the time, Forkhead was described as a nuclear protein and a putative transcriptional regulator, which had no clear homology with any class of known proteins. The gene product had a homeotic activity, notably by promoting the development of terminal segments [1]. The following discovery of the rat gene *HNF3* (hepatocyte nuclear factor 3) enabled the detection of a strongly homologous region of approximately 100 amino acids, suggested to be a DBD [2]. This discovery brought to light a previously unknown family of transcription factors carrying the so-called 'Forkhead' motif.

To date, the family of Forkhead proteins has over 2000 members identified in 108 species of animals and fungi. Interestingly, not all species involved share the same number of Forkhead genes. For instance, among Fungi, *Aspergillus flavus* has only one such gene, whereas *Saccharomyces cerevisiae* has four. This numerical diversity is also found in metazoans, with 16 Forkhead genes in *Caenorhabditis elegans*, 18 in *D. melanogaster*, 49 in the zebrafish and 50 in humans. In 2000, a phylogenetic analysis led to a unified nomenclature to simplify the identification and classification of orthologs and paralogs within the family [3]. From this point on, all factors of the family have been known as FOX (Forkhead box), and a further letter indicates their membership to a subfamily. Thus far, 19 subfamilies (A–S) have been identified [4].

The genomic distribution of Forkhead genes is not random [2,5]. For instance, in the case of the human genome, 26 of the 50 FOX genes are arranged into nine genomic clusters, whereas the rest are scattered throughout the genome. The organization of the clusters reflects the evolutionary history of vertebrate genomes [5]. Indeed, a recent study of the emergence and expansion of transcription factor families in metazoans has suggested the existence of two phylogenetically distinct groups of FOX

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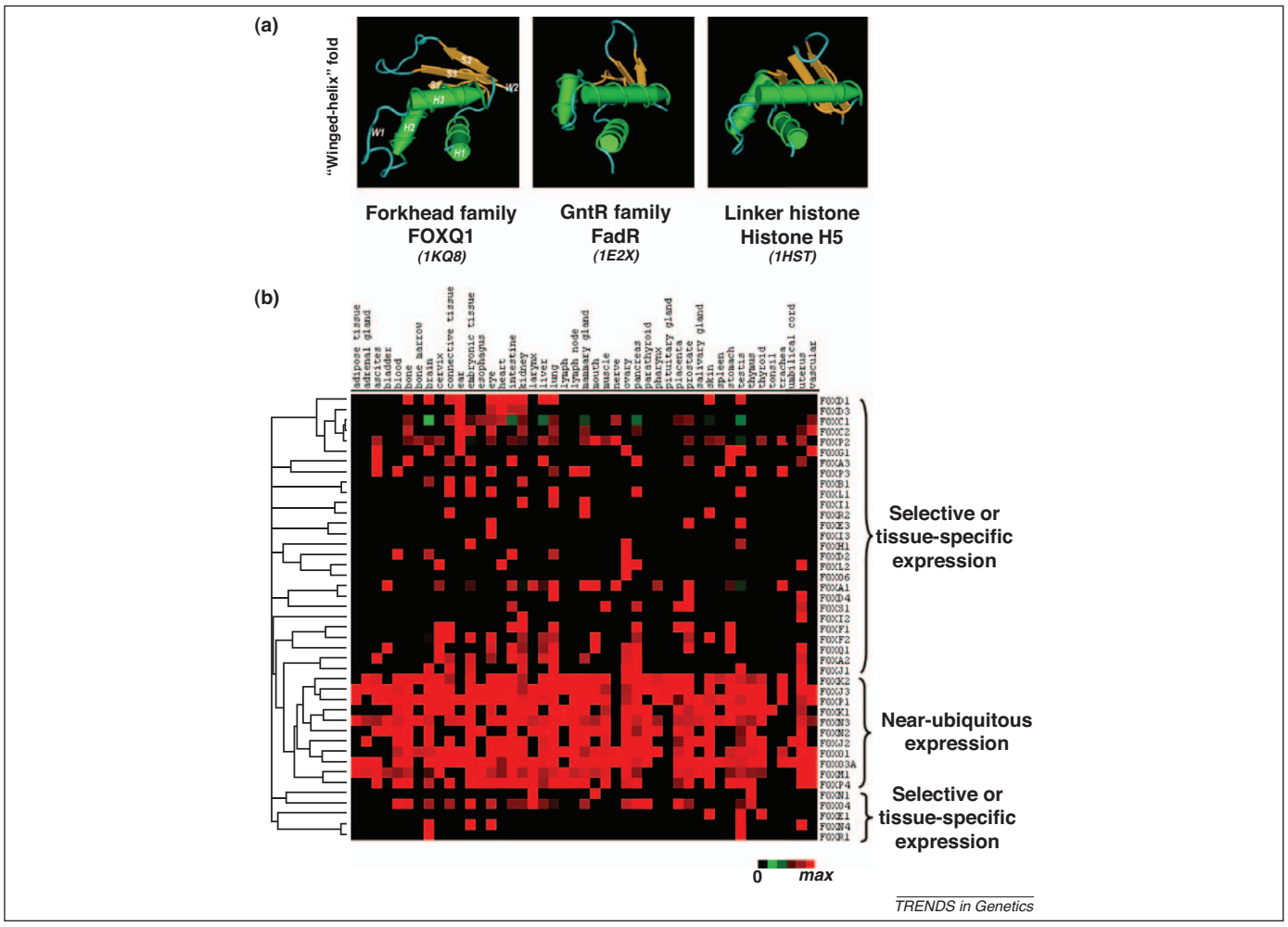

