Minireview

Emerging Roles for PAX Transcription Factors in Cancer Biology

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Abstract. Pax genes are transcription factors which act as essential control genes during the establishment of embryonic cell lineages Recent evidence now demonstrates that their expression is deregulated by different mechanisms in several tumor types including Wilms' tumors, rhabdomyosarcomas, brain tumors and lymphomas The oncogenic role of PAX proteins in tumor development is now subject of intensive investigations and may include stimulation of the rate of proliferation as well as inhibition of the apoptotic program In addition, the expression of *PAX* genes in these cancers might serve as a diagnostic and probably also prognostic tool

Key words: PAX genes — Rhabdomyosarcoma — Wilms' tumor — Oncogenes — Apoptosis

Abbreviations: EWS – Ewing's sarcoma, FKHR – forkhead related gene, HGF hepatocyte growth factor, PAX paired box, PNET – peripheral neuroectodermal tumor, RMS – rhabdomyosarcoma, RCC – renal cell carcinoma, SF – scatter factor

Introduction

During embryonic development the establishment of different cellular lineages requires the generation of a sufficient number of precursor cells which are then able to undergo terminal differentiation Usually, terminal differentiation is associated with a post-mitotic stage which means that cells are incapable of dividing The highly organized sequence of these events can only take place through the precise control of gene expression at any given stage In contrast to these normal circumstances, cancer cells have lost the ability to become post-mitotic and to differentiate In

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general, their appearance is similar to early embryonic cells and it is accompanied by the reexpression of structural as well as regulatory embryonic genes. Consequently, developmental control genes are often reexpressed in cancer cells. Since the development of fully malignant cells requires accumulation of multiple genetic changes, the question arises whether reexpression of developmental control genes plays a direct causal role in tumorigenicity or whether it is just a consequence of other genetic changes, e.g. mutations in critical growth control genes.

Here, I would like to first summarize the genetic evidence which demonstrates that *PAX* genes are essential control genes for the establishment of certain embryonic lineages. Second, newly emerging evidence will be presented which suggests that reexpression of *PAX* genes in cancer cells might play a distinct causal role in multistage carcinogenesis.

What are *Pax* genes? Only twelve years ago, the paired box was first identified as a homology region in the Drosophila segmentation genes paired and gooseberry (Bopp et al. 1986). Subsequently, *Pax* genes have been identified in species ranging from hydra to human (Sun et al. 1997). To date, nine murine as well as human Pax genes have been described (see Table 1). They encode nuclear transcription factors which have a modular structure of different functional domains. So far, three highly conserved regions have been identified in these proteins: the paired domain as primary DNA binding domain; a conserved octapeptide motif with unknown function; and a paired-type homeodomain which can serve as a second independent DNA binding region. The paired domain, 128 amino acids in length, is composed of three α-helices, two of which are located near the carboxy end and one near

Table 1. The PAX gene family

CLASS	GENE	STRUCTURE[a]	CHROMOSOMAL LOCATION[b]
I	PAX1	PD, OCT	20p11.2
	PAX9		14q12-q13
II	PAX2	PD, OCT, truncated HD	10q24.3-q25.1
	PAX5		9p13
	PAX8		2q12-q14
III	PAX3	PD, OCT, HD	2q35
	PAX7		1p36
IV	PAX4	PD, no OCT, HD	7q22-qter
	PAX6		11p13

[a]PD: paired domain; OCT: octapeptide; HD: homeodomain
[b]chromosomal location is given for human genes

the amino end of the paired domain According to these structural criteria, 4 different subgroups within this protein family can be distinguished class I proteins are composed of the paired domain and the octapeptide motif (PAX1 and PAX9), class II proteins are characterized by a truncated homeodomain (PAX2, PAX5, and PAX8), class III proteins contain all three motifs (PAX3 and PAX7), and class IV proteins are devoid of an octapeptide motif (PAX4 and PAX6) All examined PAX proteins are capable of stimulating transcription from corresponding reporter gene constructs In these experiments, transcriptional activating as well as repressing domains have been defined in several PAX proteins However, little is known so far about in vivo target genes for PAX proteins although *in vitro* selection experiments have defined recognition sequences for several PAX proteins, which appear to be unusually large (20–24bp) The paired domain recognizes a sequence characterized by a pentanucleotide GTYMC, and PAX proteins comprising a homeodomain recognize an additional sequence containing ATTA Few functional target genes have been suggested so far for PAX3, 5 and 8 These are discussed below in respect to the oncogenic role suggested for PAX proteins

