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Pax genes in embryogenesis and oncogenesis

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Abstract

The paired box genes are a family of nine developmental control genes, which in human beings (*PAX*) and mice (*Pax*) encode nuclear transcription factors. The temporal and spatial expressions of these highly conserved genes are tightly regulated during foetal development including organogenesis. *PAX/Pax* genes are switched off during the terminal differentiation of most structures. Specific mutations within a number of *PAX/Pax* genes lead to developmental abnormalities in both human beings and mice. Mutation in *PAX3* causes Waardenburg syndrome, and craniofacial-deafness-hand syndrome. The Splotch phenotype in mouse exhibits defects in neural crest derivatives such as, pigment cells, sympathetic ganglia and cardiac neural crest-derived structures. The *PAX* family also plays key roles in several human malignancies. In particular, *PAX3* is involved in rhabdomyosarcoma and tumours of neural crest origin, including melanoma and neuroblastoma. This review critically evaluates the roles of *PAX/Pax* in oncogenesis. It especially highlights recent advances in knowledge of how their genetic alterations directly interfere in the transcriptional networks that regulate cell differentiation, proliferation, migration and survival and may contribute to oncogenesis.

Keywords: transcription factor • PAX • oncogenesis • embryogenesis

Introduction

The paired box (PAX/Pax) transcription factor family encoded by developmental control genes is characterized by a highly conserved paired-box DNA-binding domain (PD). This domain was initially identified in the *Drosophila* pair-rule segmentation gene *paired* (*prd*). Since that time, PAX/Pax homologues have been discovered in numerous species from nematodes and sea urchins to human beings [1–3]. At present, nine paired box genes are known in mice (*Pax1* to *Pax9*) and human beings (*PAX1* to *PAX9*), divided into four subgroups based on two additional motifs, the presence or absence of a conserved octapeptide (OP) distal to the PD and a complete or truncated version of a homeodomain (HD) (Table 1). The temporal and spatial expressions of *PAX* genes are tightly regulated. Expression is primarily observed during embryonal development, being switched off during later phases of terminal differentiation of most structures. Specific mutations within a number of the *PAX/Pax* genes lead to a range of developmental abnormalities in both human beings and mouse. Several members of the *PAX* family, especially subgroups II (*PAX2*, *PAX5* and *PAX8*) and III (*PAX3* and *PAX7*), play key roles in human malignancies, such as renal tumours, lymphoma, medullary thyroid carcinoma,

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Table 1 Paired box transcription factor family

Subgroup/ *PAX* gene		Chromosome location		Structure				Expression during development	Syndromes/diseases associated with *PAX*/ *Pax* genes	
		Human	Mouse	PD	OP	HD	TD		Human syndromes/diseases	Mouse knock-out phenotype
I	1	20p11	2	+	+	–	+	Sclerotome, thymus Skeleton	Klippel–Feil syndrome, Jarcho–Levin syndrome, salivary gland tumour	Disturbed skeletogenesis
	9	14q12-13	12	+	+	–	+	Sclerotome, Skeleton, cranio-facial, teeth, thymus	No thymus, no parathyroid glands, no teeth, craniofacial and limb defects	Oligodontia, esophageal carcinoma, Jarcho-Levin syndrome
II	2	10q25	19	+	+	Truncated	+	CNS, kidney, eye, ear, mammary gland	Renal–coloboma syndrome (papillorenal syndrome), renal cell carcinoma, Wilms' tumour, breast cancer, Kaposi sarcoma	Renal-coloboma syndrome
	5	9p13	4	+	+	Truncated	+	CNS, B lymphoid, testis	Large cell lymphoma, lymphocytic leukaemia, medulloblastoma, neuroblastoma, astrocytoma	No B-cells, brain defects
	8	2q12-14	2	+	+	Truncated	+	CNS, kidney, thyroid	Thyroid dysplasia, thyroid follicular carcinoma, Wilms' tumour, cancer of placenta, ovarian serous tumours	Hypothyroidism, neural crest defect
III	3	2q35	1	+	+	Complete	+	CNS, NC, muscle	Waardenburg syndrome, RMS, Ewing's sarcoma	Sp, Spr, Spd, Sp1H, Sp2H, Sp4H
	7	1p36.2	4	+	+	Complete	+	CNS, NC, muscle	RMS, Ewing's sarcoma, melanoma, squamous cell lung carcinoma	Neural crest defect
IV	4	7q32	6	+	–	Complete	+	CNS, pancreas	Silver–Russell syndrome, Wolcott–Rallison syndrome, diabetes, insulinoma	No pancreatic, β, δ-cells
	6	11p13	2	+	–	Complete	+	CNS, eye, nose	Aniridia, cataract, glioblastoma multiform, anaplastic glioblastoma, astrocytic glioma	Small eye, no pancreatic α-cells, brain defects

PD, paired-box DNA-binding domain; OP, octapeptide; HD, paired-type homeodomain (absent in subgroup I PAX proteins and truncated to a single helix in subgroup II PAX proteins); TD, proline–serine–threonine-rich transactivation domain; CNS, central nervous system; NC, neural crest and RMS, rhabdomyosarcoma. Refer to text for further structural and functional details.

rhabdomyosarcoma (RMS) and melanoma [4–6]. In this review, the roles of *PAX* genes in cancer are critically evaluated, in particular those of *PAX3* and *PAX2*.

PAX proteins and embryogenesis

PAX proteins can mediate DNA binding or transcriptional activation through distinct domains. The PD that makes sequence-specific contacts with DNA is composed of 128 amino acid residues. Several PAX proteins possess a second DNA-binding domain, the paired-type HD, which consists of highly conserved 60 amino acid residues. The HD shows strong homology with similar domains in other homeobox type gene products. Strong cooperative interactions occur between the PD and HD on DNA binding [7]. However, the PD can bind to the target DNA independently and with high affinity. In contrast, an independent binding of isolated HD cannot be detected. Most PAX proteins also contain an OP motif located between PD and HD. Deletion of the OP in some PAX/Pax indicates it has a transcriptional inhibitory activity [8]. The transactivation domain (TD) is a proline, threonine- and serine-rich region at the carboxy terminus of PAX that has been shown to mediate transcriptional regulation [4, 9].

