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,
Table 1 Paired box transcription factor family

| Subgroup/ PAX gene | Chromosome location | Structure | Expression during development | Syndromes/diseases associated with PAX/ Pax genes | Human syndromes/diseases | Mouse knock-out phenotype |
|--------------------|---------------------|-----------|-------------------------------|-----------------------------------------------|--------------------------|---------------------------|
| I                  |                     |           |                               |                                               | Human syndromes/diseases | Mouse knock-out phenotype |
| I                  | 1                   | 20p11     | 2                             | ++                                            | Sclerotome, thymus       | Human syndromes/diseases  |
|                    |                     |           |                               |                                               | Skeleton                 | Mouse knock-out phenotype |
|                    | 9                   | 14q12-13  | 12                            | ++                                            | Sclerotome, Skeleton,    | Disturbed skeletogenesis  |
|                    |                     |           |                               |                                               | cranio-facial, teeth,    |                           |
|                    |                     |           |                               |                                               | thymus                   |                           |
|                    | 2                   | 10q25     | 19                            | ++                                            | Truncated                | Renal–coloba syndrome    |
|                    |                     |           |                               |                                               | CNS, kidney, eye, ear,   | (papillorenal syndrome), |
|                    |                     |           |                               |                                               | mammary gland            | renal cell carcinoma,    |
|                    |                     |           |                               |                                               |                         | Wilms’ tumour, breast    |
|                    |                     |           |                               |                                               |                          | cancer, Kaposi sarcoma   |
| II                 | 5                   | 9p13      | 4                             | ++                                            | Truncated                | Large cell lymphoma,     |
|                    |                     |           |                               |                                               | CNS, B lymphoid, testis  | lymphocytic leukaemia,   |
|                    |                     |           |                               |                                               |                         | medulloblastoma, neuro-  |
|                    |                     |           |                               |                                               |                          | blastoma, astrocytoma    |
|                    | 8                   | 2q12-14   | 2                             | ++                                            | Truncated                | Thyroid dysplasia, thy-  |
|                    |                     |           |                               |                                               | CNS, kidney, thyroid     |roid follicular carci-    |
|                    |                     |           |                               |                                               |                         | noma, Wilms’ tumour,     |
|                    |                     |           |                               |                                               |                          | cancer of placenta,      |
|                    |                     |           |                               |                                               |                          | ovarian serous tumours   |
| III                | 3                   | 2q35      | 1                             | ++                                            | Complete                | Waardenburg syndrome,    |
|                    |                     |           |                               |                                               | CNS, NC, muscle          | RMS, Ewing’s sarcoma     |
|                    | 7                   | 1p36.2    | 4                             | ++                                            | Complete                | RMS, Ewing’s sarcoma     |
|                    |                     |           |                               |                                               | CNS, NC, muscle          | melanoma, squamous       |
|                    |                     |           |                               |                                               |                          | cell lung carcinoma      |
| IV                 | 4                   | 7q32      | 6                             | ++                                            | Complete                | Silver–Russell syndrome, |
|                    |                     |           |                               |                                               | CNS, pancreas           | Wolcott–Rallison syndrome, |
|                    |                     |           |                               |                                               |                          | diabetes, insulinoma     |
|                    | 6                   | 11p13     | 2                             | ++                                            | Complete                | No pancreatic, β, α-cells |
|                    |                     |           |                               |                                               | CNS, eye, nose          |                           |

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 \( PAX6/Pax6 \) 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 \( \beta \)-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.
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 tumorigenesis is likely to be by at least partially altering the MET, PTEN or AP2 signalling pathways.

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.

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...
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 sympatheticadrenal lineage of the neural crest, and therefore primary tumours may originate in all sites of peripheral sympathetic ganglia and paranganglia [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 CGCCGTG (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 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 PAX3c 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 Pax3Δδ, 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...
or exclusion of a glutamine (Q) residue (Pax3/Q− and Pax3/Q−/H11001 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−/H11001 but both Q−/H11001 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 mesenchymal 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 epithelium [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 tumorigenesis

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 tumorigenesis [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 tumorigenesis

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-PPARgamma 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 [118]. 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 transcriptional regulation 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.

References

1. Walther C, Guenet JL, Simon D, Deutsch U, Jostes B, Goulding MD, Platchov D, Balling R, Gruss P. Pax: a murine multi-gene family of paired box-containing genes. Genomics. 1991; 11: 424–34.