Genetic Evidence for Control of Development by Pax Genes

Pax genes are expressed during development in a distinct spatial and temporal pattern Expression starts between day 8 and 10 5 p c in the mouse and can be found mainly in the central nervous system and/or the paraxial mesoderm and its

Table 2. Association of PAX genes with mouse mutants and human syndromes

GENE	SITES of EXPRESSION[a]	MOUSE MUTANTS	HUMAN SYNDROMES
PAX1	sclerotome, thymus	undulated	
PAX2	kidney, urogenital, CNS	ko – lacking kidneys	ocular-renal syndrome
PAX3	dermomyotome, neural crest, CNS	splotch	Waardenburg syndrome
PAX4	pancreas	ko – lacking pancreatic β cells	
PAX5	pro B-cells, CNS	ko – lacking B cells, brain defects	
PAX6	eye, pancreas, CNS	small eye, ko – lacking pancreatic α cells	aniridia
PAX7	dermomyotome, neural crest, CNS	ko – neural crest, defects	

[a]) only most prominent sites of expression are indicated CNS central nervous system, ko knockout No mutants are known so far for PAX8 and PAX9

derivatives. In general, Pax expression is seen in mitotically active precursor cells, although sometimes a biphasic pattern with reappearance in differentiated cells is observed. The main sites of expression of each *Pax* gene are listed in Table 2, and a more detailed summary of the expression pattern is given in Stuart et al. (1994). The first indications that Pax proteins might be important developmental control genes came from the identification of mouse mutants carrying mutations in specific *Pax* genes. The first mouse mutant identified was undulated, a mutant affecting formation of the skeleton, which carries a mutation in the *Pax1* gene (Balling et al. 1988). Mutations mainly in the paired domain of *Pax3* were found subsequently in several strains of the mouse mutant splotch which are all characterized by neural tube, neural crest, skeletal and heart muscle defects (Epstein et al. 1991). Interestingly, later it was demonstrated that the skeletal muscle defects originate in the myoblasts migrating from the somites into the extremities during myogenesis. In homozygous splotch mice these myoblasts are absent (Bober et al. 1994). Another example is given by small eye, a mouse mutant displaying underdeveloped eyes due to a mutation in *Pax6* (Hill et al. 1991). Searching for human syndromes with underlying *PAX* mutations, defects in one allele of *PAX2* have been identified in an ocular-renal syndrome, in *PAX3* for Waardenburg syndrome I and in *PAX6* for aniridia. These interesting observations prompted different researchers to generate functional null mutants through homologous recombination of several other *Pax* genes (summarized in Table 2). The major defects observed were lacking kidneys in Pax2 homozygotes, lacking pancreatic β-cells in mice missing functional Pax4, lacking mature B cells without Pax5, lacking pancreatic α-cells in Pax6 and finally neural crest defects in Pax7 knock-out mice. These experiments provide unequivocal evidence for the importance of *Pax* gene family members in the establishment of specific cell lineages during development. An intriguing concept from these experiments is that loss of function mutations in *Pax* genes almost always results in the absence of a specific cellular lineage. This is in contrast to observations made for other homeobox containing genes, like *Hox* genes, which reveal homeotic transformations of entire structures like e.g. legs into antennae. Hypothetically, Pax proteins are therefore involved either in the recruitment of precursor cells to a specific cell type, in the migration of precursor cells to the appropriate environment, or in the survival of precursor cells in their given environment. At the moment, a combination of these possibilities appears to be most likely.

The Emerging Role of Pax Genes in Oncogenesis

What is now the evidence that *PAX* genes could play a role in oncogenesis? The notion that Pax genes might act as oncogenes was first put up by the laboratory of Peter Gruss in 1993, when he and his collaborators found that Pax proteins can transform mouse fibroblasts in culture (Maulbecker and Gruss 1993). In these

experiments, ectopic expression of either Pax1, Pax2, Pax3, Pax6 or Pax8 could induce the formation of foci as well as growth of the embryonic fibroblasts in a nude mouse model. Subsequently it was found that several *Pax* genes are inappropriately expressed in human tumor cells, e.g. in Wilms' tumor (*PAX2* and *PAX8*) (Dressler and Douglass 1992; Poleev et al. 1992), rhabdomyosarcoma (*PAX3* and/or *PAX7*) (Galili et al. 1993; Schafer et al. 1994), Ewing's sarcoma (*PAX3* and/or *PAX7*) (Schulte et al. 1997), glioblastoma (*PAX5*) (Stuart et al 1995b) and thyroid cancer (*PAX8*) (Fabbro et al. 1994) (see Table 3).