PAX proteins have been implicated as regulators of embryogenesis and as crucial factors in maintaining the pluripotency of stem cell populations and cell-lineage specification during development [6, 10, 11]. Mutations of *PAX* are associated with major developmental defects. For instance, *PAX2* and *PAX8* double mutants show a complete lack of kidney formation [12]. Mutations in *PAX3* and *Pax3* cause Waardenburg syndrome and craniofacial-deafness-hand syndrome in human beings and the Splotch phenotype in mice, respectively [13, 14]. Heterozygous mutations in *PAX6/Pax6* result in eye abnormalities (microphthalmia) in human beings, mice and rats, respectively. Homozygous *Pax6*-mutant mice fail to develop eyes and nasal structures, display severe brain abnormalities and die soon after birth [15]. Key target organs or tissues of each PAX protein and its role in human diseases, including cancer, are presented in Table 1.

PAX genes and cancer

Persistent expression of *PAX* in partially differentiated tissues is associated with a block in tissue differentiation and hyperplasia. *PAX* are frequently expressed in cancer, and endogenous *PAX* gene expression is required for the growth and survival of cancer cells [16]. Pax1-3, -6 and -8 induce cellular transformation. Transfection of 3T3 cells with wild-type *Pax1, Pax3, Pax6,* or *Pax8* produced tumours in nude mice within 2 to 6 weeks; *Pax2* did so in 10 days. The tumours were well vascularized and resembled spindle cell sarcomas, with high and atypical mitotic activity and infiltration into nerve and muscle tissues, and blood vessels [17].

Several chromosomal translocations involving members of the subgroups II (*PAX5* and *PAX8*) and III (*PAX3* and *PAX7*) occur in various human cancers, suggesting altered regulation or transcriptional activity of *PAX* gene products promotes cellular transformation. Alterations in *PAX* genes of subgroups II and III are often associated with an unfavourable outcome, and knock-down of their expression in cancer cells leads to apoptosis. In contrast, *PAX* genes in subgroups I are either less often involved in cancer or their expression is indicative of a more favourable outcome. So far, *PAX1* (subgroup I) has been found by DNA microarray analysis to be up-regulated only in human salivary gland tumours [18]. The other member of subgroup I, *PAX9*, is expressed in normal epithelium of the adult human oesophagus and is absent or significantly reduced in the majority of invasive carcinomas and pre-cancerous epithelial dysplasias [19].

With regard to subgroup IV, overexpression of *PAX4* has been linked to insulinoma and lymphoma. *PAX4* is expressed in the early pancreas but later expression is restricted to β-cells and is absent in mature islets [20]. *PAX4* is highly expressed in human insulinomas [21]. *In vitro* studies show that PAX4 controls insulinoma cell survival through up-regulation of the anti-apoptotic gene, *BCL-XL* [22]. Demethylation in the promoter region of *PAX4*, leading to its overexpression, has been observed in primary lymphoma [23]. The forced expression of *PAX4* gene in HEK293 and SHSY/610 cell lines enhances cell growth. Thus ectopically expressed *PAX4* may have oncogenic roles *in vivo* by de-regulating cell proliferation and survival signals. *PAX6* is expressed throughout the pancreatic bud during embryogenesis but not in the mature pancreas. The expression of *PAX6* occurs in primary pancreatic adenocarcinomas and cell lines [24]. The overexpression of *Pax6* in transgenic mice promotes ductal and islet cell proliferation and the subsequent development of pancreatic cystic adenoma [25]. However, PAX6 exerts a tumour suppressor function that limits the growth of glioblastoma cells. High levels of *PAX6* expression are correlated with improved prognosis in malignant astrocytic gliomas, whereas low levels are associated with an unfavourable outcome [26]. PAX6 suppresses the invasiveness of glioblastoma cells by repressing the expression of *MMP2* [27]. Methylation and silencing of *PAX6* has been observed in breast cancers [28]. Recently, it was reported that *Pax6*-transduced (non-neuronal) Hela cells express neuron-specific genes and *Pax6* expression is a strong signal for induction of cell migration [29]. Whether PAX6/Pax6 confers positive or negative effects on cell proliferation and migration would seem to depend on the cell type.

PAX3 gene in embryogenesis and cancer

PAX3 is located on chromosome 2q35. Full-length *PAX3* consists of 10 exons encoding a 510 amino acid residue protein. Human PAX3 protein is 98% identical to the mouse orthologue [30]. Studies in *Xenopus* show that both *Pax3* and *Zic1* are independently required for neural crest (NC) differentiation. The cooperative functions of *Pax3* and *Zic1* determine NC cells fate [31]. *PAX3* expression is necessary for proliferation and migration of NC cells and muscle cell precursors in the dorsal dermomyotome. It is also involved in developmental pathways that lead to melanocytes and

neurons originating from the NC, and mature skeletal myocytes from the dorsal dermomyotome. Accordingly, PAX3 is implicated in the pathogenesis of tumours associated with these tissues, including RMS, melanoma and neuroblastoma. The following section will focus on the functions of PAX3 and the roles of its isoforms in myogenesis, melanogenesis, neurogenesis and related oncogenesis.

