

Review Article

PAX3: A Molecule with Oncogenic or Tumor Suppressor Function Is Involved in Cancer

Ashok Arasu,¹ Sengottuvelan Murugan,² Musthafa Mohamed Essa ³,
Thirunavukkarasu Velusamy ¹ and Gilles J. Guillemin ⁴

¹Department of Biotechnology, School of Biotechnology and Genetic Engineering, Bharathiar University, Coimbatore 641046, Tamil Nadu, India

²Endocrine Research Facility, Department of Animal Science, Rutgers University, New Brunswick-08901, NJ, USA

³Department of Food Science and Nutrition, CAMS, Sultan Qaboos University, Muscat, Oman

⁴Neuroinflammation Group, Faculty of Medicine and Health Sciences, Deb Bailey MND Research Laboratory, Macquarie University, NSW, 2109, Australia

Correspondence should be addressed to Musthafa Mohamed Essa; drmdessa@gmail.com,
Thirunavukkarasu Velusamy; bharathiarasu@gmail.com, and Gilles J. Guillemin; gilles.guillemin@mq.edu.au

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Metastasis is the most deadly aspect of cancer and results from acquired gene regulation abnormalities in tumor cells. Transcriptional regulation is an essential component of controlling of gene function and its failure could contribute to tumor progression and metastasis. During cancer progression, deregulation of oncogenic or tumor suppressive transcription factors, as well as master cell fate regulators, collectively influences multiple steps of the metastasis cascade, including local invasion and dissemination of the tumor to distant organs. Transcription factor PAX3/Pax3, which contributes to diverse cell lineages during embryonic development, plays a major role in tumorigenesis. Mutations in this gene can cause neurodevelopmental disease and the existing literature supports that there is a potential link between aberrant expression of PAX3 genes in adult tissues and a wide variety of cancers. PAX3 function is tissue-specific and could contribute to tumorigenesis either directly as oncogene or as a tumor suppressor by losing its function. In this review, we discuss comprehensively the differential role played by PAX3 in various tissues and how its aberrant expression is implicated in disease development. This review particularly highlights the oncogenic and tumor suppressor role played by PAX3 in different cancers and underlines the importance of precisely identifying tissue-specific role of PAX3 in order to determine its exact role in development of cancer.

1. Introduction

Cancer is a disease that is characterized by uncontrolled cell growth, proliferation, migration, and invasion of abnormal cells resulting in aggregation of these cells to form tumors in organ. There are several etiologies for the development of cancer, ranging from environmental to genetic causes. Mutations that inhibit the normal function of oncogenes, tumor suppressor genes, and apoptosis genes can lead to uncontrollable cell growth. Changes in molecular mechanisms that regulate stem cell differentiation were also known to be a strong factor associated with development of cancer [1]. The paired box (PAX: human)/(Pax: mouse)

gene family is known to be associated with developmental functions and play a crucial role in cellular proliferation, differentiation, migration, and tissue development [2]. PAX proteins are subclassified according to the additional DNA-binding homeodomain or octapeptide region, which can serve as a binding motif for the protein cofactor for effective inhibition of downstream gene transcriptions [3]. The role of PAX/Pax protein as transcriptional activators or repressors has been demonstrated through their interaction with other transcription factors to induce target promoters [4]. There is a possible link between the anomalous expression of PAX genes in adult tissues and a wide class of cancers by promoting tumorigenesis as an oncogene or by failing to perform its role

as a tumor suppressor [5, 6]. PAX proteins may be useful as a biomarker in the diagnosis of cancers. PAX genes have an oncogenic ability when constitutively expressed, either as a part of a fusion gene or as a whole gene [7–12].