Table 3. PAX genes associated with cancer

GENE	CANCER	TYPE OF GENETIC CHANGE	TARGET GENES
PAX2	Wilms' tumor, renal cell carcinoma	overexpression	p53, WT1
PAX3	rhabdomyosarcoma Ewing's sarcoma	translocation t(2,13), overexpression	c-met, myoD (?), myf-5 (?)
PAX5	lymphoma, glioblastoma	translocation t(9,14), overexpression	p53, CD19, IgHC
PAX7	rhabdomyosarcoma Ewing's sarcoma	translocation t(1,13), overexpression	similar to PAX3 ?
PAX8	Wilms' tumor, tyroid cancer	overexpression	N-CAM, p53, bcl-2, WT1, thyroperoxidase, thyroglobulin

What might be the cause of this deregulated expression of *PAX* genes? In some instances, deregulation might reflect persistent expression in the precursor cell type from which the tumor developmentally originates, caused by the failure to downregulate the PAX genes at appropriate times of development. However, "true" overexpression might also be produced by a) loss of critical cis-control regions, b) alterations of trans-acting upstream regulators, or c) in situ amplification of the gene locus.

Even more compelling was the finding that *PAX* genes participate in specific chromosomal translocations. The specific presence of such chromosomal abnormalities, being at least diagnostically important for some tumor types, suggests that the genes involved in the translocation event are causally involved in the development of cancer. This concept has proven true for a number of very well characterized genes such as c-myc (t(8;14)), bcr-abl (t(9;22)) or bcl-2 (t(14;18)) and is now generally accepted. Remarkably, three different *PAX* gene family members are affected

by chromosomal translocations as well: *PAX3* and *PAX7* in the pediatric skeletal muscle derived tumor alveolar rhabdomyosarcoma, and *PAX5* in a subtype of B-cell lymphoma (discussed in more detail below).

At this point the available experimental evidence suggests, either directly or indirectly, a role of PAX proteins in tumorigenicity. Hence, one would like to know at what stage and through which mechanisms PAX proteins will contribute to multistep tumor development. Since they generally act as transcription factors, the activation of specific target genes is certainly a crucial mechanism. However, at this point it cannot be excluded that other mechanisms like protein-protein interactions play an important role as well. Nevertheless, since not much is currently known about protein interactions involving PAX proteins, I will discuss in the next sections the possible target genes of some PAX proteins and how they might give us a clue to the role of PAX proteins in cancer cells. Interestingly, different mechanisms might apply for class II and class III PAX proteins. These insights might also help to shed light on the mechanisms resulting in some of the phenotypes observed in genetic experiments.

Class II PAX Genes in Cancer: Involvement of p53

PAX2 and PAX8 in Wilms' tumor and thyroid cancer

The first *PAX* gene described to be expressed in a tumor of embryonic origin was *PAX2* in the epithelial components of Wilms' tumor (Dressler and Douglass 1992) which is a common pediatric malignancy. In the adult, renal cell carcinomas (RCC) are presumed to have the proximal tubule epithelium as their origin. Hence, it was not surprising that a high percentage of RCC cell lines and primary tumors were found to express *PAX2* as well (Gnarra and Dressler 1995). Another *PAX* gene expressed in the developing kidney is PAX8. Again, high levels of *PAX8* expression were found in Wilms' tumors (Poleev et al. 1992). Unfortunately, expression of *PAX8* in RCC has not been investigated. Interestingly, higher expression of the *PAX8* gene is also found in thyroid neoplasms where its increasing levels were correlated with lower risk papillary carcinomas. This indicates that PAX genes could serve as diagnostic markers in some instances. However, the diagnostic value of class II *PAX* genes in cancer patient management, especially in Wilms' tumors and renal cell carcinomas, still has to be evaluated.

PAX5 in brain tumors and lymphomas

The *PAX5* gene has been implicated in some brain tumors and in a type of B-cell lymphoma. Two studies have linked the expression of PAX5 to brain tumors. Kozmik et al. (1995) found deregulated expression in a large proportion of medulloblastoma, a pediatric brain tumor. Although other *PAX* genes were occasionally expressed in some primary tumor samples of the neuroectodermal lineage, *PAX5*

was found most consistently and, more importantly, its expression was absent from normal neonatal and adult human cerebellum. In a second study Stuart et al. (1995b) described inappropriate expression of *PAX5* in astrocytomas originating in the forebrain. In their study, they found that an increased expression of *PAX5* is associated with higher grade malignancy and higher expression levels of the epidermal growth factor receptor. Although this study suggests that PAX5 may prove to be useful as a diagnostic indicator of malignancy in astrocytomas, no subsequent report has been published so far corroborating these findings.