PAX3 in myogenesis and RMS

Skeletal muscles are formed from the paraxial mesoderm surrounding the neural tube. Cells of the dermomyotome exhibit early, restricted patterns in expression of *Pax3* and *Pax7*, and then develop into skeletal muscles of the trunk and limbs. Pax3 is involved in induction and migration of myoblast precursors, and in the expression of the muscle-specific transcription factors, MyoD, Myf-5 and myogenin (Fig. 1A). However, Pax3 is down-regulated when muscle tissue begins to differentiate and the muscle-specific transcription factors are activated [32, 33]. Ectopic expression of *Pax3* prevents the myogenic differentiation of myoblasts into myotubes, which might involve the cooperation of *Msx1* and *Notch* genes [34–37]. In chicken embryos, the expression of *Msx1* overlaps with *Pax3* in migrating limb muscle precursors and Msx1 antagonizes the myogenic activity of Pax3 [38]. *Msx2*, another *Msx* homeobox gene family member, was found to be an immediate downstream effector of Pax3. Pax3 represses *Msx2* expression in the development of the murine cardiac neural crest [39]. Pax3 potentiates the migration of hypaxial muscle precursors by directly modulating the expression of c-Met tyrosine kinase receptor [40, 41]. Interestingly, in muscle tumours, which often harbour an activated PAX3, *c-MET* is up-regulated [42]. Therefore, both muscle development and tumourigenesis involve regulation of the MET pathway by PAX3. In P19 carcinoma cells, Wnt3 up-regulates *Pax3* expression, which in turn, activates *Six1*, *Eya2* and *Dach2*. This is followed by the down-regulation of *Pax3* and activation of *MyoD* and myogenin expression [43, 44]. Thus evidence supports a role for Pax3 as a controller of a cascade of transcriptional events that are necessary and sufficient for skeletal myogenesis. This role deserves greater study from both scientific and clinical viewpoints. This hypothesis is also supported by observations that *PAX3/Pax3* mutations are associated with limb muscle hypoplasia in Waardenburg syndrome patients and Splotch phenotype mice, respectively [33, 45]. *PAX3* and *PAX7* have similar structures and patterns of expression. Despite this, a lack of *PAX3* expression during embryogenesis may not be compensated for by *PAX7* or other genes [46, 47]. On the other hand, the distinct roles of Pax3 and Pax7 in regenerative myogenesis of adult mammals suggest that Pax3 may not compensate for Pax7 in postnatal muscle development [48]. However, the function of Pax7 in specification of postnatal myogenic satellite cells is still controversial [49, 50]. The interaction between Pax3 and Pax7 needs further clarification.

RMS is the most frequent soft tissue tumour in children under 15 years old. It develops as a consequence of disruption to the regulation of the growth and differentiation of myogenic precursor cells. In contrast to normal myogenic cells, RMS tumour cells remain in cell cycle and usually fail to differentiate completely into muscle cells.

Embryonal RMS (ERMS) and alveolar RMS (ARMS) are two major subtypes [51, 52]. A number of molecular genetic lesions are implicated in the development of RMS. The amplification of genes, such as *PAX3/7-FKHR*, *MYCN*, *MDM2* and *CDK4*, is a characteristic feature of ARMS. Specific chromosomal gains, including chromosomes 2, 8, 12 and 13, are associated with ERMS. In addition, the disruption of some genes, for example *IGF2*, *P16*, *TP53* and of the HGF/c-MET signalling pathway has been implicated in the progression of RMS [42, 53–55]. Unlike normal muscle, a CpG island within *PAX3* is hypermethylated in the majority of ERMS but not in most ARMS. This CpG methylation is inversely correlated with *PAX3* expression [51].

Chromosomal translocations are characteristic of ARMS: with t(2;13)(q35;q14) and t(1:13)(q36;q14) occurring in about 75% and 25% of sufferers, respectively (Fig. 1B). The translocations lead to production of two fusion proteins: PAX3-FKHR and PAX7-FKHR. A microarray study of RMS identified a novel variant translocation t(2;2)(q35;q23), which generates a fusion protein composed of PAX3 and the nuclear receptor co-activator, NCOA1 [56]. The PAX3-NCOA1 protein is a transcriptional activator with similar transactivation properties to PAX3-FKHR. PAX3-FKHR shows 10–100 times the transactivating and transforming capacity of wild-type PAX3. The Fas death domain-associated protein (Daxx) represses the transcriptional activity of Pax3 by approximately 80% but Pax3-FKHR is unresponsive to this repressive effect [57]. Daxx-mediated repression of Pax3 is inhibited by the nuclear body associated protein PML [58]. PAX3-FKHR induces the expression of a large set of genes involved in myogenesis, such as *MyoD1*, *myogenin* and *Six1*. Other downstream targets are *BCL-XL*, *FKHR*, *TGF-*α, *PDGF-*α receptor, insulin-like growth factor (*IGF*)*-1* receptor, as well as genes that are not normally targets of wild-type PAX3 [51, 59]. A comparison of the gene expression profiles using microarrays revealed an overexpression of putative PAX3-FKHR target genes, such as *DCX*, *CNR1*, in PAX3-FKHR positive ARMS, relative to that in PAX3-FKHR negative ERMS [60]. The role(s) of PAX3/7-FKHR in promoting the different molecular pathogeneses of ARMS and ERMS remains to be tested and would surely prove to be a fruitful topic of study.

PAX3-FKHR can induce cellular transformation and prevent apoptosis [61, 62]. Furthermore, it shows oncogenic effects, predominantly at relatively low levels although suppression of growth occurs at higher levels [59]. The two DNA-binding domains of PAX3-FKHR, that is PD and the HD, are functionally separate influencing the control of growth suppression and transformation, respectively. In a landmark study, the mouse myoblast C2C12 cell line was transfected singly with cDNA for *Pax3*, *PAX3-FKHR*, *IGF-II* or cotransfected with *IGF-II* plus *Pax3* or with *IGF-II* plus *PAX3-FKHR* genes. All transfectants showed altered morphologies, a lack of differentiation and higher proliferation rates *in vitro* [63]. Moreover, the subcutaneous injection of C2C12 transfectants into nude mice produced tumours.