Figure 1. Molecular characteristics of Forkhead transcription factors. **(a)** Examples of 'winged helix' proteins (SCOP classification n°46785) from diverse families. **(i)** the Forkhead transcription factor FOXQ1 (reported in white letters: positions of helices, strands and wings of the domain). **(ii)** the bacterial repressor FadR of the GntR family. **(iii)** Linker histone H5. PDB accession numbers for the structures are given in parentheses below each structure view. Notice the high similarity in the three-dimensional organization of these protein domains, which could have evolved from a common ancestral structure. **(b)** Expression patterns of human Forkhead transcription factors. This figure was built using 'electronic northern blot' data from Unigene EST profiles. Patterns were subjected to an unsupervised hierarchical clustering using Cluster3.0, using absolute correlation as a distance measure. Distinct types of expression pattern can be observed: a group of near-ubiquitously expressed FOX genes emerges, in contrast to most Forkheads, which exhibit selective to more tissue-specific expression patterns (<half of tested tissues). Close paralogs from particular FOX subfamilies do not necessarily share common expression patterns.

genes: an ancestral paralog group present in the common ancestor of metazoans and Fungi (Group II), and one that emerged as a metazoan specific-class (Group I) [6]. The evolution of the Forkhead family of transcription factors has been reviewed in depth elsewhere [4,6].

Finally, in teleost fish, owing to recent whole-genome duplication, most of the chordate FOX factors are found in duplicate. However, evidence suggests that evolutionary divergence has already had a role in reallocating functions and expression patterns of recently duplicated paralogs, as was shown in the case of *FoxL2A* and *FoxL2B* in the rainbow trout and pufferfish [7].

The Forkhead domain is a DBD

The first structure of a Forkhead domain (FHD) complexed to a target DNA sequence resolved by X-ray diffraction crystallography was that of FOXA3/HNF3γ [8]. Since then, the structure of several other FHDs has been resolved, and these are similar to that of FOXA3 [9]. The FHD contains three N-terminal α-helices (H1–3), three β-strands and two loops (W1–2) towards its C-terminal region (Figure 1a). The peculiar folding of the loops around the helices, which is reminiscent of butterfly wings, is the reason for the 'winged helix' nickname of the domain. An additional α-helix (H4) is sometimes found between H2 and H3 in some Forkheads. The interaction of the FHD with specific sequences essentially involves the H3 helix (or recognition helix) and the major groove of DNA [8]. In addition, the DNA-binding specificity of Forkheads strongly depends on the variable region at the junction of helices H2 and H3 and wings W1 and W2, which interact with bases in the minor groove of DNA [9]. The study of the interaction between FOXA1 and its target sequences has shown that wings modulate the DNA-binding affinity and specificity of the FHD [10]. Several *in vitro* studies enabled the identification of a DNA consensus sequence of seven base pairs recognized by Forkhead factors [5′-(G/A)(T/C)(A/C)AA(C/T)A-3′ [11]]. Known *in vivo* binding sites indicate that Forkheads can bind sequences with some degree of degeneracy. Moreover, bases flanking the core sequence are important in defining the specificity of the interaction between the transcription factor and the DNA sequence [9,10].

