The Pax-5 Gene: A Pluripotent Regulator of B-cell Differentiation and Cancer Disease

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Abstract

The Pax-5 oncogene encodes a potent transcription factor that plays a key role in B-cell development and cancerous processes. In normal B-lymphopoiesis, Pax-5 accomplishes a dual function by activating B-cell commitment genes while concomitantly repressing non-B-lineage genes. Given the pivotal importance of Pax-5-mediated processes in B-cell development, an aberrant regulation of Pax5 expression has consistently been associated with B-cell cancers, namely, lymphoma and lymphoblastic leukemias. More recently, Pax-5 gene expression has been proposed to influence carcinogenic events in tissues of nonlymphoid origin by promoting cell growth and survival. However, in other cases, Pax-5 products have opposing effects on proliferative activity, thus redefining its generally accepted role as an oncogene in cancer. In this review, we attempt to summarize recent findings about the function and regulation of Pax-5 gene products in B-cell development and related cancers. In addition, we present new findings that highlight the pleiotropic effects of Pax-5 activity in a number of other cancer types. Cancer Res; 71(24); 7345–50. © 2011 AACR.

Introduction

Pax-5, or B-cell–specific activator protein, was initially characterized in the context of B-cell commitment, where it was shown to be essential for B-cell maturation beyond the early pro-B stage (1). Recent developments have led to the identification of Pax-5 expression in several non–B-cell cancers and to a better understanding of the upstream activators and downstream effectors that revolve around this transcription factor. We begin this review with an emphasis on malignant diseases other than B-cell–derived malignancies in which Pax-5 has been shown to be deregulated. Subsequently, we discuss the key factors that activate, interact with, or are transcriptionally regulated by Pax-5, and we highlight how these interactions are important for tumor formation and metastasis.

Pax-5 in Development and Cancer

Role of Pax-5 in development

The paired box (Pax) genes encode for a family of developmentally regulated transcription factors that serve central functions in cell differentiation and tissue development (2). Pax-5 is required for B-cell commitment, as evidenced by the maintenance of pluripotency observed in Pax-5–deficient pro-B cells (1). Additionally, Pax-5 ensures the maintenance of B-cell identity during subsequent differentiation. At the onset of B lymphopoiesis, Pax-5 repressors are overcome by early B-cell factor-1 (EBF-1)–mediated chromatin remodeling, which ultimately activates Pax-5 expression and initiates B-cell commitment (3). Pax-5 is initially expressed at a pro-B stage and is maintained until plasma cell differentiation occurs, during which time it activates the transcription of genes belonging to the pre–B-cell receptor signaling network as well as a series of secondary transcription factors that further strengthen the commitment to the B-cell lineage (4, 5). Concomitantly, Pax-5 also represses B-cell lineage–inappropriate genes (4). Pax-5 is thus considered as a central regulator of the B-cell gene expression program.

In addition to its role in B cells, Pax-5 has been linked to the development of other tissue types. During mouse embryogenesis, Pax-5 expression is observed in the mesencephalon and the spinal cord (2). Accordingly, Pax-5 null mice have altered morphogenesis of the midbrain. In this context, Pfeffer and colleagues (6) showed that Pax-5 expression is activated by structurally related Pax-2 and other homeodomain proteins through direct binding of an enhancer sequence within the Pax-5 gene. They also showed that Pax-5 was expressed in a specific temporal and spatial pattern during the formation of the midbrain–hindbrain boundary of transgenic mouse embryos, which is an essential step preceding the development of the midbrain and cerebellum in the vertebrate embryo.

Pax-5 transcription is regulated through the activation of 2 known alternative promoters. This generates transcripts with distinct first exons, 1A and 1B, with otherwise identical in-frame sequences. Moreover, alternative splicing events throughout the transcript generate multiple protein isoforms (7). However, due to the inherent challenges of isoform variant analysis, not much is known about the contribution of the Pax-5 variant proteins in normal and cancerous contexts. As a
result, their respective functional significance is poorly understood. However, recent studies have begun to shed light on the expression of Pax-5 protein variants as well as their putative function in both normal and malignant B cells, as discussed further below.