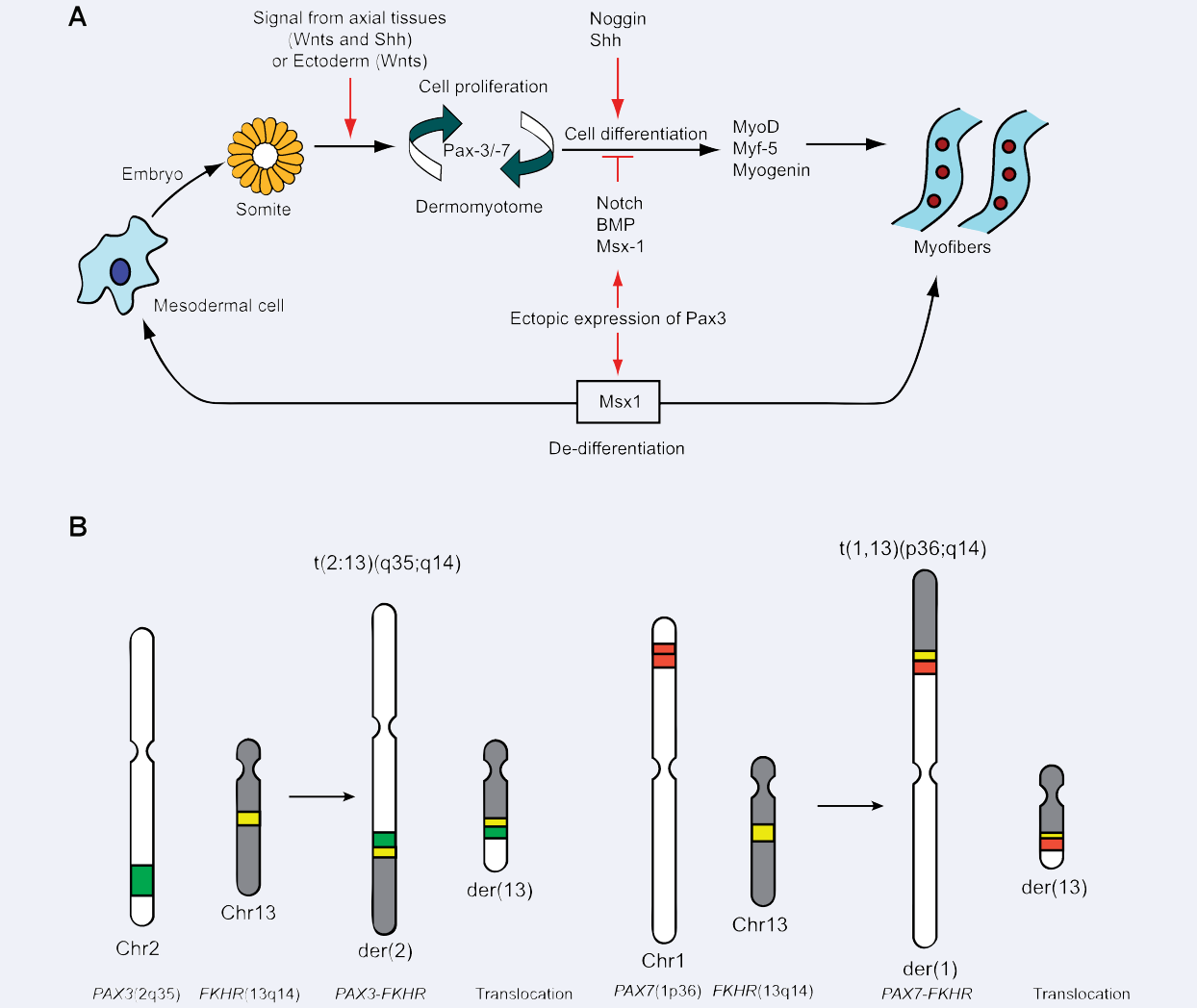

Fig. 1 (**A**) PAX3 and PAX7 are involved in myogenesis during embryonic development. The ectopic expression of PAX3 prevents terminal myogenic differentiation, possibly by regulating Msx1 and Notch signalling. (**B**) Schematic representation of the chromosomal translocations involving *PAX3*/*PAX7* and *FKHR*, which are known to result in alveolar rhabdomyosarcoma.

Tumours derived from *IGF-II* and *PAX3-FKHR* cotransfected cells were composed of undifferentiated cells showing most angiogenesis, least apoptosis and invaded normal muscle tissues. Schaaf *et al.* found that *IGF-II* is expressed at higher levels in RMS than in normal muscles [64], suggesting that PAX3 or PAX3-FKHR interact with IGF-II to play a critical role in RMS development, which is summarized in Fig. 2. Recently, the ability of PAX3 and PAX3-FKHR to promote RMS cell survival by regulating the expression of *PTEN* or *TFAP2B* was demonstrated [65, 66]. The involvement of PAX3 and PAX3-FKHR in RMS tumourigenesis is likely to be by at least partially altering the MET, PTEN or AP2 signalling pathways.

PAX3 in melanogenesis and melanoma

Melanocytes are dendritic pigment-producing cells that originate from non-pigmented precursors of the NC melanoblasts. Several transcription factors, including PAX3 and microphthalmia-associated transcription factor (MITF), are involved in this transformation. Pax3 is required to expand a pool of committed melanoblasts or restricted progenitor cells early in development, whereas MITF facilitates melanoblast survival within and immediately after, migration from the dorsal neural tube [67]. The expression of Pax3 is probably necessary, but not sufficient, for maintaining