The FHD is also responsible for nuclear import. Indeed, the existence of two nuclear localization sequences (NLS) in the FHD was demonstrated for FOXF2, FoxA2, FOXE1 and Foxp3 [2,12,13]. These NLS are located at both ends of the FHD, one in H1 and the other in W2. The C-terminal NLS (typically RK-rich) is highly conserved, suggesting an involvement of common nuclear import mechanisms for all factors, whereas the N-terminal NLS is not systematically present [12].

Whereas the FHD is rather well conserved, flanking regions, which contain effector domains (transactivation and/or transrepression, partner interactions, etc.) are poorly conserved. Some Forkheads contain homopolymeric repeats in these regions, such as polyGln (e.g. FOXP2) or polyAla (e.g. FOXE1 or FOXL2). These kinds of amino acid run can be mildly polymorphic (e.g. FOXE1 [14]) but are usually stable. Strong variations in repeat length can disrupt protein function and are pathogenic (e.g. FOXP2 [15] and FOXL2 [16,17]). Although they are frequent in transcription factors, the function of these low-complexity domains is not clear. It has been previously suggested that such stretches participate in the modulation of transcription factor activity [18].

Interestingly, recent evidence has shown that, in addition to their conventional regulation of gene expression as transcription factors, Forkhead factors also influence gene expression in unconventional manners (Box 1).

Box 1. Unconventional regulation of gene expression by Forkhead factors.

A winged helix fold similar to a FHD, but lacking W2, is found in linker histones H1 and H5 (SCOP classification n°46827). Many studies suggest that this structural similarity has a functional meaning. Not surprisingly, FOXA1 is known to direct nucleosome positioning at the albumin enhancer by binding nucleosomes, similarly to histones H1 and H5 [2]. However, unlike that of H1 and H5, FOXA1 binding results in an opening of chromatin and in the transcriptional reactivation of the locus. Interestingly, a more recent study has confirmed and extended this early observation. Indeed, FoxA1 was shown to mediate chromatin opening and facilitate binding of the glucocorticoid receptor at the *MMTV* promoter, and induce a context-specific response to ligand binding [58].

Accumulating experimental evidence indicates that the ability of Forkhead factors to affect chromatin structure is a more general characteristic. For instance, FOXP3 is capable of inducing repression of the interleukin-4 (*IL4*) locus by inducing an inhibitory remodelling of the surrounding chromatin structure [59]. In response to hormonal stimulation, FOXE1 can bind to its target site in the thyroperoxidase promoter, even if the latter is protected by a nucleosome. This causes chromatin decompaction and facilitates the access of other transcription factors that regulate thyroperoxidase gene expression [60]. FOXI1 is able to bind to compacted mitotic chromosomes and alter the local condensation state of chromatin sites [61]. Finally, FOXO1 was also found to be able to bind to condensed nucleosomes [62]. In particular, it can bind to the compacted insulin growth factor binding protein 1 (*IGFBP1*) promoter and this disrupts the interaction between DNA and histones, resulting in chromatin remodelling and transcriptional reactivation [63].

These experiments suggest that Forkhead transcription factors also regulate gene expression in an unconventional way for transcription factors, by regulating the local state of chromatin at target loci, independently of their abilities as direct transcriptional activators/repressors.

Regulation and target specification of Forkhead factors

A first regulation level by expression pattern regulation

With the expansion of the FOX family in Metazoa, these genes acquired different functions, targets and expression patterns. 'Electronic northern blot' Unigene EST profiles have been used to explore expression patterns of human Forkhead genes (available for 43 FOX genes). An unsupervised hierarchical clustering of expression data revealed the existence of two clear types of expression pattern: (i) a near-ubiquitous expression pattern (11 FOX genes); and (ii) an expression that ranges between more selective to tissue specific (32 FOX genes; Figure 1b). This probably reflects the relative breadth of processes and functions regulated by each Forkhead transcription factor.