2. Noll M. Evolution and role of Pax genes. Curr Opin Genet Dev. 1993; 3: 595–605.

3. Mansouri A, Hallonet M, Gruss P. Pax genes and their roles in cell differentiation and development. Curr Opin Cell Biol. 1996; 8: 851–7.

4. Chi N, Epstein JA. Getting your Pax straight: Pax proteins in development and disease. Trends Genet. 2002; 18: 41–7.

5. Robson EJ, He SJ, Eccles MR. A Partnerama of PAX genes in cancer and development. Nat Rev Cancer. 2006; 6: 52–62.

6. Lang D, Powell SK, Plummer RS, Young KP, Ruggeri BA. Pax genes: roles in development, pathophysiology, and cancer. Biochem Pharmacol. 2007; 73: 1–14.

7. Apuzzo S, Gros P. Cooperative interactions between the two DNA binding domains of PAX3: helix 2 of the paired domain is in the proximity of the amino terminus of the homeodomain. Biochemistry. 2007; 46: 2984–93.

8. Eberhard D, Jimenez G, Heavey B, Busslinger M. Transcriptional repression by Pax5 (BSAP) through interaction with corepressors of the Groucho family. EMBO J. 2000; 19: 2292–303.

9. Robichaud GA, Nardini M, Laflamme M, Cuperlovic-Culf M, Ouellette RJ. Human Pax-C-terminal isoforms possess distinct transcription activating properties and are differentially modulated in normal and malignant B cells. J Biol Chem. 2004; 279: 49956–63.

10. Buckingham M. Skeletal muscle progenitor cells and the role of Pax genes. C R Biol. 2007; 330: 530–3.

11. Lin HT, Kao CL, Lee KH, Chang YL, Chiou SH, Tsai FT, Tsai TH, Sheu DC, Ho LL, Ku HH. Enhancement of insulin-producing cell differentiation from embryonic stem cells using pax4-nucleofection method. World J Gastroenterol. 2007; 13: 1672–7.

12. Bouchard M, Souabni A, Mandler M, Neubuser A, Busslinger M. Nephric lineage specification by Pax2 and Pax8. Genes Dev. 2002; 16: 2958–70.

13. Chalepakis G, Goulding M, Read A, Strachan T, Gruss P. Molecular basis of Splotch and Waardenburg Pax-3 mutations. Proc Natl Acad Sci USA. 1994; 91: 3685–9.

14. Machado AF, Martin LJ, Collins MD. Pax3 and the splotch mutations: structure, function, and relationship to teratogenesis, including gene-chemical interactions. Curr Pharm Des. 2001; 7: 751–85.

15. Hill RE, Favor J, Hogan BL, Ton CC, Saunders GF, Hanson IM, Prosser J, Jordan T, Hastie ND, van Heyningen V. Mouse small eye results from mutations in the gene Pax4. Nature. 1991; 354: 522–5.

16. Muratovska A, Zhou C, He S, Goodyer P, Eccles MR. Paired-Box genes are frequently expressed in cancer and often required for cancer cell survival. Oncogene. 2003; 22: 7989–97.

17. Maulbecker CC, Gruss P. The oncogenic potential of Pax genes. EMBO J. 1993; 12: 2361–7.

18. Francisco F, Carinci F, Tosi L, Scapoli L, Pezzetti F, Passerella E, Evangelisti R, Pastore A, Pelucchi S, Piattelli A, Rubini C, Fironi M, Carinci P, Volinia S. Identification of differentially expressed genes in human salivary gland tumors by DNA microarrays. Mol Cancer Ther. 2002; 1: 533–8.

19. Gerber JK, Richter T, Kremmer E, Adamski J, Höfler H, Balling R, Peters H. Progressive loss of PAX9 expression correlates with increasing malignancy of dysplastic and cancerous epithelium of the human oesophagus. J Pathol. 2002; 197: 293–7.

20. Sosa-Pineda B. The gene Pax4 is an essential regulator of pancreatic beta-cell development. Mol Cells. 2004; 18: 289–94.

21. Miyamoto T, Kakizawa T, Ichikawa K, Nishio S, Kajikawa S, Hashizume K. Expression of dominant negative form of Pax4 in human insulinoma. Biochem Biophys Res Commun 2001; 282: 34–40.