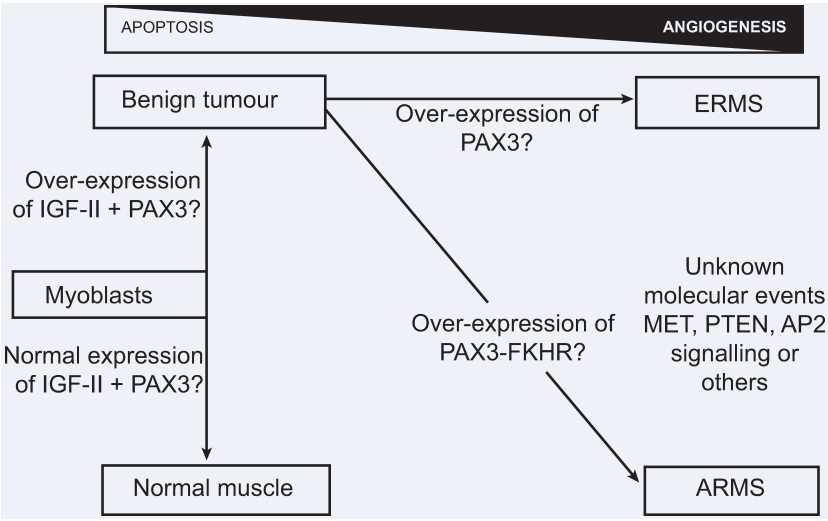

Fig. 2 A diagrammatic representation summarizing how PAX3 and the chimeric protein, PAX3-FKHR could promote the development of rhabdomyosarcoma (ERMS, embryonal rhabdomyosarcoma and ARMS, alveolar rhabdomyosarcoma).

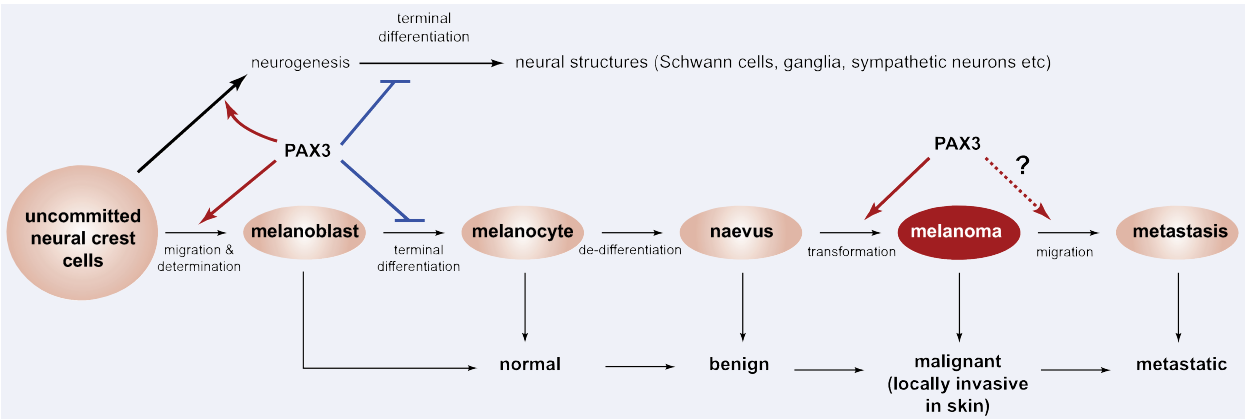

Fig. 3 The common origin of melanocytes and nerve cells from the neural crest and the development and progression of normal melanocytes to metastatic melanoma. The roles of PAX3 in neurogenesis, melanogenesis and melanoma development are proposed.

melanocytes in their differentiated state [68]. PAX3 promotes melanocyte lineage commitment while simultaneously preventing their differentiation (Fig. 3) [69]. Furthermore, PAX3 alone or in synergy with SOX10, activates *MITF* [70]. Failures in this regulation arising from *PAX3* mutations cause the auditory–pigmentary symptoms in Waardenburg syndrome 1 patients [71].

Melanocytes can develop into cutaneous and ocular melanoma. Pigmented ocular tumours can also develop from proliferating cells of the retinal pigment epithelium [72]. The incidence and mortality of cutaneous melanoma have increased at annual rates of 2–3% worldwide over the last 30 years, with the greatest increases seen in elderly men [73]. Melanomas can clinically progress through four subtypes: benign naevi to dysplastic naevi then radial and vertical growth phase melanoma and finally metastatic melanoma (Fig. 3) [74]. Dysplastic naevus has been suggested as the precursor of cutaneous melanoma.

The transition of melanocytes into clinically characterized melanoma is associated with changes in the function of numerous genes. Melanoma susceptibility genes are *CDKN2A* and *CDK4*, those for growth factors, such as *bFGF*, *PDGF* and *EGF*, and for proteins in signalling pathways, especially *MAPK*, *STAT* and *Nodal* [75–78]. Several transcription factors, such as PAX3, MITF, SOX10, c-MYC, PTEN, RAS and c-RET, also play roles in the pathogenesis of melanoma [79]. PAX3 is a key transcription factor in regulating the expression of a variety of melanocytic genes [69, 80].

PAX3 is expressed in primary melanomas and melanoma cell lines but not in the surrounding normal tissues or skin sections. Transfection of melanoma cells with antisense *PAX3* oligonucleotides triggers cell death by inducing apoptosis [81, 82]. The down-regulation of Pax3 in interleukin-6 receptor/interleukin-6 induced melanoma cells (B16F10.9) is linked to arrested growth and transdifferentiation to a glial cell phenotype. Pax3 reduction

also induces a loss of melanogenesis, followed by a sharp decrease in *MITF* mRNA and gene promoter activity [83]. Recently, PAX3 was identified as a regulator of the melanoma susceptibility and progression genes *SCF*, *TGF-β*, *MUC18*, *RhoC* and *TIMP3* using microarray analyses [84, 85]. In quiescent cells, the retinoblastoma tumour suppressor protein, pRB, in its unphosphorylated state, interacts with the transcription factor E2F and inhibits the transcription of E2F-responsive genes, which are essential for cell cycle progression. PAX3 interacts strongly with pRB [86].

PAX3 in neurogenesis and neuroblastoma

Pax3 is expressed in the developing nervous system during early neurogenesis (Fig. 3) [87]. The induction of *Pax3* in P19 embryonal carcinoma stem cells is closely linked to subsequent neuronal differentiation [88]. Koblar *et al.* found that Pax3 regulates the generation of sensory neurons from precursors that originate from the NC [89]. They demonstrated that *Pax3* mRNA was initially expressed in all NC cells but was later restricted to neurons. The addition of FGF2 to the cultures significantly increased *Pax3* mRNA expression in NC cells and resulted in increased neurogenesis.