**Pax-5 in B-cell malignancies**

Given its pivotal regulatory function in B-cell development, Pax-5 can be considered an attractive candidate for involvement in B-cell oncogenesis, and accordingly, its aberrant expression is correlated with aggressive subsets of B-cell non-Hodgkin lymphoma (8). Furthermore, in lymphoplasmacytoid lymphoma, the Pax-5 gene is often implicated in a recurrent chromosomal rearrangement that places most of the protein coding sequences under transcriptional control of the potent promoters of the immunoglobulin heavy chain (IgH) gene leading to elevated Pax-5 transcription (9). To gain knowledge about Pax-5 chromosomal translocations in human tumors, Souabni and colleagues (10) reconstructed a human t(9;14) translocation in a germine knockout mouse by inserting a Pax-5 minigene into the IgH locus (IgHP5ki). Consequently, forced Pax-5 expression in the hematopoietic lineage interfered with normal T-cell development, which led to the formation of immature T-lymphoblastic lymphomas in IgHP5ki mice, suggesting that the T-lymphoid lineage is also particularly sensitive to the oncogenic program of Pax-5. Although the oncogenic contributions of Pax-5 in B-cell canerous lesions have yet to be completely elucidated, it seems that downstream perturbation of the B-cell receptor signaling network of genes can, at least in part, account for its role in B lymphomagenesis (11).

Pax-5 deregulation is also associated with certain types of acute leukemia, particularly B-cell precursor acute lymphoblastic leukemia (B-ALL). Pax-5 has been shown to be closely correlated with this form of leukemia and may become a reliable marker for the diagnosis of this disease. Fluorescence in situ hybridization studies are revealing an increasing number of chromosomal translocations involving Pax-5 in B-ALL that result in the expression of novel chimeric proteins. Some of Pax-5’s fusion partners encode themselves for transcription factors, such as ETV6 (12), or encode for functionally diverse proteins, such as JAK2 tyrosine kinase (13). Thus far, in all reported Pax-5 fusions, the chimeric proteins retain the N-terminal DNA-binding domain of Pax-5; consequently, these proteins are likely to retain the putative DNA-binding and target specificity, albeit with altered regulatory properties. This is exemplified by an ectopic expression study of the Pax-5-ETV6 fusion protein that showed that normally activated Pax-5 target genes become dominantly repressed by the fusion protein in vitro, leading to apoptotic resistance in affected cells (14).

Although studies have shown that in some instances Pax-5 genetic lesions result in expression of proteins with dominant oncogenic functions, as discussed above, other reports have provided evidence in support of haploinsufficiency-inducing Pax-5 mutations in B-ALL. In particular, Mulligan and colleagues (15) identified a series of Pax-5 copy number changes in a large-scale screening of genetic alterations in B-ALL patient samples. Specifically, 57 of the 242 samples analyzed were found to have genetic alterations resulting in loss of function of Pax-5. In contrast to a proposed tumor-promoting model commonly described for Pax-5, the tumor-suppressor/haploinsufficiency model is corroborated by the works of Cobaleda and colleagues (16) and Heltemes-Harris and colleagues (17), which support Pax-5 tumor-suppressor properties in B-cell malignancies. These dichotomous events mediated by Pax-5 in cancer cells (pro-oncogenic versus tumor suppressor) appear to be context dependent. Further investigation is thus needed to clarify what specific intracellular environment will dictate Pax-5 function in cancer processes. As will be discussed throughout this review, dichotomous functions of Pax-5 in cancer are commonly observed and are not limited to B-cell malignancies.

**Pax-5 expression in nonhematopoietic cancers**

Pax-5 expression has also been observed in a growing number of cancers of nonhematopoietic origin. Recently, Pax-5 expression has been linked to nonlymphoid cancers such as astrocytomas, medulloblastomas, neuroblastomas, oral carcinomas, colorectal carcinomas, cervical carcinomas, bladder carcinomas, and small-cell lung carcinomas. In most cases, Pax-5 expression correlates with increasing disease malignancy. Furthermore, recent findings have shown that Pax-5 expression is present in breast cancer (18, 19) and is thought to be involved in metastatic progression (20). Taken together, these results add to the growing body of evidence that implicates Pax-5 activity in diverse nonhematopoietic solid tumor malignancies.