Interestingly, all 11 Forkhead members that display a near-ubiquitous expression pattern in humans belong to the ancestral FOX group mentioned above (Group II [6]). In addition, although some Group II FOX genes are not expressed near ubiquitously (only seven in the data set; e.g. *FOXP2*), at least one of their close paralogs from the same FOX subfamily are near ubiquitous (e.g. *FOXP1* and *FOXP4*). This suggests that, among Group II FOX genes, the near-ubiquitous ones should have retained ancestral functions that are necessary in most cells, whereas newly duplicated paralogs developed more tissue-specific functions. The latter is also likely to be true for the more recent subgroup of Forkhead transcription factors (Group I), which emerged in metazoans, along with the evolutionary emergence of novel tissue types and embryonic layers.

Close paralogs within specific FOX subfamilies do not necessarily share common expression patterns. For instance, *FOXO6* expression seems highly specific (e-northern blot profile displays a signal only in 'nerve' tissue), whereas *FOXO1* and *FOXO3a* expression is near ubiquitous. Furthermore, *FOXR1* is mainly expressed in testis and brain tissue, whereas its close paralog *FOXR2* is mostly expressed in skin, mammary glands and connective tissue (Figure 1b). The existence of some tissue specificity in the expression of close FOX paralogs provides a first level of explanation of how they can regulate distinct processes, and of the various phenotypes that their misregulation can provoke (see below).

Forkhead homo- and heterodimerization and higher order interactions

Forkhead factors tend to bind to DNA as monomers [8,9,19]. However, exceptions have been documented. This is the case for FOXP2 and FOXK1, for which co-crystal structures of the FHD with DNA have indicated an interaction with their target sequence as homodimers [20,21]. Forkhead factors have also been shown to regulate gene expression as Forkhead heterodimers. For instance, transcription regulation of cell proliferation inhibitor *p21Cip1* (*Cdkn1a*) involves cooperation between FoxO3a and FoxG1 [22], whereas that of the estrogen receptor gene requires cooperation between FoxO3a and FoxM1 [23]. In addition, Forkhead factors often act in concert with other protein partners, such as transcription factors from other families, such as SMAD3 [effector of the transforming growth factor (TGF)-β pathway], STAT3 or HOXA5 [24,25].

Interestingly, not all partners of Forkhead proteins are transcription factors. Heterodimerization with partners can be covalent, as shown between FOXO4 and acetyltransferase p300 following disturbances in the cellular redox balance, which induces disulfide bond formation between the proteins [26]. FOX proteins also interact with co-activators, co-repressors and even other kinds of protein. For instance, FoxO1 can be recruited to Pml nuclear bodies and interact directly with Pml proteins, which enables it to regulate expression of *NeuroD* in pancreatic β-cells [27]. The cooperation of Forkheads with each other and with other proteins represents another layer of complexity in the regulation that they can undergo and provide.

The high degree of conservation of the Forkhead DBD and target consensus sequence might be problematic in terms of specific target regulation, but interaction with partner proteins is likely to have a key role in target selection [11]. Interaction of Forkhead factors with partners through the FHD itself is likely to result in regulation of processes common to many FOX proteins (e.g. apoptosis), and interactions through divergent regions should elicit more-specific functions [11,25].

Posttranslational modifications and the activity of Forkhead transcription factors: FOX proteins as molecular integrators

Accumulating evidence has revealed that Forkhead factors are the recipients of many posttranslational modifications. Indeed, they can be regulated by the phosphorylation of serine, threonine or tyrosine residues [28–30]. A control of activity through the balance of acetylation and/or deacetylation of lysines has also been observed for several members of the family [28,31,32]. In addition, a recent study has shown that Forkhead can undergo arginine methylation or serine/threonine *O*-GlcNAcylation [33–35]. Conjugation to proteins of the ubiquitin/ubiquitin-like family has also been reported [36]. The specific consequences of these posttranslational modifications are numerous, and all affect, to some degree, the activity, cellular localization and/or stability of Forkhead transcription factors.