22. Brun T, Duhamel DL, Hu He KH, Wolffheim CB, Gauthier BR. The transcription factor PAX4 acts as a survival gene in INS-1E insulinoma cells. Oncogene. 2007; 26: 4261–71.

23. Li Y, Nagai H, Ohno T, Ohashi H, Murohara T, Saito H, Kinosita T. Aberrant DNA demethylation in promoter region and aberrant expression of mRNA of PAX4 gene in hematologic malignancies. Leuk Res. 2006; 30: 1547–53.

24. Lang D, Mascarenhas JB, Powell SK, Haledouga J, Nelson M, Ruggeri BA. PAX6 is expressed in pancreatic adenocarcinoma and is downregulated during induction of terminal differentiation. Mol Carcinog. 2008; 47: 148–56.

25. Yamaoka T, Yano M, Yamada T, Matsushita T, Moritani M, Il S, Yoshimoto K, Hata J, Itakura M. Diabetes and pancreatic
tumours in transgenic mice expressing Pax6. Diabetologia. 2000; 43: 332–9.

26. Zhou YH, Wu X, Tan F, Shi YX, Glass T, Liu TJ, Wathen K, Hess KR, Gumin J, Lang F, Yung WK. Pax6 suppresses growth of human glioblastoma cells. J Neurooncol. 2005; 71: 223–9.

27. Mayes DA, Hu Y, Teng Y, Siegel E, Wu X, Panda K, Tan F, Yung WK, Zhou YH. Pax6 suppresses the invasiveness of glioblastoma cells and the expression of the matrix metalloproteinase-2 gene. Cancer Res. 2006; 66: 9809–17.

28. Ballesta E, Paz MF, Valle L, Wei S, Fraga MF, Espada J, Cigudosa JC, Huang TH, Esteller M. Methyl-CpG binding proteins identify novel sites of epigenetic inactivation in human cancer. EMBO J. 2003; 22: 6335–45.

29. Cartlier L, Laforge T, Feki A, Arnaudeau S, Dubois-Dauphin M, Krause KH. Pax6-induced alteration of cell fate: shape changes, expression of neuronal alpha tubulin, postmitotic phenotype, and cell migration. J Neurobiol. 2006; 66: 421–36.

30. Barber TD, Barber MC, Cloutier TE, Friedman TB. Pax3 gene structure, alternate splicing and evolution. Gene. 1999; 237: 311–9.

31. Sato T, Sasai N, Sasai Y. Neural crest determination by co-activation of Pax3 and Zic1 genes in Xenopus ectoderm. Development. 2005; 132: 2355–63.

32. Bailey P, Holowacz T, Lassar AB. The origin of skeletal muscle stem cells in the embryo and the adult. Curr Opin Cell Biol. 2001; 13: 679–89.

33. Lamey TM, Koenders A, Ziman M. Pax genes in myogenesis: alternate transcripts add complexity. Histol Histopathol. 2004; 19: 1289–300.

34. Odelberg SJ, Kolhoff A, Keating MT. Differdetermination of mammalian myotubes induced by msx1. Cell. 2000; 103: 1099–1045.

35. Miller KA, Barrow J, Collinson JM, Davidson S, Lear M, Hill RE, Mackenzie A. A highly conserved Wnt-dependent TCF4 binding site within the proximal enhancer of the anti-myogenic Msx1 gene supports expression within Pax3-expressing limb bud muscle precursor cells. Dev Biol. 2007; 311: 665–73.

36. Luo D, Renault VM, Rando TA. The regulation of Notch signaling in muscle stem cell activation and postnatal myogenesis. Semin Cell Dev Biol. 2005; 16: 612–22.

37. Kitamura T, Kitamura YI, Funahashi Y, Shawber CJ, Castrillon DH, Koliappar R, DePinho RA, Kitajewski J, Accili D. A Foxo/Notch pathway controls myogenic differentiation and fiber type specification. J Clin Invest. 2007; 117: 2477–85.

38. Bendall AJ, Ding J, Hu G, Shen MM, Abate-Shen C. Msx1 antagonizes the myogenic activity of Pax3 in migrating limb muscle precursors. Development. 1999; 126: 4965–76.