PAX3, a member of PAX gene family, plays a critical role in cell proliferation, differentiation, and migration during embryonic development of cells [13]. PAX3 contains all three complete structural domains: a paired domain, homeodomain, and octopeptide, which is a characteristic of PAX family of proteins. PAX3 interacts with other transcription factors through its paired domain (PD) and recruits them to target promoters [14, 15]. PAX3 is expressed as several isoforms such as PAX3a, PAX3b, PAX3c, PAX3d, PAX3e, PAX3g, and PAX3h [16–19]. Each of these isoforms are generally involved in developmental function, however, with specific functions that are unique to them in different tissues [19]. PAX3 expression is necessary for proliferation and migration of neural crest (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. Acceleration of proliferation, migration, and survival of neural crest cells are also regulated by PAX3 [20]. PAX3/Pax3 (human/mouse) is a developmentally expressed transcription factor in embryo and is rapidly switched off during terminal differentiation [21–23]. PAX3 is necessary not only for the proper specification of these developing tissues, but also for cell survival. Homozygous loss of PAX3 in mouse leads to several neural crest and neural tube defects, attributable to induction of p53-dependent apoptosis [5, 24–28]. PAX3 is necessary for neural crest-derived peripheral nervous system development (PNS) and its reexpression in differentiated neural crest cells is suspected to be involved in the development of peripheral nervous system tumor, neuroblastoma. A gain of function mutation in PAX3 is associated with several diseases such as Waardenburg syndromes, craniofacial-deafness-hand syndromes, alveolar rhabdomyosarcomas, and neuroblastomas [3, 29]. Being an important developmental factor, the expression of PAX3 gradually decreases as the tissues differentiate. However, its reexpression in differentiated adult tissues that require PAX3 function during embryonic development leads to several tumors [3, 15].

More recently, PAX3 was observed to be overexpressed in various types of cancer tissues and cell lines like neuroblastomas, glioblastomas, melanomas, and gastric cancers, suggesting that it may function as an oncogene in these cancers [30–36]. In rhabdomyosarcomas, PAX3-FOXO1 fusion increases the transcriptional activity of PAX3 by constitutive nuclear localization [37]. However, in thyroid cancer, it plays a completely different role as a tumor suppressor. Loss of function of PAX3 due to decreased expression caused by hypermethylation is reasoned out as an underlying cause for development of thyroid cancer [36]. Based on these facts, it is important to determine the functions of PAX3 specific to tissue type and then understand its role in disease pathogenesis. This review will mainly discuss about the specific roles played by PAX3 in different tissue types and then will focus on specific examples where PAX3 has contradictory

function as either oncogene or tumor suppressor, and is implicated in development of cancer.

2. PAX3 and Its Role in Development and Diseases

PAX3 is a very well-characterized protein that is expressed in the neural crest (NC) [38]. It is also one of the most well-characterized transcription factors of the NC and has been extensively studied in NC induction during development, cardiac NC, and melanocyte lineages [38]. The physiological function of PAX3 has been well understood through characterizing the mutations that are reported in mice and humans. PAX3 plays a major role during early skeletal muscle formation in the embryo, while PAX7 predominates during postnatal growth and muscle regeneration in the adult [39]. PAX3 is expressed during early migration of NC and in somitic cells along the neuronal crest cell migratory path [40]. PAX3 is generally extinguished in migratory neural crest cells as they differentiate but maintained in the melanocyte lineage and also sustained in cultured neural crest-derived stem cells [41], in later stages of development in various lineages. The timing of expression in early neural crest and subsequent downregulation as neural crest-derived cells differentiate also implicates PAX3 as an important factor for maintaining progenitor populations. In fact, persistent PAX3 misexpression in cranial neural crest cells resulted in cleft palate and other craniofacial defects, ocular defects, and perinatal lethality [42]. Another phenotype associated with persistent PAX3 expression is that bone morphogenetic protein- (BMP-) induced osteogenesis is blocked via upregulation of the PAX3 target *Sostdc1*, a soluble BMP inhibitor. This highlights the role PAX3 plays in maintaining an undifferentiated state by blocking responsiveness to differentiation signals [42]. PAX3 function well-characterized in the neural crest is the availability of a series of mutations in the murine *PAX3* gene. These alleles represent a range of null, severely hypomorphic, and mildly hypomorphic forms of PAX3. In addition to neural crest defects, PAX3 and PAX7 mutants display neurulation defects and altered somitogenesis [39]. PAX3 and *versican* have mutually exclusive expression pattern and when PAX3 is lost, the *versican* (VCAN) expression domain is expanded, correlating with an absence of migrating neural crest cells lateral to the neural tube [43]. Where PAX3 and *Zic1* expression overlap, they act together to specify the neuroectoderm to adopt a neural crest fate [44, 45] and activate *Slug* expression in a Wnt-dependent manner [24]. Multiple experiments demonstrate that PAX3 and *Zic1* together are both necessary and sufficient to specify neural crest [44, 45]; PAX3 is upregulated in the dorsal neural crest in response to Wnt signaling and also depends on bone morphogenetic protein (BMP) signaling. Downregulation of PAX3 occurs when the neural crest migration starts [46, 47].