At the molecular level, posttranslational modifications can fine-tune binding efficiency and/or specificity to different DNA target sequences [19,37]. More surprisingly, accumulating evidence also indicates that posttranslational modifications can help specify FOX factors to particular target gene subsets in response to environmental signals [28–30,32,33]. For instance, the sole acetylation status of FOXO factors regulates whether they promote cell survival or apoptosis in response to cell stress [28]. These observations have led to the hypothesis of the existence of a posttranslational modification code for transcription factors, which would be at the root of target selection after integration of different extracellular signals [38].

Forkhead factors: key actors of biological processes

Forkhead factors: effectors of major signalling pathways

Forkhead factors can act as terminal effectors of many major signal transduction pathways. These notably include the TGF-β cascade, the mitogen-activated protein kinase (MAPK) pathway, the Sonic-Hedgehog (Shh) pathway, the Wnt/β-catenin pathway and the insulin/insulin growth factor (IGF) pathway. The interactions and the biological significance of Forkhead factors in key signalling pathways are summarized in Table 1.

Table 1. The interactions and biological significance of Forkhead factors in key signalling pathways

Signalling pathway	KEGG reference pathway	FOX protein and its interaction(s) with the signalling pathway	Refs
Hedgehog	04340	FoxA1: induction of expression in neural tube; activation of Shh transcription; transcriptional effector	[2,67–70]
		FoxC2: induction of expression in presomitic mesoderm; transcriptional effector	
		FoxD2: induction of expression in presomitic mesoderm	
		FoxE1: transcriptional effector	
		FoxF1: induction of expression; mediation of endoderm–mesoderm signalling; terminal transcriptional effector	
		FoxF2: terminal transcriptional effector	
		FoxL1: mediation of endoderm–mesoderm signalling; transcriptional effector	
Insulin/IGF	04910	FoxA2: terminal target; activity inhibition via AKT/PKB	[28,71,72]
		FoxM1: inhibition of expression by IGF-1 in cochlea	
		FoxO: terminal targets; activity inhibition via AKT/PKB	
MAPK	04010	FoxB: *Caenorhabditis elegans* LIN-31; effector in vulva progenitor cells	[2,73–75]
		FoxO4: activation upon stress	
		FoxO1: modulation of gluconeogenic targets	
		FoxM1: regulation of invasivity and anchorage-independent growth	
TGF-β/SMAD	04350	FoxH1: transcriptional effector	[2,22,24,76–79]
		FoxG1: transcriptional effector	
		FoxL2: transcriptional effector	
		FoxO3a: transcriptional effector	
		FoxP3: transcriptional effector	
Wnt/β-catenin	04310	FoxL1: inhibition of Wnt signalling	[25,80–82]
		FoxL2: interaction with Wnt4 signalling in gonadal development	
		FoxO: heterodimerization with β-catenin upon cell stress	
		FoxN1: induction of expression through Wnt5a in hair follicles	

The fact that many Forkhead proteins act as terminal effectors of several key signalling pathways supports the hypothesis that they can act as molecular integrators of extracellular signals. Indeed, they might constitute 'nodes' in cellular networks, allowing cross-talk between seemingly parallel signalling pathways, and thus more efficient and adequate responses to environmental fluctuations. These signalling cascades most often regulate FOX activity through posttranslational modification, as described above.

The key position of many Forkhead factors in major signalling pathways is in line with the severity of the phenotypes associated with their mutation or misregulation (see below). This also suggests that Forkhead gene deregulation should have adverse effects not only during development, but also in the adult life (Box 2).

Box 2. Forkhead factors during development and in adult tissues.

Many Forkhead factors are involved in fate determination of different mesodermal cell populations after gastrulation, and throughout organogenesis [4,39]. Accordingly, expression of Forkhead factors is often restricted to specific tissues, where they have major roles in the determination and/or differentiation of different cell types. Many Forkhead factors that control morphogenesis and differentiation during embryonic development also perform other functions in the adult, such as controlling carbohydrate and/or lipid metabolism, stress response or energy homeostasis. This can be assimilated to a situation of 'gene sharing' (i.e. when a single polypeptide can perform distinct functions according to the biological contexts). For instance, FOXA2 controls development of liver and pancreas in the embryo, and is later involved in the secretion of insulin in the differentiated pancreas, and in modulating gluconeogenesis and bile production by mature hepatocytes [54]. Another example is provided by Foxl2, which has a key role in ovarian determination and development during embryogenesis [64]. In the adult ovary, Foxl2 expression is crucial to maintain the differentiated ovarian state [65] and, in addition, it regulates steroidogenesis and stress responses [66].