**Pax-5 Functions in Oncogenic Processes**

**Pax-5 as a driver of tumor progression**

Findings suggest that Pax-5 function is associated with hallmark cancer cell processes such as proliferation, apoptosis, and phenotype transitioning. In mouse B-cell lymphoma cell lines, Pax-5 stimulates cell growth, an effect that is reversed upon pharmacologic inhibition of B-cell receptor components activated by Pax-5 (11). Similarly, Pax-5 protein expression positively correlates with proliferation of neuroblastoma cells (21) and likely is involved in human growth hormone–induced proliferation of breast carcinoma cells, because nuclear translocation and activity of Pax-5 are increased upon autocrine stimulation (19). Furthermore, Pax-5 has been linked to cell survival, mainly through its involvement in apoptosis pathways. Recently, it was shown that individual Pax-5 isoforms may have specific effects in apoptosis (22). In a B-ALL cell model using a novel ribozyme-derived isoform–specific knockdown system, Robichaud and colleagues (22) showed that a reduction of the Pax-5B isoform reduces cell viability by inducing apoptotic cell death. They also found that the loss of Pax-5B isoform expression led to an increase in Pax-5A expression, and they suggested an autoregulatory effect between the 2 distinct promoter–driven isoforms. The observation of interregulating capabilities between Pax-5 variants has also been made in murine models (23). A link between Pax-5 expression and apoptosis was also revealed in a study of multiple myeloma cell lines, in which the authors found that
Pax-5 overexpression promoted apoptosis events (24). Other studies suggest a growth-inhibitory function for Pax-5. Of note, Pax-5 slightly decreases proliferation rates in hepatocellular carcinoma cell lines (25) as well as in selected breast carcinoma cell lines (18). In general, Pax-5 seems to confer a progrowth effect in lymphoid cells and an inhibitory effect in nonlymphoid tissues.

**Dichotomous roles of Pax-5 in metastasis**

In addition to its involvement in proliferation and cell fate events, Pax-5 has been implicated in tumor metastasis. Findings in a neuroblastoma cell model revealed that Pax-5 overexpression led to an increase in colony formation in soft agar (21). Furthermore, Pax-5 directly activates transcription of c-Met (26), a receptor tyrosine kinase involved in cell motility and angiogenesis in small-cell lung cancer. These results implicate Pax-5 as a promoter of aggressiveness in certain cancer types.

In contrast, Vidal and colleagues (18) showed that Pax-5 reduces colony formation and promotes epithelial behavior in breast carcinoma cells. They also reported that recombinant Pax-5 overexpression in invasive MDA-MB231 cells reduced migration and motility. The same study showed that siRNA-mediated depletion of endogenous Pax-5 in MCF-7 cells enhanced cell migration. These findings suggest that Pax-5 contributes to the mesenchymal–epithelial transition, a process that is required for successful colonization of tumors to secondary metastatic locations. Indeed, cancer cells are thought to transiently undergo phenotype transitioning to accommodate the different stages of the metastatic process. Accordingly, mesenchymal properties are advantageous to cancer cells for extracellular matrix degradation and invasion toward blood vessels, whereas an epithelial phenotype favors metastatic tumor formation and recapitulation of primary tumor characteristics. Of interest, a gene expression microarray analysis revealed a 100-fold overexpression of Pax-5 in breast cancer cells that metastasized to lymph nodes compared with the primary tumor (27). Moreover, Pax-5 has been shown to suppress the expression of the proinvasive focal adhesion kinase, reminiscent of the mesenchymal–epithelial transition (20). Collectively, these findings suggest the involvement of Pax-5 in breast cancer and its progression to metastasis.

The seemingly conflicting functions exhibited by Pax-5 appear to be highly dependent on precise cellular contexts and suggest a complex spectrum of functions for Pax-5 and possibly its numerous isoforms in different cell backgrounds. Further studies aimed at elucidating the downstream signaling pathways regulated by Pax-5 will likely prove to be the key in understanding its role in cancer cells.

**Pax-5 Signaling in Cancer**

**Upstream regulation**

High-throughput gene expression analysis has led to the identification of various downstream Pax-5 effectors. However, the upstream signaling networks that are involved in aberrant Pax-5 activity in cancer processes remain largely unidentified. Although genomic instability seems to be at the root of aberrant Pax-5 expression in B-cell malignancies, so far no translocations involving the Pax-5 locus have been found in characterized small-cell lung carcinoma cell lines (26), suggesting an alternate mechanism for Pax-5 ectopic expression. The exploration of specific signaling events that activate Pax-5 is critical for our understanding of the pathogenesis of these cancers and may lead to the identification of potential therapeutic strategies aimed at targeting the Pax-5 pathway.

A recent study identified metastasis-associated protein 1 (MTA1) as a regulator of Pax-5 transcription (28). Although its role in metastasis is unclear, MTA1 expression strongly correlates with invasiveness and angiogenesis in many cancers, including breast cancer. A study using chromatin immunoprecipitation revealed MTA1 binding to an enhancer sequence within the Pax-5 gene in MCF-7 breast carcinoma cells (28). This interaction was subsequently investigated in B-cell lymphoma, where MTA1 directly induces transcription of Pax-5, an effect that is dependent on acetylation of MTA1. Of interest, histone acetyltransferase (HAT) p300 interacts with and acetylates Pax-5 to enhance its transcriptional activity. Taken together, these findings are consistent with an involvement of Pax-5 in metastasis, and they underscore acetylation as an important posttranslational modification that regulates Pax-5 transcription and activity.