In about 2% of non-Hodgkin lymphomas, a specific translocation t(9;14)(p13; q32) fuses the *PAX5* upstream region to the immunoglobulin heavy chain locus, bringing the potent Eμ enhancer close to the *PAX5* gene (Busslinger et al. 1996). Consequently, this translocation event results in deregulation of *PAX5* gene expression which increases the level of PAX5 protein and forces it to be present at a time during B-cell differentiation when the endogenous gene usually is switched off. This observation is very interesting since it represents not simply a case of possible reexpression of an embryonic gene and therefore points towards a causal role of PAX5 in the development of this and probably other tumors.

Possible molecular mechanisms

In this section, I would like to discuss the molecular mechanisms by which class II Pax proteins might contribute to tumor development. An intriguing clue to this question was provided by experiments from the laboratory of Peter Gruss. These authors demonstrated that all three class II Pax proteins, in contrast to other Pax family members, can repress transcription of the tumor suppressor protein p53 (Stuart et al. 1995a). p53 seems to be a direct target of these PAX proteins since they are able to bind to a site within the untranslated first exon of p53. These data suggest that loss of p53 function brought about by PAX mediated transcriptional repression contributes to tumor initiation or progression. The two main critical actions of wild type p53, when expressed in tumor cells containing mutated p53, are growth arrest at the G1 phase of the cell cycle and induction of apoptosis (Attardi et al. 1996). The growth arrest function of p53 seems to be mediated, at least partially, by the cell cycle regulator p21. Interestingly, downregulation of PAX2 (by means of antisense oligonucleotides) in renal cell carcinoma and of PAX8 in thyroid cancer cells results in growth inhibition (Gnarra and Dressler 1995; Rossi et al. 1995). These results are therefore in agreement with the proposed role of p53 in this system. However, the involvement of the p21 protein has not been investigated. The second function of p53 in this respect, apoptosis, is mediated at least partially by the bcl-2 family protein bax, which is a pro-apoptotic bcl-2 family member; it is transcriptionally upregulated by p53. One would therefore expect that failure to induce bax (as a result of PAX mediated repression of p53) leads to continued expression of bcl-2 homodimers and hence failure to induce apoptosis.

In this scenario, PAX proteins would indirectly modulate apoptosis through p53. Interestingly and extending this observation, it was just recently suggested that PAX8 (the only PAX protein tested in this assay) can stimulate transcription of bcl-2 *in vitro* (Hewitt et al. 1997). Hence one might speculate that also a more direct control of bcl-2 proteins by PAX family members is contributing to p53 modulated apoptosis. In summary, it seems likely that an entire set of target genes rather than only repression of p53 may combine to contribute to the oncogenic activity of class II PAX proteins. Supporting this conclusion experimental evidence has identified in the past an additional tumor suppressor gene whose expression is modulated by PAX2, namely the Wilms' tumor suppressor gene WT1 (McConnell et al. 1997).

Class III PAX Genes in Cancer: The Role of Growth Factor Receptors

PAX3 and PAX7 in rhabdomyosarcoma and other soft tissue sarcomas

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in children, accounting for about 5% of all cases of childhood cancer. A comparison of the expression of some skeletal muscle genes in RMS to normal skeletal myoblasts suggests that RMS represents the progeny of cells arrested during a restricted period of skeletal muscle development. RMS can be grouped into two main histological subtypes, the more common embryonal (70–80%) and the more aggressive alveolar subtype. The latter subtype is characterized by a consistent translocation between chromosomes 2 and 13 (t(2;13)(q35;q14)) resulting in the generation of a fusion protein between *PAX3* and a forkhead domain transcription factor called *FKHR* (Galili et al. 1993; Shapiro et al. 1993). Incidentally, a second translocation event t(1;13) fuses a similar part of *PAX7* to the same *FKHR* gene portion (Davis et al. 1994). In both instances, the transactivation domains of the *PAX* genes are replaced by the transactivation domain of *FKHR* generating a transcriptionally more potent fusion protein (Bennicelli et al. 1995). Interestingly, the mechanims leading to overexpression of the fusion proteins seem to be distinct in both cases. For the *PAX3-FKHR* translocation a copy-number independent process appears to operate whereas the overexpression of the PAX7-FKHR protein results from fusion gene amplification (Davis and Barr 1997). These different overexpression mechanisms most likely ensure a critical level of the fusion protein necessary for oncogenic function. Both translocation events are useful diagnostic tools distinguishing alveolar RMS from other small round cell tumors (Kelly et al. 1997).