39. Kwang SJ, Brugger SM, Lazik A, Merrill AE, Wu LY, Liu YH, Ishii M, Sangiorgi FO, Rauchman M, Sucov HM, Maas RL, Maxson RE Jr. Msx2 is an immediate downstream effector of Pax3 in the development of the murine cardiac neural crest. Development. 2002; 129: 327–38.

40. Epstein JA, Shapira DN, Cheng J, Lam PY, Maas RL. Pax3 modules expression of the c-Met receptor during limb muscle development. Proc Natl Acad Sci USA. 1996; 93: 4213–8.

41. Relaix F, Polimeni M, Rocancourt D, Schafer BW, Buckingham M. The transcriptional activator Pax3-FKHR rescues the defects of Pax3 mutant mice but induces a myogenic gain-of-function phenotype with ligand-independent activation of Met signaling in vivo. Genes Dev. 2003; 17: 2950–65.

42. Chen Y, Takita J, Mizuguchi M, Tanaka K, Ida K, Koh K, Igarashi T, Hanada R, Tanaka Y, Park MJ, Hayashi Y. Mutation and expression analyses of the MET and CDK2A genes in rhabdomyosarcoma with emphasis on MET overexpression. Genes Chromosomes Cancer. 2007; 46: 348–58.

43. Petropoulos H, Skjerjanc IS. Beta-catenin is essential and sufficient for skeletal myogenesis in P19 cells. J Biol Chem. 2002; 277: 15393–9.

44. Shen MM, Arcara R, Perez-Polo JR, De Giovanni C, Ingram WA, Cigudosa JC, Mendelsohn C, dePinho RA. The PAX3-FKHR oncoprotein is unresponsive to the Pax3-associated repressor hDaxx. EMBO J. 1999; 18: 3702–11.

45. Lehembre F, Muller S, Pandolfi PP, Dejean A. Regulation of Pax3 transcriptional...
activity by SUMO-1-modified PML. Oncogene. 2001; 20: 1–9.

59. Xia SJ, Barr FG. Analysis of the transforming and growth suppressive activities of the PAX3-FKHR oncoprotein. Oncogene. 2004; 23: 6864–71.

60. Loe M, Ahn EH, Mercado GE, Chuai S, Edgar M, Pawel BR, Oshen A, Barr FG, Ladanyi M. Global gene expression profiling of PAX3-FKHR fusion-positive alveolar and PAX-FKHR fusion-negative embryonal rhabdomyosarcomas. J Pathol. 2007; 212: 143–51.

61. Bernasconi M, Rempis A, Fredericks WJ, Rauscher FJ, 3rd, Schafer BW. Induction of apoptosis in rhabdomyosarcoma cells through down-regulation of PAX proteins. Proc Natl Acad Sci USA. 1996; 93: 13164–9.

62. Lam PY, Sublett JE, Hollenbach AD, Roussell MF. The oncogenic potential of the Pax3-FKHR fusion protein requires the Pax3 homeodomain recognition helix but not the Pax3 paired-box DNA binding domain. Mol Cell Biol. 1999; 19: 594–601.

63. Wang W, Kumar P, Epstein J, Helman L, Moore JV, Rauscher FJ, 3rd, Schafer BW. Induction of apoptosis in rhabdomyosarcoma cells through downregulation of PAX proteins. Proc Natl Acad Sci USA. 1996; 93: 13164–9.

64. Schaaf GJ, Ruijter JM, van Ruissen EJ, Millar SE, Jonkers J, Goedemaker JH, Wessels L, van Loo P, van Hagen A, Broxterman HJ, Van Den Abbeele AD. Interaction of the pRB-family proteins with factors containing paired-like homeodomains. Oncogene. 1998; 16: 227–36.

65. Bignami S, Colombo D, Tandri G, Leme R, Dallara V, Gratacos M, Turchi T, Dioguardi N, Albertini F. The transcription factor II and PAX3-FKHR cooperate in the oncogenic potential of PAX3, PAX3c, PAX3e and PAX3g isoforms in melanocytes by microarray analysis. Int J Cancer. 2007; 120: 1223–31.

66. Wiggan O, Taniguchi-Sidle A, Hamel PA. Interaction of the pRb-family proteins with factors containing paired-like homeodomains. Oncogene. 1998; 16: 227–36.