Recent evidence provides reason to suspect that the JAK2/STAT5 pathway is involved in Pax-5 regulation. One study revealed a STAT5 binding motif within the Pax-5 gene and showed that phosphorylated STAT5 enhances activation of the Pax-5 promoter in pre-B cells (29). Subsequently, another report indicated that JAK2 activation induces DNA-binding ability and nuclear compartmentalization of Pax-5 in breast carcinoma cells (19). Taken together, these results suggest 2 means by which the JAK2/STAT5 pathway could potentially modulate Pax-5 activity in cancer cells: (i) through the regulation of Pax-5 localization and (ii) through the activation of STAT5-mediated transcription (Fig. 1). On the other hand, an inverse correlation between Pax-5 and STAT5 was recently reported by Heltemes-Harris and colleagues (17), who described a mechanism in which reduced expression of Pax-5 and EBF1 synergizes with STAT5 overactivation to initiate B-ALL. A number of studies have implicated aberrant activation of STAT5 in breast and hematopoietic cancers. Further investigation will help clarify the relationship between JAK2/STAT5 signaling and Pax-5, and the potential of the JAK2/STAT5 pathway as a target for inhibition.

**Interacting partners**

Several proteins have been reported to interact directly with Pax-5. Of note, Pax-5 recruits the Ets-1 oncprotein and enhances its DNA-binding ability at the promoter of the mb-1 gene (30). The Ets-1 protein is a transcription factor, and its aberrant expression is associated with numerous cancers in which it acts to promote the transcription of genes involved in tumor invasiveness and angiogenesis. Of interest, Ets-1 stimulates c-Met transcription (31), which is also a transcriptional target of Pax-5 (ref. 26; see above). Moreover, both Ets-1 and Pax-5 are expressed in astrocytomas and breast cancer cell lines (18, 31). Although the physiologic relevance of the
Pax-5/Ets-1 interaction has not been investigated beyond the normal B cell, it would be of great interest to explore possible interactions between these 2 proteins in a carcinogenic model to assess any synergistic effect that could contribute to oncogenesis.

The death-domain–associated protein Daxx also interacts with Pax-5 (32). Daxx localizes to both cytoplasmic and nuclear compartments. In the former, it binds to transmembrane protein FAS and potentiates FAS-induced apoptosis. In the latter, it acts as a transcriptional coregulator with diverse transcription factors. A yeast 2-hybrid assay identified Daxx as a potential coregulator of Pax-5, and further analysis revealed that it either enhanced or repressed Pax-5 transcriptional activity depending on the cell context (32). Further study will help elucidate the interaction between these 2 proteins and their effect on the transcription of downstream targets.

Pax-5 has also been shown to interact with the tumor-suppressor retinoblastoma protein (33). The hypophosphorylated form of retinoblastoma protein interacts with Pax-5, as shown by coimmunoprecipitation experiments in B cells. Curiously, this finding was not explored in more detail, and further clarification of this interaction may reveal a sequestering of active retinoblastoma protein by Pax-5, thus presenting a means by which Pax-5 could contribute to proliferative events.

An interesting link between the Pax-5 interacting proteins discussed herein is that they are all elements of promyelocytic leukemia protein nuclear bodies. These dynamic nuclear structures regulate many cellular processes, such as apoptosis, proliferation, and senescence. It is compelling to think that Pax-5 might contribute to tumorigenesis by competitively interfering with key components of promyelocytic leukemia protein nuclear bodies and negatively influencing their tumor-suppressive properties.

**Downstream targets**

Many studies have aimed to identify downstream effectors of Pax-5 that could account for its oncogenic role. Stuart and colleagues (34) explored a possible relationship between Pax-5 and p53 expression in human astrocytomas, and they observed an inverse correlation between Pax-5 mRNA and p53 expression in this malignancy. Additionally, a Pax-5 binding site exists within the p53 transcript, and Pax-5 overexpression reduces p53 expression levels in NIH3T3 mouse fibroblasts. In contrast, Pax-5 activates p53 promoter-driven luciferase in MCF-7 cells (18).