Forkhead factors, hereditary diseases and cancer

The key role of Forkhead factors in embryonic development is illustrated by the consequences of their mutations and/or deregulation in humans and by the severity of phenotypes affecting multiple tissues in knockout mouse models. Currently, mutations in 11 FOX genes have been linked to human genetic diseases (Table 2). Although specificities are observed, phenotypic consequences common to the mutations of two or more Forkhead genes are rather frequent. Indeed, mutations of four Forkhead genes (*FOXC1*, *FOXC2, FOXE3* and *FOXL2*) lead to developmental abnormalities of the ocular region [39], whereas mutations in *FOXP3* and *FOXN1* are responsible for severe immune defects [2,39]. Premature ovarian failure can occur as a result of the mutations of *FOXO3a* or *FOXL2* [40,41]. Finally, mental retardation, autism and speech disorders are observed as a consequence of *FOXG1*, *FOXP1* and *FOXP2* mutations [39,42–45].

There is no general rule about the mode of inheritance of diseases caused by Forkhead gene mutations. Diseases

Table 2. Human genetic diseases associated with mutations in FOX genes and corresponding phenotypes of the invalid murine orthologs

Human gene	Associated mutation-induced phenotype(s) in humans	OMIM	Orthologous gene knockout/in phenotype in mouse model	Refs
FOXC1	Iridogoniodysgenesis type 1 (glaucoma and iris hypoplasia); Axenfeld–Rieger syndrome type 3	601090	Perinatal lethality; numerous developmental anomalies, notably in the eye region	[2,39]
FOXC2	Lymphedema-distichiasis syndrome (lymphoedema of the limbs, double rows of eyelashes and ptosis)	602402	Pre- and perinatal lethality; skeletal and cardiovascular defects; and numerous developmental anomalies, notably in the eye region	[2,39]
FOXE1	Bamforth–Lazarus syndrome (hypothyroidism, spiky hair, cleft palate and choanal atresia); cleft lip and palate	602617	Lethality within 48 hours of birth; cleft palate; and abnormal development of thyroid gland	[2,39,83]
FOXE3	Primary congenital aphakia; anterior segment mesenchymal dysgenesis (with Peters anomaly)	601094	Viable and fertile; severe cataract and degeneration of lens epithelium	[2,39]
FOXG1	Congenital variant of Rett syndrome; mental retardation, cerebral malformations and microcephaly	164874	Perinatal lethality; severe reduction of the size of cerebral hemispheres and abnormal telencephalon development	[2,44,45,84]
FOXL2	BPES and telecanthus; premature ovarian failure	605597	High perinatal lethality; craniofacial abnormalities, with eyelid hypoplasia; anomalies in the development and differentiation of the ovary; females can be either sub- or infertile; partial to total ovary to testis transdifferentiation	[17,39,41,65,85]
FOXN1	T-cell immunodeficiency, congenital alopecia and nail dystrophy	600838	Alopecia and T-cell immunodeficiency, owing to athymia	[2,39]
FOXO3A	Premature ovarian failure	602681	Viable; minor defects in glucose uptake; overproliferation of helper T-cells; age-dependent female infertility owing to premature activation and depletion of ovarian follicles	[40,57]
FOXP1	Mental retardation, with language impairment and autistic features	605515	Early lethality; defects in immune and cardiovascular systems and movement; abnormal development of lungs and oesophagus	[42,43,86,87]
FOXP2	Mental retardation, with language impairment and autistic features	605317	Early lethality; motor problems; absence of ultrasonic vocalizations in the young	[39,88–91]
FOXP3	Immunodysregulation, polyendocrinopathy and enteropathy, x-linked (IPEX)	300292	Overproliferation of CD4+CD8- T-cells; infiltrations in various organs; increase in levels of various cytokines	[2,39]