Neuroblastoma is the commonest extracranial solid tumour in childhood. It is a disease of the sympathicoadrenal lineage of the neural crest, and therefore primary tumours may originate in all sites of peripheral sympathetic ganglia and paraganglia [90, 91]. Many genetic abnormalities have been implicated in neuroblastomas, including amplification of the *N-MYC* oncogene [92]. Overexpression of transfected *N-MYC* induces cellular transformation. For example, transgenic mice overexpressing *N-MYC* in NC-derived tissues frequently develop neuroblastomas. However, a reduced expression of *N-MYC* in cultured human neuroblastoma cell lines decreases proliferation and induces differentiation [93]. Abnormally elevated expression of *PAX3* has also been found in some neuroblastoma cell lines and tumours [94]. Deletion and mutagenesis experiments have shown that *Pax3* contains the inverted E box sequence CGCGTG (or CACGCG) in the 5′ promoter region, which responds to regulation by N-Myc and c-Myc. Mouse N-Myc and c-Myc directly activate the *Pax3* promoter and the ectopic expression of *N-Myc* and *c-Myc* increases *Pax3* expression [95]. Whether PAX3 initiates pathogenesis of neuroblastoma or N-MYC induces neuroblastoma by modulating *PAX3* expression, is a question worthy of further investigation.

PAX3 splicing and tumours

The PAX3 isoforms are designated as PAX3a, PAX3b, PAX3c, PAX3d, PAX3e, PAX3g and PAX3h (Fig. 4) [30, 96, 97]. PAX3a and PAX3b are encoded by exons 1–4 and lack the HD and the carboxy terminal TD. *PAX3c*, *d* and *e* contain 8, 9 and 10 exons, respectively, and their isoforms possess intact HD and TD. Both *PAX3c*

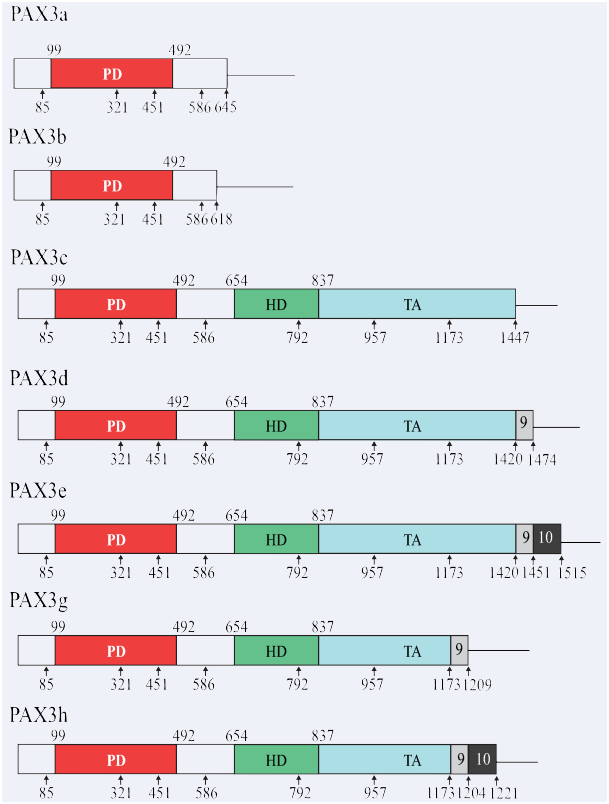

Fig. 4 PAX3 isoforms. The diagram illustrates the structure of PAX3 isoforms a-h DNA-binding domains: PD, paired domain; HD, homeodomain and TD, transactivation domain (not to scale). Lower vertical arrows and numbers indicate the nucleotide position of exon boundaries and the additional exons 9 and 10 are also indicated.

and *PAX3d* are evolutionarily conserved in human beings and mice. Intron 8 is retained in *PAX3c* transcript and translation proceeds from exon 8 for five codons into intron 8 before reaching a stop codon. Intron 8 is spliced in *PAX3d*, and translation proceeds from exon 8 to exon 9. The predicted amino acid residues of PAX3c and PAX3d are identical except at the extreme carboxy termini. *In vitro* DNA-binding and transactivation studies suggested that PAX3d is functionally similar to PAX3c [30, 46]. PAX3g and PAX3h are truncated isoforms of PAX3d and PAX3e, respectively [97], both lack part of the TD encoded by exon 8.

Alternative transcripts of *PAX3* have been identified in a variety of tissues, including human adult skeletal muscle and mouse embryos. Pax3g, also named Pax3Δ8, occurs in primary mouse myoblasts [98]. A transient transfection assay demonstrated that Pax3g was transcriptionally inactive, although its presence effectively inhibited the activity of Pax3d, presumably by competing with it for Pax3-binding sites. A further alternative splicing occurs in *Pax3* at the intron 2/exon 3 junction and results in the inclusion

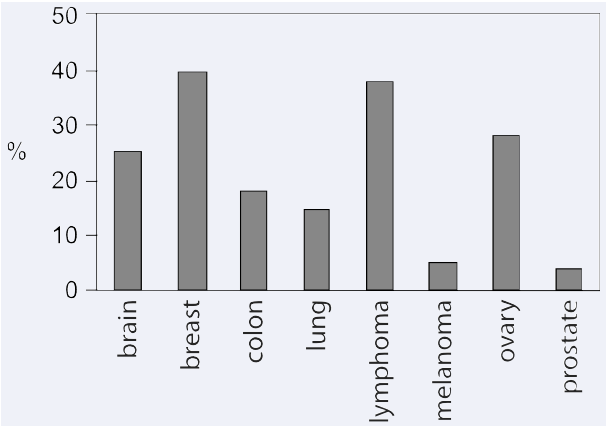

Fig. 5 Immunohistochemistry is used to screen PAX2 expression in eight common tumour types including tumours of brain, breast, colon, lung, ovary, prostate, lymphoma and melanoma. Percentages of human tumours expressing PAX2 are shown (adapted from [16]).

or exclusion of a glutamine (Q) residue (Pax3/Q^+ and Pax3/Q^- forms, respectively) in the linker between the amino and carboxy terminal paired box domains [99]. The Pax3/Q^- form has stronger binding affinity and higher transcriptional activity than the Pax3/Q^+ but both $Q^{+/-}$ forms are co-expressed in equal abundance during multiple developmental stages in mice. A functional study of PAX3 isoforms in mouse melanocytes *in vitro* demonstrated they differ in biological functions [100]. PAX3c, d and h promote melanocyte proliferation, migration, transformation and survival. Other isoforms have negative or no discernable effects on melanocytes.