In the absence of functional PAX3 (PAX3 mutants), the neural crest cells migrating to the peripheral nervous system undergo premature neurogenesis (evidenced by increased *Brn3* positive staining in neural tube explants), perhaps due to a change in the regulation of genes such as *Hes1* and

Ngn2 (needed for differentiation and proliferation), which PAX3 regulates by directly binding to their promoters. In this role, PAX3 may couple migration with neural crest cells maintenance and neurogenesis [48]. *In vitro*, a different phenomenon is seen. PAX3 is initially expressed in all neural crest cells from culture but is subsequently only retained in neurons. PAX3 homozygous mutant neural crest cultures had an 80% reduction in the capacity to generate sensory-like neurons. Downregulation of PAX3 in dorsal root ganglia inhibited 80–90% of newly generated sensory neurons but had no effect on survival of sensory neurons or precursors [49]. Mutation of PAX3, as occurs in PAX3 homozygotes, results in development of heart defects including persistent truncus arteriosus (PTA), signs of cardiac failure [25, 50, 51], and defects of the aortic arches, in addition to thymus, thyroid, and parathyroid defects, ultimately leading to embryonic lethality. PAX3 inhibits p53-dependent apoptosis in the dorsal neural tube to regulate its closure [28] and is also important for normal development and migration of the cardiac NC to the developing heart [25]. In addition to its function as a standalone transcription factor, several downstream molecules of other important pathways can act as a coactivator in mediating the transcriptional effects of PAX3. The downstream effector molecules of the Hippo pathway Taz and Yap65 play an important role in neural crest development and deletion of these proteins in neural crest resulted in embryo-lethal neural crest defects and decreased expression of the Pax3 target gene, *Mitf* [52].

The role of PAX3 as a transcription factor is well established in the development of melanocytes during embryogenesis and has recently been characterized as a molecular switch in the initiation of mature melanocytes formation [32]. Interestingly, PAX3 is one of the key molecules in melanogenesis that is absent or greatly reduced in the bulbs of white hair when compared to black hair, further reinforcing its role in melanocyte stem cell populations [53]. Among various PAX3 isoforms, PAX3c is the most studied and well-characterized isoform; in mouse melanocytes *in vitro*, the effect of PAX3 on proliferation, migration, survival, and transformation varies depending on the isoform, with some isoforms having opposite effects [20].

Evidence supports a role for PAX3 as a controller of a cascade of transcriptional events that are necessary and sufficient for skeletal myogenesis [54, 55]. PAX3 is downregulated when muscle tissue begins to differentiate and the muscle specific transcription factors are activated [54, 56]. Ectopic expression of PAX3 prevents the myogenic differentiation of myoblasts into myotubes, which might involve the cooperation of *Mx1* and *Notch* genes [57, 58].

Aberrant expression of PAX proteins is implicated in developmental defects. Specific mutations within a number of PAX/ Pax (human/mouse) genes lead to a range of developmental abnormalities in both human beings and mouse. Many studies proved that PAX3 loss of function mutations are involved in either type 1 or type 3 Waardenburg syndrome [59–65]. PAX3/Pax3 (human/mouse) mutations are associated with limb muscle hypoplasia in Waardenburg syndrome patients and *Splotch* phenotype mice, respectively [54, 55].

Interestingly, PAX3 mutations were typically not found in families with other neurocristopathies [64]. Mutations that partially abolish the activity of PAX2, PAX3, and PAX6 were known to cause dysmorphic syndromes with developmental abnormalities [66–70]. PAX2 and PAX8 double mutants show a complete lack of kidney formation due to reduced activity of these proteins [71].

3. PAX3 Plays an Oncogenic Role in Various Types of Tumors

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 tumors, lymphoma, medullary thyroid carcinoma, rhabdomyosarcoma (RMS), and melanoma [5, 72–74]. Transfection of 3T3 cells with wild-type Pax1, Pax2, Pax3, Pax6, or Pax8 produced tumors in the nude mice within 2 to 6 weeks; the tumors were well vascularized and resembled spindle cell sarcomas, with high and a typical mitotic activity and infiltration into nerve and muscle tissues and blood vessels [75]. PAX genes in subgroups I (PAX1 and PAX9) are less often involved in cancer or their expression is indicative of a more favorable outcome [5]. PAX/Pax (human/mouse) proteins, therefore, serve as tumor markers in several cancers such as rhabdomyosarcoma, melanoma, neuroblastoma, and Ewing's sarcoma [2, 6, 76]. While PAX expression is very rare in adult tissues, findings support that its expression may be involved in maintaining pluripotency and survival of stem cell populations. Either continuing or recurring PAX expression is essential to provide pools of progenitor cells for tissue regeneration upon injury. In cancer cells, achieving self-sufficiency in growth signals and unrestricted replicative potential are required to be able to survive in potentially adverse microenvironments during tumor progression. There are numerous studies that prove that PAX genes play important roles in conferring growth and survival advantages to cancer cells and that they regulate cell plasticity [2]. Conceptually, cell proliferation and differentiation are placed at opposite ends of the "spectrum" of tumor progression. PAX genes, such as PAX8, could play a key role in balancing these processes [2].