Table 3. Forkhead transcription factor misregulation and cancerogenesis

Human gene	Mechanism(s) of dysregulation	Consequence(s) of dysregulation	Role	Refs
FOXA1	Somatic hypermethylation of promoter and loss of expression	Proliferation and independence of survival to growth factors, promoting tumourigenesis	Tumour suppressor	[80]
FOXC1	Overexpression in metastatic breast cancer	Increased invasive properties and metastatic potential of tumour cells	Oncogene	[80]
FOXC2				
FOXG1	Increased activity and expression, notably through gene amplification in medulloblastoma, hepatoblastoma and epithelial ovarian cancer	Inhibition of P21-CIP1 expression and loss of TGF-β-induced cytostasis	Oncogene	[80,92–94]
FOXL1	Loss of function is associated with gastrointestinal tumourigenesis	Activation of Wnt/β-catenin antigen-presenting cells; dysregulation of cell proliferation; modifier of tumourigenesis in mice	Tumour suppressor	[80]
FOXL2	Loss of expression or recurrent somatic mutation in ovarian granulosa cell tumours; somatic hypermethylation in colorectal cancer tissue and cells	Decreased ability of cells to enter apoptosis; increased aggressivity and mitotic index of tumour cells	Tumour suppressor	[95–97]
FOXM1	Increased expression and activity in tumours, notably through 12p13 amplification in various cancer types	Activation of cyclin A expression; increased unscheduled proliferation; increased genomic instability	Oncogene	[80]
FOXN3	Decreased expression by gene deletion in mouth, larynx and hepatic carcinoma	Reactivation of genes promoting malignant transformation	Tumour suppressor	[98–102]
FOXO1	Inactivation via an increase of PI3k/AKT signaling; gene deletion; involvement in oncogenic protein fusions, with paired box (PAX)-3, -7 or mixed-lineage leukaemia (MLL)	Unscheduled proliferation, apoptosis resistance to, and independence of, growth factor signalling; androgen independence of prostate cancer	Tumour suppressor	[48,80,103]
FOXO3				
FOXO4		Triple invalidation in the mouse causes severe leukemia		
FOXP1	Overexpression of short oncogenic isoforms after recurrent translocations in leukemia and gastric tumours	Poor prognosis for patients	Oncogene	[80,104,105]
	Loss of expression in breast and colorectal cancer	Expression is associated with better prognosis for patients	Tumour suppressor	
FOXP3	Increased expression in regulatory T cells around cytokine-secreting tumours (e.g. TGF-β)	Role in escape of immunosurveillance	Oncogene	[80,106,107]
	Loss of expression in aggressive breast cancer; somatic inactivation in prostate cancers	Mouse invalidation provokes an abnormal proliferation of lymphocytes and predisposes mice to breast cancer	Tumour suppressor	
		Transcriptional repression of SKP2 and cMYC oncogenes; limits tumour cell proliferation		

associated with mutations of *FOXE1* and *FOXN1* are autosomal recessive. The syndrome associated with mutations of *FOXP3* is transmitted in a recessive X-linked way, whereas in the remaining cases, transmission is autosomal dominant [39]. The importance of Forkhead gene dosage is particularly clear in the case of pathogenic mutations of *FOXC1*, where eye anomalies are observed in cases of both locus deletion or duplication [39].

An increasing number of studies have revealed links between the deregulation of Forkhead factors and the process of malignant transformation (Table 3). Indeed, most Forkhead transcription factors are involved to some extent in embryonic development and they retain an ability to regulate cell differentiation, proliferation and apoptosis during adulthood. Thus, the necessity to bypass or hijack these factors for 'successful' tumourigenesis seems to be a rather inescapable step. In fact, many Forkheads have already been suggested to act as either tumour suppressors or oncogenes. Many mechanisms of deregulation have been described, including gene amplification, somatic mutation or locus epigenetic remodelling (Table 3).