PAX3 splicing variants show distinct expression profiles in different tumours. Compared to *PAX3a*, a high proportion of neuroblastomas and RMS express *PAX3b*, although the levels of both are low. Truncated isoforms of *PAX6* act in a dominant negative manner when co-expressed with wild-type *PAX6* [101]. Similar results have been found with *PAX4* and *PAX5*. Thus PAX3a and PAX3b might interact in a similar way with other PAX3 isoforms to regulate their functions.

PAX3d is present in Ewing's sarcoma, melanoma and ERMS cell lines but is generally absent in neuroblastoma [46]. *PAX3c* and *PAX3d* are preferentially expressed in melanocytes, melanoma cell lines and melanoma tissues but only faintly in testes, muscle, brain and brain tumours and are absent in the other normal tissues and cancer cell lines [102]. *PAX3d* is the main isoform present in ARMS cell lines tested although *PAX3c* is expressed strongly in half of them (our unpublished data). With regard to ERMS cell lines, *PAX3c* and *PAX3d* are expressed at low-to-moderate levels [46].

Thus the published literature suggests that PAX3 isoforms may have significant roles in the development and progression of tumours of NC origin, specifically, PAX3c and PAX3d in melanomas and PAX3g and PAX3h in neuroblastomas [46, 94, 97, 100, 102]. PAX3a and PAX3b may function by regulating the transactivation properties of the other isoforms, as indeed, do the truncated isoforms of PAX4, PAX5 and PAX6. The precise roles of these PAX3 spliced variants in normal developmental processes and in oncogenesis remain to be elucidated, and surely warrants an extended series of investigations.

PAX2 in tumourigenesis

A comprehensive immunohistochemical study involving 54 cancer cell lines and 406 primary tumour tissues representing eight common tumour types, found 90% of cancer cell lines and 25% of tumours of brain, breast, colon, lung, ovary, prostate, lymphoma and melanoma are *PAX2* positive (Fig. 5). *PAX2* RNA interference induces apoptosis in tumour cells [16]. It was demonstrated that inhibition of *PAX2* expression in prostate cancer cells results in cell death [103]. Similarly, transfection of antisense *PAX2* into Kaposi sarcoma cells results in reduced cell motility, invasiveness and cell death [104] indicating that the endogenous expression of *PAX2* is needed for their growth and survival.

PAX2, apart from being crucial to the development of the kidney, eye and mammary gland, is involved in paediatric nephroblastoma or Wilms' tumour. This arises from the primitive metanephric blastema. Wilms' tumour is frequently associated with precursor lesions, known as nephrogenic rests that are the foci of normal embryonal cells persisting into postnatal life. These may regress, remain dormant, develop into benign adenomatous structures or progress to Wilms' tumour [105]. Most cases of Wilms' tumour are sporadic, but 5–10% are hereditary. The latter are thought to result from a germ cell mutation, followed by loss of heterozygosity in a single somatic cell [106]. Germline and somatic mutations and deletions affect the Wilms' tumour suppressor gene, *WT1*, which is a major genetic disposing factor in children with Wilms' tumour. Both *PAX2* and *WT1* are normally expressed in tissues undergoing mesenchyme to epithelial transition. During renal development, PAX2 and WT1 regulate the expression of each other [107]. WT1 is a negative regulator of *PAX2*. As the level of *WT1* increases, that of *PAX2* decreases. High levels of *WT1* are first seen in the proximal region of the S-shaped body, the glomerular region, where *PAX2* expression first declines [108]. The addition of *WT1* to cells with a chloramphenicol acetyl transferase reporter gene under the control of *Pax2* promoter sequences showed a five-fold reduction in its transcription [109]. Pax2 can transactivate *WT1* and inhibition of *Pax2* has been shown to repress *WT1* expression and block nephron differentiation [110].

Renal cell carcinoma (RCC) is thought to arise from proximal tubules in kidney. It often accompanies Von Hippel Lindau (VHL) syndrome, which is the result of mutations in or deletion of the *VHL* tumour suppressor gene. Over 70% of cell lines derived from RCCs with deletions/mutations of *VHL* express *PAX2* [111]. Indeed, *PAX2* is expressed in all renal tumour subtypes, except

transitional cell carcinomas, with papillary RCC expressing the highest level [112]. Moreover, the extent of *PAX2* expression is correlated with proliferation index and is significantly higher in patients with metastatic disease.

PAX2 has an anti-apoptotic function in embryonic renal cells [113]. The inhibition of *PAX2* expression in RCC cell lines triggers growth inhibition and cell death [111]. An increased in *PAX2* expression protects cells against high NaCl concentration-induced apoptosis [114]. The mechanism of PAX2-mediated protection from cell death is unknown. The mesenchyme of dysplastic kidneys fails to express the anti-apoptosis gene, *BCL-2.* Consequently increased apoptosis is seen in the mesenchyme which reduces precursor cell survival. In contrast, *BCL-2* is expressed in the dysplastic epithelia [108]. This is ectopic expression, because ureteric bud derivatives do not normally express *BCL-2*, therefore, little or no apoptosis occurs in the dysplastic epithelium, enhancing cell proliferation. Because enhanced expression of PAX3 or PAX3/FKHR stimulates transcription of *BCL-XL* [115], *PAX2* might also play a role regulating the expression of *BCL-2*.