67. Goulding MD, Chalepakis G, Deutsch U, Ersselius JR, Gruss P. Pax-3, a novel murine DNA binding protein expressed during early neurogenesis. EMBO J. 1991; 10: 1135–47.

68. Pruitt SC. Discrete endogenous signals mediate neural competence and induction in P19 embryonal carcinoma stem cells. Development. 1994; 120: 3301–12.

69. Koblar SA, Murphy M, Barrett GL, Underhill A, Gros P, Bartlett PF. Pax-3 regulates neurogenesis in neural crest-derived precursor cells. J Neurosci Res. 1999; 56: 518–30.

70. Schiller GM, Jorgenson AL, Miller AJ, Mihm MC Jr. Melanoma susceptibility and progression of melanoma. Oncogene. 2003; 22: 3087–91.

71. Tandri G, Colombo D, Bignami S, Leme R, Dallara V, Chalepakis G, Turchi T, Dioguardi N, Albertini F. The transcription factor II and PAX3-FKHR cooperate in the oncogenic potential of PAX3, PAX3c, PAX3e and PAX3g isoforms in melanocytes by microarray analysis. Int J Cancer. 2007; 120: 1223–31.

72. Polsky D, Cordon-Cardo C. Oncogenes in melanoma. Oncogene. 2003; 22: 3087–91.

73. Miller AJ, Mihr MC Jr. Melanoma. N Engl J Med. 2006; 355: 51–65.

74. Thompson JF, Scolyer RA, Keeford RF. Cutaneous melanoma. Lancet. 2005; 365: 687–701.

75. Polsky D, Cordon-Cardo C. Oncogenes in melanoma. Oncogene. 2003; 22: 3087–91.

76. de Snoo FA, Hayward NK. Cutaneous melanoma susceptibility and progression genes. Cancer Lett. 2005; 230: 153–66.

77. Rodolfo M, Daniotti M, Vallacchi V. Embryonic and tumorigenic pathways converge via Nodal signaling; role in melanoma aggressiveness. Nat Med. 2006; 12: 925–32.

78. Vance KW, Dummer R, Schafer BW. Induction of apoptosis in rhabdomyosarcoma cells through downregulation of PAX proteins. Proc Natl Acad Sci USA. 1996; 93: 13164–9.

79. Li HG, Wang Q, Li HM, Kumar S, Parker C, Slovin M, Kumar P, Pax3 and Pax3-FKHR promote rhabdomyosarcoma cell survival through downregulation of PTEN. Cancer Lett. 2007; 253: 215–23.

80. Ebauer M, Wahleitner M, Niggl F, Schäfer BW. Comparative expression profiling identifies an in vivo target gene signature with TFA2P2 as a mediator of the survival function of PAX3/FKHR. Oncogene. 2007; 26: 7267–81.

81. Humansky TJ, Hayes DJ, Chiu LY, Ziff EB. Transcription factors in melanocyte development: distinct roles for Pax-3 and Mitf. Mech Dev. 2001; 101: 47–59.

82. Prince S, Iling N, Kidson SH, SV-40 large T antigen reversibly inhibits expression of tyrosinase, TRP-1, TRP-2 and Mitf, but not Pax-3, in conditionally immortalized mouse melanocytes. Cell Biol Int. 2001; 25: 91–102.

83. Lang D, Lu MM, Huang L, Engleka KA, Zhang M, Chu EY, Lipner S, Skoultchi A, Millar SE, Epstein JA. Pax3 functions at a nodal point in melanocyte stem cell differentiation. Nature. 2005; 433: 884–7.

84. Bondurand N, Pingault V, Goerich DE, Lemort N, Sock E, Le Caigene C, Wegner M, Goossens M. Interaction among SOX10, PAX3 and MITF, three genes altered in Waardenburg syndrome. Hum Mol Genet. 2000; 9: 1907–17.

85. Watanabe A, Takeda K, Ploplis B, Tachibana M. Epistatic relationship between Waardenburg syndrome genes MITF and PAX3. Nat Genet. 1998; 18: 283–6.

86. Foletti A, Ackermann J, Schmidt A, Hummler E, Beermann F. Absence of fibroblast growth factor 2 does not prevent tumor formation originating from the RPE. Oncogene. 2002; 21: 1841–7.