In addition to these translocation events, both PAX3 and PAX7 were found to be deregulated in embryonal RMS cell lines (Schäfer et al. 1994). However, this finding must still be confirmed in clinical tumor samples and possible expression patterns must be compared to clinical data. Interestingly, PAX3 and probably PAX7 expression can also be demonstrated in Ewing's sarcoma (EWS), an-

other childhood sarcoma (Schulte et al. 1997). Additionally, in these experiments, PAX3 expression was observed in patients with peripheral neuroectodermal tumors (PNET) and one glioblastoma cell line. These data indicate that deregulation of PAX3 might be a more widespread feature in tumor biology, and certainly warrant a broadened search for other tumors expressing PAX3, especially in glioblastomas.

Possible molecular mechanisms

In the first place the distinct appearance of the PAX3/7-FKHR fusion proteins in alveolar rhabdomyosarcoma strongly suggests that the fusion proteins have oncogenic function. Experimental evidence supporting this hypothesis then comes from studies in chicken embryo fibroblasts, where ectopic expression of PAX3-FKHR via a retroviral vector leads to transformation as judged by anchorage independent growth (Scheidler et al. 1996). However, similar experiments in mouse fibroblasts and myoblasts failed to induce growth in soft agar despite clear enhancement of the cellular proliferation rate (own results), suggesting that the oncogenic potential of the fusion protein is influenced by the cell background. Nevertheless, it is highly likely that the fusion proteins contribute to oncogenesis. Hence, what are the mechanisms mediating this activity? To gain some insight into this, we developed a strategy using antisense oligonucleotides to downregulate either the PAX3-FKHR fusion protein, PAX3, or PAX7 in RMS cells. Following antisense treatment, leading to a downregulation of the targeted PAX proteins, all RMS cell lines tested underwent substantial induction of apoptosis (Bernasconi et al. 1996). Since the PAX3-FKHR fusion protein functions as transcriptional activator, with binding specificity similar to PAX3 but higher transactivational potential (Fredericks et al. 1995), one might speculate that the oligonucleotide induced repression of specific PAX target genes plays a role in the anti-apoptotic function of PAX proteins in tumor cells. Consequently, the question arises what these target genes might be.

Table 3 lists the possible target genes identified so far for PAX3. Two of these potential target genes are the myogenic regulatory proteins myoD and myf5 (Maroto et al. 1997; Tajbakhsh et al. 1997). These genes generally act as inducers of muscle differentiation and are therefore not suitable as candidate genes with oncogenic potential. Indeed, it is not at all clear from the literature, if these genes are direct targets of PAX3, since expression of PAX3 in neural tube and neural crest cells does not induce myoD or myf5 activation.

The single direct target gene for PAX3 which has been reported so far is c-met, a receptor tyrosine kinase having the hepatocyte growth factor/scatter factor (HGF/SF) as its known activating ligand. Interestingly, c-met was originally identified as an oncogene by transfection studies (Cooper et al. 1984) and as the deregulated product of a translocation t(1;7) found frequently in human gastric carcinomas (Soman et al. 1991). In addition, both germline and somatic mutations in the tyrosine kinase domain have been identified in papillary renal carcinomas

(Schmidt et al 1997) Hence, at least three different pathways seem to enhance the activity of c-met a translocation event, mutations, and overexpression of PAX3 or PAX3-FKHR leading to overexpression of c-met It seems therefore likely that c-met expression participates in the formation of muscle tumors This conclusion is supported by recent data suggesting that both increased expression of PAX3 (Epstein et al 1995) or of c-met (Anastasi et al 1997) are capable of inhibiting differentiation in cultured myoblasts Intriguingly, expression of the cytoplasmic portion of met in hepatocytes is able to block apoptosis (Amicone et al 1997), suggesting that c-met might be one of the mediators of the observed anti-apoptotic effects of class III PAX genes In a different approach to search for additional media tors of this anti-apoptotic action, we have recently identified the insulin-like growth factor 1 receptor as a possible target of PAX3 (unpublished observation) Similarly to c-met, this receptor mediates tyrosine kinase signalling and is able to prevent cells from entering apoptosis (O'Connor et al 1997) In conclusion, the available data suggest that activation of tyrosine kinase receptors might be a common theme mediating the oncogenic action of class III PAX genes