PAX3, which plays a critical role during neural crest development, especially in progenitor cell populations, also plays a role in tumor formation in these tissue types. Many human neuroectodermal tumors express PAX3 [18, 34, 77, 78]. The role of PAX3 and its isoforms in myogenesis, melanogenesis, neurogenesis, and related oncogenesis have been well established (Figure 1). PAX3 is specifically detected in most neural crest-derived Ewing's family tumors and in most peripheral neuroectodermal tumors [34], small cell lung cancer [18], and most primary cultured melanomas [18, 34, 79]. Screening of Ewing's sarcoma and peripheral neuroectodermal tumor specimens and its derived cell lines showed elevated PAX3 expression [34]. Deactivation of p53 during neural tube closure and cardiac neural crest development by stimulating its ubiquitination and degradation requires PAX3 function, but not as a transcriptional regulator

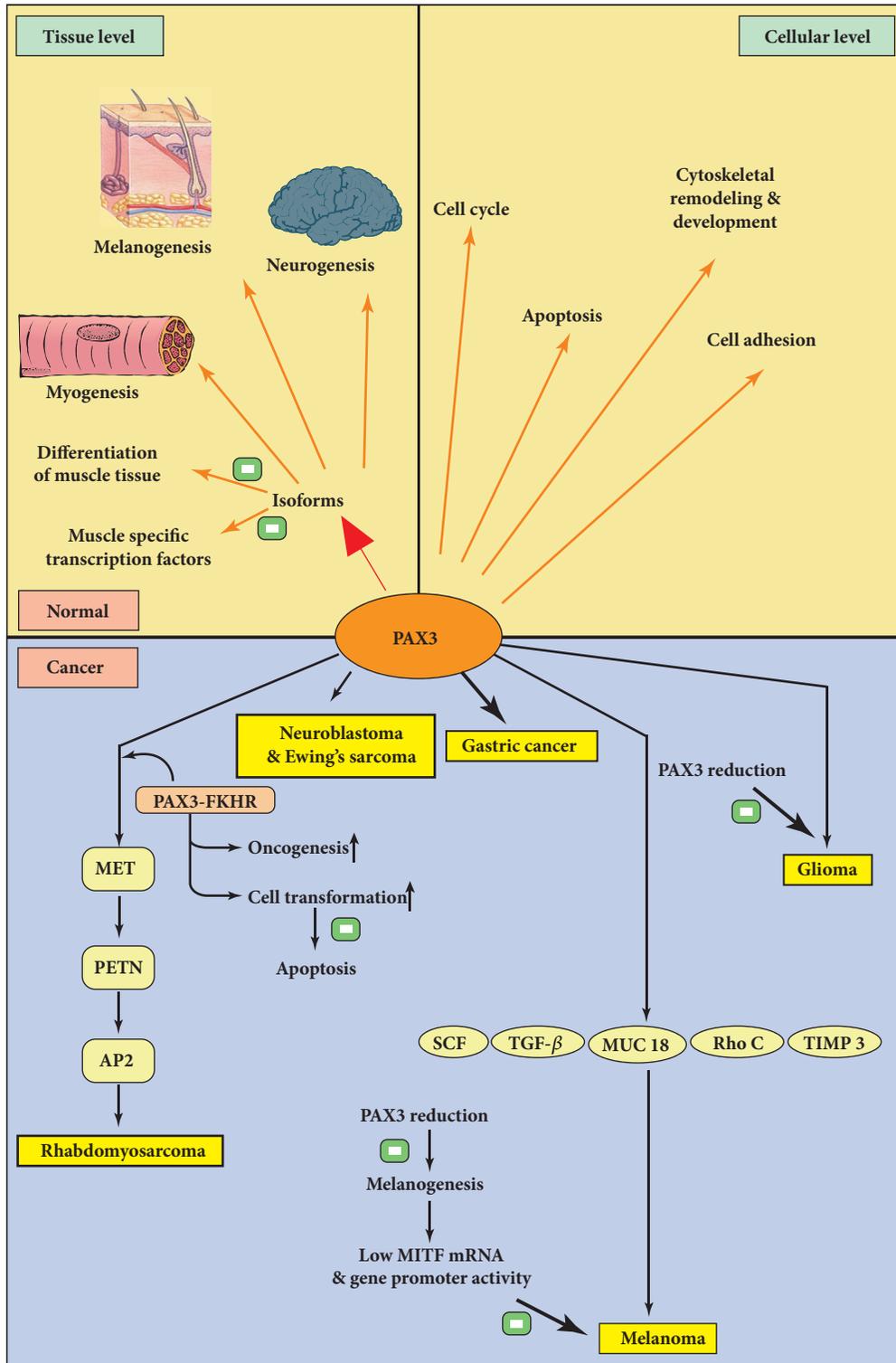


FIGURE 1: Oncogenic role of PAX3 in various types of cancer. PAX3 isoforms play a role in myogenesis, melanogenesis, and neurogenesis. PAX3 is involved in physiological events such as cell cycle, apoptosis, cell adhesion, and cytoskeletal remodelling and development. Overexpression of PAX3 inhibits the differentiation of muscle tissues and muscle specific transcription factors. While overexpression of PAX3 was observed in various types of cancers, PAX3-FKHR leads to tumorigenesis in rhabdomyosarcoma through altering the MET, PTEN, or AP2 signaling pathways. PAX3-FKHR can induce cellular transformation and prevent apoptosis. Reduction of PAX3 prevents development of glioma and also induces a loss of melanogenesis in melanoma. MITF mRNA and gene promoter activity is also decreased because of the PAX3 reduction. Melanoma susceptibility and progression genes like SCF, TGF- β , MUC18, RhoC, and TIMP3 can be regulated by PAX3 and it leads to melanoma.

TABLE 1: Pax3 transcription factor plays a dual role in various types of cancers.

Cancer Type	Effects	Role of PAX3