87. Miller AJ, Mihr MC Jr. Melanoma. N Engl J Med. 2006; 355: 51–65.

88. Thompson JF, Scolyer RA, Keeford RF. Cutaneous melanoma. Lancet. 2005; 365: 687–701.

89. Polsky D, Cordon-Cardo C. Oncogenes in melanoma. Oncogene. 2003; 22: 3087–91.

90. de Snoo FA, Hayward NK. Cutaneous melanoma susceptibility and progression genes. Cancer Lett. 2005; 230: 153–66.

91. Rodolfo M, Daniotti M, Vallacchi V. Genetic progression of metastatic melanoma. Cancer Lett. 2004; 214: 133–47.

92. Topczewska JM, Postovit LM, Margaryan NV, Sam A, Hess AR, Wheaton WW, Nickoloff BJ, Topczewski J, Hendrix MJ. Embryonic and tumorigenic pathways converge via Nodal signaling; role in melanoma aggressiveness. Nat Med. 2006; 12: 925–32.

93. Vance KW, Godin CR. The transcription network regulating melanocyte development and melanoma. Pigment Cell Res. 2007; 17: 318–25.

94. Blake JA, Ziman MR. Pax3 transcripts in melanoblast development. Dev Growth Differ. 2005; 47: 627–35.

95. Schill OA, Kamarashev J, Murmann O, Geersten R, Dummer R, Schäfer BW. Pax3 is expressed in human melanomas and contributes to tumor cell survival. Cancer Res. 2001; 61: 823–6.

96. He SJ, Stevens G, Braithwaite AW, Eccles MR. Transfection of melanoma cells with antisense PAX3 oligonucleotides additively complements cisplatin-induced cytotoxicity. Mol Cancer Ther. 2005; 4: 996–1003.

97. Kamaraj AK, Bertolotto C, Chebath J, Revel M. Pax3 down-regulation and shut-off of melanogenesis in melanoma B16/F10.9 by interleukin-6 receptor signaling. J Biol Chem. 2002; 277: 15132–41.

98. Mayani CS, George D, Freilich L, Milian EJ, Mania-Farnell B, McLone DG, Bremer EG. Microarray analysis detects novel Pax3 downstream target genes. J Biol Chem. 2001; 276: 49299–309.

99. Wang Q, Kumar S, Milsios N, Slevin M, Kumar P. Investigation of downstream target genes of PAX3c, PAX3e and PAX3g isoforms in melanocytes by microarray analysis. Int J Cancer. 2007; 120: 1223–31.

100. Wiggan O, Taniguchi-Sidle A, Hamel PA. Interaction of the pRb-family proteins with factors containing paired-like homeodomains. Oncogene. 1998; 16: 227–36.

101. Goulding MD, Chalepakis G, Deutsch U, Ersselius JR, Gruss P. Pax-3, a novel murine DNA binding protein expressed during early neurogenesis. EMBO J. 1991; 10: 1135–47.
96. Tsukamoto K, Nakamura Y, Niikawa N. Isolation of two isoforms of the PAX3 gene transcripts and their tissue-specific alternative expression in human adult tissues. Hum Genet. 1994; 93: 270–4.

97. Parker CJ, Shawcross DG, Li H, Wang QY, Harrington CS, Kumar S, MacKie RM, Prime W, Rennie IG, Sisley K, Kumar P. Expression of PAX3 alternatively spliced transcripts and identification of two new isoforms in human tumors of neural crest origin. Int J Cancer. 2004; 108: 314–20.

98. Pritchard G, Grosveild G, Hollenbach AD. Alternative splicing of PAX3 produces a neurotrophin-like inactive protein. Gene. 2003; 305: 61–9.

99. Vogan KJ, Underhill DA, Gros P. An alternative splicing event in the Pax-3 paired domain identifies the linker region as a key determinant of paired domain DNA-binding activity. Mol Cell Biol. 1996; 16: 6677–86.

100. Wang Q, Kumar S, Stevin M, Kumar P. Functional analysis of alternative isoforms of the transcription factor PAX3 in melanocytes in vitro. Cancer Res. 2006; 66: 8574–80.

101. Singh S, Tang HK, Lee JY, Saunders GF. Truncation mutations in the transactivation region of PAX6 result in dominant-negative function. Proc Natl Acad Sci USA. 2005; 102: 503–8.