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**Figure 1.** Novel cancer-associated signaling pathways that regulate Pax-5 expression and activity. Aberrant receptor tyrosine kinase activation by human growth hormone leads to activation of JAK2 and nuclear translocation of Pax-5, both of which are inhibited by AG490, a specific JAK2 inhibitor. JAK2 also phosphorylates (P) STAT5, which induces its translocation to the nucleus, resulting in Pax-5 transcription. HAT activity is also important for Pax-5 regulation because MTA1 transcriptionally activates Pax-5 upon acetylation (Ac), whereas HAT p300 acetylates Pax-5 to increase target gene transcription. Among the Pax-5 target genes are many cancer-associated proteins, such as p53, hTERT, and mesenchymal markers, including matrix metalloproteinase 2 (MMP-2), vimentin, and c-Met. The Pax-5–mediated transcriptional control of these target genes is highly dependent on cellular context and is discussed throughout this review.
Recently, telomerase reverse transcriptase (hTERT) was identified as a Pax-5 transcriptional target (35). The hTERT gene encodes for the catalytic subunit of the telomerase enzyme and is the limiting factor for its activity. Telomerase reverse transcriptase expression is inactivated in differentiated somatic cells, but it is restored in many immortalized tumor cells. Of interest, it was shown that Pax-5 directly activates hTERT transcription in a Burkitt lymphoma cell line through binding of a Pax-5 consensus sequence within the first exon of the hTERT gene (35). This finding suggests an additional means by which Pax-5 could enhance cell survival in cancer.

Large-scale microarray studies have enabled the identification of many Pax-5 activated and repressed target genes involved in a variety of biologic processes not commonly ascribed to Pax-5 function. For example, Schebesta and colleagues (36) found that Pax-5 modulates the transcription of genes involved in migration and adhesion in B cells. Murine Pax-5-positive pro-B cells have decreased migration and increased adhesion compared with Pax-5-/- pro-B cells. These findings are consistent with the observations of Vidal and colleagues (18) discussed above, and they present specific target genes through the modulation of which Pax-5 may manifest phenotypic transitioning in metastasis. The latter study also demonstrates the effect of Pax-5 on mesenchymal marker expression. In MCF-7 cells, Pax-5 decreases MMP-2, vimentin, and fibronectin transcript levels. Moreover, immunostaining of tumor sections reveals that Pax-5 promotes the localization of β-catenin to the site of cell–cell contact, thus increasing adhesion at intercellular junctions.

Pridans and colleagues (37) identified a series of Pax-5–modulated target genes with functions in cell motility and adhesion in B cells. Their data revealed the repression of an adhesion molecule, Embigin, by Pax-5. This finding is in slight contrast to the reports mentioned above, which support a proadhesion function for Pax-5. Similarly, Pax-5 activates transcription of a potent promotility oncogene in small-cell lung cancer cells (26). These are clear illustrations of how Pax-5 can stimulate the transcription of genes with opposing cellular activities in a context-dependent fashion.

The complexity of Pax-5 signaling is increased at many levels. First is the expression of multiple Pax-5 protein variants through alternative splicing events. Numerous studies have shown that many of the Pax-5 isoforms maintain DNA-binding ability but have different or truncated regulatory domains (7). Consequently, expressed alternatively spliced variants of the Pax-5 gene have the ability to alter the transactivation potential of the target gene’s expression. These findings underscore the importance of establishing the identity of the Pax-5 isoform and the ratios present in cells when attempting to understand Pax-5 pathways. Another determining factor is the cellular environment. Many of the conflicting results discussed herein are likely due to the cellular environment and its functions where Pax-5 is expressed. In this context, a recent study revealed that Pax-5 overexpression leads to an increase of CD19 transcription in B-AML cell lines but not in epithelial cells, where the chromatin of the CD19 promoter is inaccessible (38). Collectively, these findings might provide some insight into why such functional variability of Pax-5 is observed among different cells types, as well as offer promising avenues of research that could ultimately lead to the development of therapeutic interventions.

Conclusions

Pax-5 is recognized as an important contributor to normal hematopoiesis and organogenesis in complex organisms. Furthermore, the involvement of Pax-5 in B-cell cancers and other malignant diseases is generally accepted. However, much remains to be elucidated with regard to its precise function in all cancer types. The role of Pax-5 in the metastatic process is one such example that will likely garner strong interest in the future. At the molecular level, knowledge about the upstream pathways that influence Pax-5 expression and its subsequent downstream targets will not only lead to a better understanding of the complex role of Pax-5 and other Pax-related genes but also provide significant insight into the molecular biology of cancer. The involvement of Pax-5 in signaling cascades (e.g., Jak/STAT) and chromatin remodeling components (e.g., MTA1) sheds light on the lesser-known epigenetic functions associated with this transcription factor (25, 37) and may eventually lead to promising new strategies for cancer therapy. Overall, the interesting research avenues currently under study will help clarify the multiple roles of Pax-5.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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