Conclusions and Future Directions

In this review, I presented evidence supporting a role for members of the paired-box containing transcriptional regulators in oncogenesis Figure 1 summarizes these experiments in a hypothetical model PAX proteins of classes II and III influence tumor development, at least, through enhancing the rate of proliferation as well as reducing the amount of apoptosis The pathways by which these actions are mediated might involve the tumor suppressor protein p53, the anti-apoptotic protein bcl-2, and possibly several tyrosine kinase growth factor receptors At the moment, it is not clear if both classes of PAX proteins can activate all of the above suggested target proteins, except for p53 which seems to be repressed only by class II proteins In our model, only known target proteins are indicated, although it

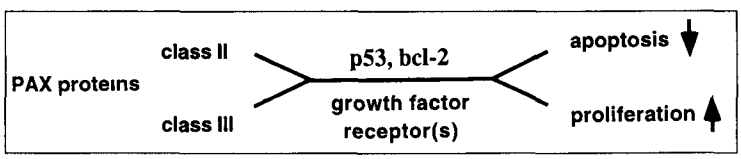

Figure 1. Hypothetical model for the role of Pax proteins in oncogenesis Pax transcription factors appear to increase the rate of proliferation and decrease the amount of apoptosis in tumor cells These actions might be brought about through regulation of different target genes like p53, bcl-2, and receptor tyrosine kinases Activation of these target genes might or not be specific for one Pax gene, an issue which still has to be investigated in most cases

seems very likely that additional genes will be identified in the near future which are regulated by PAX proteins. One possible strategy towards this goal might be the use of gridded cDNA arrays combined with specific induction of PAX proteins in different cell types. A second limitation of the model is the restriction to target proteins. It is likely that other mechanisms like protein-protein interactions play a role in mediating the observed actions of PAX proteins. A fascinating example in this respect is the recent demonstration that the retinoblastoma tumor suppressor protein pRB can interact with paired-like homeodomain proteins like Pax-3 (Wiggan et al. 1998). The work by Wiggan et al. has demonstrated that binding of pRB to PAX3 causes repression of activated transcription from the c-met promoter. This suggests that functional pRB can repress part of the oncogenic action of PAX3. Again, it will be interesting to see if such an interaction is restricted to PAX3 only, and more protein-protein interactions will certainly be identified in the near future.

It is interesting to note that oncogenic transcription factors other than PAX are also involved in the control of cell death. The best studied is c-myc which promotes cell proliferation in the presence of growth factors and cell death in the absence of such factors. Induction of apoptosis in this case is mediated by a receptor-ligand interaction; c-myc can stimulate transcription of fas (CD95)(Hueber et al. 1997) which triggers apoptosis by binding fas ligand. Hence, c-myc regulates and lies upstream of these receptor. Likewise, at least some PAX proteins stimulate transcription, and therefore lie upstream, of particular growth factor receptors which can contribute to cell survival by a receptor-ligand interaction. It will be necessary to determine if these regulatory circuits are unique to cancer cells or, as probably could be expected, are important during development as well.

Although it is certainly very simplified, one can ask what the implications of the presented model for the treatment and diagnosis of cancer patients would be. In the tumor types where PAX genes have been identified, it might be possible to functionally inactivate these transcriptional regulators to improve treatment, possibly in combination with existing chemotherapy protocols. This could be accomplished through a number of approaches like treatment with sequence specific antisense oligonucleotides, expression of hammerhead ribozymes, or dominant negative mutants. Considering the example of rhabdomyosarcoma, it might even be more rewarding to inhibit the activity of PAX3 than to inactivate e.g. several growth factor receptor pathways one at a time, since PAX3 seems to effect several of these pathways simultaneously (c-met and IGF-1R). The current technical advances in drug development and gene therapy seem to make these possibilities a realistic option for the future. However, it will be very important to clearly define in which tumor types of both adult and pediatric origin PAX proteins are deregulated. A prerequisite to establish a deregulation pattern is of course a general account of PAX gene expression in human. Such a broadened search might be greatly facilitated by the development of highly specific antibodies. Finally, these experiments

would also allow to establish a possible correlation between the expression of PAX proteins and clinical prognosis. Perhaps then, PAX proteins might turn out to not only be essential for developmental processes but also for cancer diagnostics and treatment.

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