102. Dong HY, Liu W, Cohen P, Mahle CE, Poppe B, De Paepe P, Michaux L, Moreau E, Cavazzini F, Yigit N, Van Dijk E, Limbergen H, De Paepe A, Praet M, De Coene R, Cozma D, Yu D, Hodawadekar S, Metzgar MH, Paterson J, Erikson J, Marafioti T, Monroe JG, Atchison ML, Azvolinsky A, Grande S, Tobias JW, Thomas-Tikhonenko A. B cell activator PAX5 transcription is enhanced by STAT5 in the early stage of B cells. Eur J Immunol. 2003; 33: 1824–9.

103. Bromberg J. Stat proteins and oncogene-s. J Clin Invest. 2002; 109: 1139–42.

104. Cozma D, Yu D, Hodawadekar S, Azvolinsky A, Grande S, Tobias JW, Metzgar MH, Paterson J, Erikson J, Marafioti T, Monroe JG, Atchison ML, Thomas-Tikhonenko A. B cell activator PAX5 promotes lymphomagenesis through stimulation of B cell receptor signaling. J Clin Invest. 2007; 117: 2602–10.

105. Beckwith JB, Kiviat NB, Bonadio JF. WT1 is a modifier of the Pax2 mutant phenotype: cooperation and interaction between WT1 and Pax2. Oncogene. 2003; 22: 8145–55.

106. Winnay PJ, Risdon RA, Sams VR, Dressler GR, Woolf AS. The PAX2 transactivation factor is expressed in cystic and hyperproliferative dysplastic epithelia in human kidney malformations. J Clin Invest. 1996; 98: 451–9.

107. Ryan G, Steele-Perkins V, Morris JF, Rausher FJ, 3rd, Dressler GR. Repression of Pax-2 by WT1 during normal kidney development. Development. 1995; 121: 867–72.

108. Davies JA, Ladomery M, Hohenstein P, Michael L, Shafe A, Spraggon L, Hastie N. Development of an siRNA-based method for repressing specific genes in renal culture and its use to show that the WT1 tumour suppressor is required for nephron differentiation. Hum Mol Genet. 2004; 13: 235–46.

109. Gnarra JR, Dressler GR. Expression of Pax-2 in human renal cell carcinoma and growth inhibition by antisense oligonucleotides. Cancer Res. 1995; 55: 4092–8.

110. Daniel L, Lechevalier E, Giorgi R, Siches H, Zattara-Cannoni H, Figarella-Branger D, Coulange C. Pax-2 expression in adult renal tumors. Hum Pathol. 2001; 32: 283–7.

111. Torban E, Eccles MR, Favor J, Goodyer MA, Donald CD, Rennie IG. Expression of Pax-5 alternatively spliced transcripts and identification of two new WT1 transcripts and their tissue-specific alteration in human tumors of neural crest origin. J Natl Cancer Inst. 1972; 48: 1015–20.

112. Kozmik Z, Sure U, Ruedi D, Busslinger M, Aguzzi A. Deregulated expression of PAX5 in medulloblastoma. Proc Natl Acad Sci USA. 1995; 92: 5709–13.

113. Mullighan CG, Goorha S, Radtke I, Miller CB, Coustan-Smith E, Dalton JD, Girtman K, Mathew S, Ma J, Pounds SB, Su X, Pui CH, Relling MV, Evans WE, Shurtleff SA, Downing JR. Genome-wide analysis of genetic alterations in acute lymphoblastic leukemia. Nature. 2007; 446: 758–64.

114. Stuart ET, Hafner R, Oren M, Gruss P. Loss of p53 function through PAX-mediated transcriptional repression. EMBO J. 1995; 14: 5638–45.

115. Baumann Kubetzko FB, Di Paolo C, Maag C, Meier R, Schäfer BW, Betts DR, Stahel RA, Himmelmann A. The PAX5 oncogene is expressed in N-type neuroblastoma cells and increases tumorigenicity of a S-type cell line. Carcinogenesis. 2004; 25: 1383–46.

116. Torlakovic E, Slipicevic A, Robinson C, DeCoteau JF, Alston GC, Vyberg M, Chibbar R, Fiorenese VA. Pax-5 expression in nonhematopoietic tissues. Am J Clin Pathol. 2006; 126: 798–804.

117. Cobaleda C, Jochum W, Busslinger M. Conversion of mature B cells into T cells by dedifferentiation to uncommitted progenitors. Nature. 2007; 449: 473–7.