Forkhead factors and ageing

It has been proposed that ageing and cancer are two sides of the same coin [46]. This is compatible with the fact that, in addition to their misregulation and/or mutation in cancer, several Forkhead factors have also been implicated in ageing modulation. Indeed, the FoxO factors of *C. elegans* and *D. melanogaster* act as crucial regulators of longevity and stress response [47]. However, the conservation of the role of FoxO factors in the mammalian regulation of longevity, notably using knockout mice models, was disappointing, as no clear phenotype of accelerated ageing was observed [48]. The recent availability of mice carrying deletions of the three paralogs ($Foxo1^{-/-}$/$Foxo3a^{-/-}$/$Foxo4^{-/-}$) has shown that Foxo factors were partially redundant with regard to their function as tumour suppressors, but no data on longevity could be obtained owing to cancer-induced premature death [48]. However, several genetic association studies in independent human populations have revealed significant associations between longevity and SNPs linked to *FOXO1* and *FOXO3a* [49–52]. These studies are consistent with a conserved role of the

FOXO subfamily of Forkhead transcription factors in longevity regulation.

FoxA was recently shown to have a key role in lifespan extension by caloric restriction in the nematode [53]. In mammals, FOXA factors have a key role in liver organogenesis and metabolism in the adult, although conservation of a role in longevity modulation remains to be proven [54]. FOXM1 is also emerging as a potential regulator of ageing, as it is strongly downregulated in ageing fibroblasts and in prematurely aged fibroblasts from patients with progeria [55]. Thus, a FOXM1 deficiency could favour the appearance of early-ageing phenotypes. This is consistent with the fact that overexpression of Foxm1 rescues age-associated degeneration of hepatic tissue in older mice [56].

Forkhead factors have also been linked to specific organ ageing. For instance, homo- and heterozygous *Foxo3a* knockout mice display accelerated depletion of their follicular pool, which is similar to premature ovarian ageing [57]. Interestingly, allelic variants of *FOXO3a* have been identified in human patients with premature ovarian failure [40,57]. *FOXL2* mutations in humans can also lead to premature ovarian failure either as a part of the blepharophimosis, ptosis, epicanthus inversus syndrome (BPES) phenotype or as an isolated phenotype [17,41].

Concluding remarks

The involvement of members of the Forkhead transcription factor superfamily in many signalling pathways and in human disease has attracted much interest during the two decades that have followed their discovery. The blooming research on FOX factor regulation, notably by posttranslational modification, and their implications in physiology and pathology, bears witness to the complexity and importance of this family. Moreover, it is now clear that Forkhead transcription factors control many aspects of developmental and cellular processes, which is the result of a functional specialization of family members.

Despite the existence of a documented dedicated function for each Forkhead transcription factor, evidence also suggests that some have retained a degree of functional redundancy (whether by common ancestry or convergence). Indeed, as discussed above, only with the triple invalidation of *Foxo* genes did a long-predicted cancer phenotype appear in the mouse [48], even if distinct phenotypes were induced by single invalidations (metabolism for *Foxo1*, ovarian failure for *Foxo3a*, etc.). Similarly, only with the invalidation of both *Foxc1* and *Foxc2* does a phenotype of aborted somitogenesis appear in mice [2]. These kinds of observation indicate that redundancy between Forkhead transcription factors could provide, to some extent, a source of functional robustness for key processes. Although this has been documented for close Forkhead paralogs (i.e. from the same subfamily), mutations in more divergent Forkhead genes have also been found to affect similar processes and/or organs (e.g. effect of *FoxO3a* and *FoxL2* mutations and/or invalidation on ovarian function, effects of *FoxG1*, *FoxP1* and *FoxP2* on brain function, etc.). These instances could also indicate a degree of functional overlap and/or redundancy of more divergent Forkhead transcription factors. Obvious differences in overall phenotypes provoked by invalidation or mutation of particular genes from the Forkhead family have long captivated the attention of the research community, thus preventing the search for functional redundancies among more divergent paralogs. However, combinations of slightly hypomorphic alleles of such Forkhead genes might induce disease phenotypes in humans, acting as QTL. Uncovering such variants would be a big step towards understanding the functional interdependencies of different FOX members, and their potential implications in more complex pathologies.

Consequently, in addition to unravelling in more detail the specificities of members of the Forkhead family, future research should also try to further the understanding of the potential redundancies, notably by studying the phenotypes of mice carrying heterozygous or homozygous invalidations of FOX genes suspected to share partially redundant roles. These kinds of study could help uncover unsuspected common core roles of FOX genes.

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