PAX5 in tumourigenesis

The chromosomal rearrangement t(9;14)(p13;q32), which results in the fusion of *PAX5* to genes for immunoglobulin heavy chain, has been reported in a subset of non-Hodgkin's lymphomas (NHL) [116]. *PAX5* expression occurs in various types of benign and malignant tumours including B-cell NHL, Hodgkin lymphomas, Merkel cell carcinoma, small cell carcinoma, neuroendocrine carcinomas and medulloblastomas [117, 118]. *PAX5* is the most frequent site of somatic mutations in acute lymphoblastic leukaemia [119]. Mutations of *p53* are associated with astrocytomas and glioblastomas. The expression of *PAX5* in astrocytomas is inversely proportional to the expression of *p53*, and *PAX5* can bind to the *p53* promoter, repressing its activity [120]. *PAX5* expression is also identified in N-type neuroblastoma cells (a malignant subset), but is absent in S-type cells (a benign subset). The forced expression of *PAX5* in S-type cells has been shown to confer N-type characteristics such as increased rate of proliferation and the ability to form colonies in soft agar [121]. Recently, it was confirmed that *PAX5* is expressed in poorly differentiated neuroendocrine tumours but never in well-differentiated classic carcinoid tumours [122]. However, loss of *Pax5* in mature B cells can initiate lymphoma development in mice despite their advanced differentiation [123]. This implies that PAX5/Pax5 has cell type specific effects on cell differentiation. *PAX5* transcription is enhanced by STAT5 in the early stages of B cell development. Indeed, a STAT-binding motif has been shown to occur in the *PAX5* promoter [124]. Constitutive activation of STAT5 is associated strongly with tumourigenesis [125]. The expression of dominant-negative *Pax5* in murine lymphomas and *PAX5* knockdown in human lymphoma negatively affects cell expansion [126]. Hence PAX5 may contribute to oncogenesis through the STAT signalling pathway and/or by the direct inhibition of *p53* expression.

PAX8 in tumourigenesis

PAX8 is expressed in most non-invasive urothelial neoplasia but not in normal adult urothelial epithelium [127]. It is highly expressed in epithelial ovarian cancer but expression appears absent in the precursor ovarian surface epithelia of healthy individuals [128]. The translocation, t(2;3)(q13; p25) results in the fusion of *PAX8* and peroxisome proliferator-activated receptor gamma (*PPARgamma*) genes. The production of the PAX8-PPARgamma fusion protein contributes to neoplasia by acting as a dominant-negative inhibitor of wild-type PPARgamma [129]. The occurrence of the *PAX8-PPARgamma* is thought to be restricted to follicular tumours (adenomas and carcinomas) of the thyroid. A subset of the follicular variant of papillary thyroid carcinoma harbours the *PAX8-PARgamma* translocation, which is significantly associated with multi-focality and vascular invasion [130]. Foukakis *et al.* demonstrated that the Ras effector NORE1A, a putative tumour suppressor, is suppressed in follicular thyroid carcinomas with a *PAX8-PPARgamma* fusion [131]. An *in vitro* study has shown that PAX8-PPARgamma stimulates thyroid cell viability but inhibits thyroid-specific gene expression [132]. Thus it appears possible that the fusion protein may contribute to the malignant transformation of thyroid follicular cells by modulating the Ras signalling pathway.

PAX and the treatment of cancer

PAX proteins may be useful tools in the diagnosis of cancers. For example, PAX2 immunostaining has been used to identify nephrogenic adenoma [133]. This staining has been recommended as a method to distinguish between metastatic ovarian serous papillary carcinoma and primary breast carcinoma [134]. PAX5 immunohistochemistry is employed in the diagnosis and sub-classification of lymphomas [135].

Genetic alterations to members of the *PAX* family directly interfere in transcriptional networks leading to oncogenesis by regulating tumour cell survival, proliferation and migration [16, 22, 23, 65, 81, 114]. Thus some of the *PAX* genes are potential targets for gene-based cancer therapy. Also, gene-specific therapies, such as antisense oligonucleotides or RNA interference-based treatments have progressed to clinical trials although these types of treatment have potential problems [136, 137]. However, the *in vitro* transfection of tumour cells with appropriate *PAX* gene antisense oligonucleotide or RNA interference molecules induce cell apoptosis or reduce cell proliferation or migration [16, 81, 82, 104, 111, 126].

PAX fusion proteins, for example PAX3-FKHR, PAX7-FKHR, PAX8-PPARgamma, have critical roles in malignant transformation. Recently, Broeke *et al.* identified a PAX-FKHR fusion protein breakpoint epitope in ARMS that induced a human cytotoxic T-lymphocyte line to kill ARMS cells [138]. Thus, PAX fusion proteins are potential targets of immunotherapy-based treatments for some malignancies. PAX3d is a melanocyte/melanoma-specific immunogenic antigen capable of inducing IgG antibodies [102]. Hence the PAX3d isoform may be a useful target for the development of immunotherapy in patients with melanoma. It is apparent that much work is still required to elucidate the role(s) of PAX/Pax in normal and disease cells. However, the benefits to patients of such studies are likely to be considerable.

Summary

In summary, PAX/Pax transcription factors control organ development and tissue differentiation during embryogenesis. They are involved in regulating cell differentiation, proliferation, migration and the survival of different cell types through transactivation of target genes. *PAX* genes clearly have pivotal roles in the oncogenesis of several human tumours. The use of microarray investigations has been of enormous value in unravelling the mechanisms of oncogenesis associated with *PAX* genes [18, 56, 60, 64, 66, 84, 85, 129]. The vast increases in knowledge of PAX-related activities provided by such studies are being exploited to identify potential new targets for cancer treatment.

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