118. Hirokawa S, Sato H, Kato I, Kudo A, EBF-regulating Pax5 transcription is enhanced by STAT5 in the early stage of B cells. Eur J Immunol. 2003; 33: 1824–9.

119. Marafioti T, Monroe JG, Atchison ML, Thomas-Tikhonenko A. B cell activator PAX5 expression occurs in renal medullary epithelial cells in vivo and in cell culture, is osmoregulated, and promotes osmotic tolerance. Proc Natl Acad Sci USA. 2005; 102: 503–8.

120. Margue CM, Bernasconi M, Barr FG, Schafer BW. Transcriptional modulation of the anti-apoptotic protein BCL-XL by the paired box transcription factors PAX3 and PAX5/FKHR. Oncogene. 2000; 19: 2921–9.

121. Cai Q, Dmitrieva NI, Ferraris JD, Brooks HL, van Balkom BW, Burg M. Pax-2 expression occurs in renal medullary epithelial cells in vivo and in cell culture, is osmoregulated, and promotes osmotic tolerance. Oncol Rep. 2007; 104: 331–7.

122. Bowen NJ, Logani S, Dickerson EB, Kapa LB, Akhtar M, Benigno BB, McDonald JF. Emerging roles for PAX8 in ovarian cancer and endosalpingeal development. Gynecol Oncol. 2007; 104: 331–7.
129. Giordano TJ, Au AY, Kuick R, Thomas DG, Rhodes DR, Wilhelm KG Jr, Vinco M, Misek DE, Sanders D, Zhu Z, Ciampi R, Hanash S, Chinnaiyan A, Clifton-Bligh RJ, Robinson BG, Nikiforov YE, Koenig RJ. Delineation, functional validation, and bioinformatic evaluation of gene expression in thyroid follicular carcinomas with the PAX8-PPARG translocation. Clin Cancer Res 2006; 12: 1983–93.

130. Castro P, Rebocho AP, Soares RJ, Magalhães J, Roque L, Trovisco V, Vieira de Castro I, Cardoso-de-Oliveira M, Fonseca E, Soares P, Sobrinho-Simães M. PAX8-PPARgamma rearrangement is frequently detected in the follicular variant of papillary thyroid carcinoma. J Clin Endocrinol Metab. 2006; 91: 213–20.

131. Foukakis T, Au AY, Wallin G, Geli J, Forsberg L, Clifton-Bligh R, Robinson BG, Lui WO, Zedenius J, Larsson C. The Ras effector NORE1A is suppressed in follicular thyroid carcinomas with a PAX8-PPARgamma fusion. J Clin Endocrinol Metab. 2006; 91: 1143–9.

132. Espadinha C, Cavaco BM, Leite V. PAX8PPARgamma stimulates cell viability and modulates expression of thyroid-specific genes in a human thyroid cell line. Thyroid. 2007; 17: 497–509.

133. Herlitz LC, Tong GX, Hamele-Bena D, Greenebaum E. Nephrogenic adenoma identified on urine cytology using PAX-2 immunostaining. Diagn Cytopathol. 2008; 36: 47–9.

134. O’Connor SM, Bhargava R, Karabakhtsian R, Dabbs DJ, Chivukula M. Use of PAX-2 antibody to distinguish metastatic ovarian serous papillary carcinoma from primary breast carcinoma. Mod Pathol. 2008; 21: 216A.

135. Feldman AL, Dogan A. Diagnostic uses of Pax5 immunohistochemistry. Adv Anat Pathol. 2007; 14: 323–34.

136. Aagaard L, Rossi J. RNAi therapeutics: principles, prospects and challenges. Adv Drug Deliv Rev. 2007; 59: 75–86.

137. Iorns E, Lord CJ, Turner N, Ashworth A. Utilizing RNA interference to enhance cancer drug discovery. Nat Rev Drug Discov. 2007; 6: 556–68.

138. van den Broeke LT, Pendleton CD, Mackall C, Helman LJ, Berzofsky JA. Identification and epitope enhancement of a PAX-FKHR fusion protein breakpoint epitope in alveolar rhabdomyosarcoma cells created by a tumorigenic chromosomal translocation inducing CTL capable of lysing human tumors. Cancer Res. 2006; 66: 1818–23.