Rhabdomyosarcoma		PAX3-FKHR can induce cellular transformation and prevent apoptosis by altering the MET, PTEN or AP2 signalling pathways [55, 57, 58, 87, 89, 90, 92].
Melanoma		Regulating the expression of a variety of melanocytic genes, followed by a decrease in MITF mRNA and gene promoter activity and progression genes SCF, TGF- β , MUC18, RhoC and TIMP [84, 85]
Neuroblastoma	Oncogenic effects	Inhibition of PAX3 expression significantly decreased the attachment of S-type SH-EPI cells to extra-cellular matrix proteins, fibronectin, laminin and collagen IV and therefore downregulation of PAX3 reduces migration and invasion [31].
Glioblastoma		PAX3 was upregulated in the glioma SHG-44 cells promoted tumor formation <i>in vivo</i> cell lines, while its knockdown resulted in decreased cell proliferation, invasion and induced apoptosis in these cells. PAX3 expression in glioblastoma U-87MG cells suppressed tumorigenicity [30].
Gastric cancer		MiR-206 significantly suppressed gastric cancer cell invasion and metastasis both <i>in vitro</i> and <i>in vivo</i> by down regulating PAX3. <i>In vivo</i> metastasis assay shows that overexpression of PAX3 promotes invasiveness and pulmonary metastasis [35].
Urothelial cancer		FOXO3a negatively regulates Twist1 and Y-box-binding protein 1 (YB-1), and positively regulated E-cadherin in With FOXO3a PAX3 acts as a prognostic factor [94].
Thyroid cancer	Suppressor	PAX3 a tumor suppressor in thyroid cancer, through inhibiting the activities of PI3K/Akt and MAPK signaling pathways and promoting transcription factor FOXO3a.). The ectopic expression of PAX3, up-regulated the expression of ZIC1, a zinc-finger transcription factor, which in turn increases FOXO3a transcriptional activity [36].
Rhabdomyosarcoma		Overexpression of miR-29 and 206 downregulates the expression of cell cycle genes, induce cell cycle arrest through stabilizing PAX3 [6].
Alveolar rhabdomyosarcoma		PAX3-FOXO1 up-regulate <i>Noxa</i> (Promote activation of <i>caspases</i> and apoptosis) [95].

indicating a novel role for PAX3 in human diseases, such as in neural crest-derived cancers and Waardenburg syndrome types 1 and 3 [80].

Neuroblastoma is an extracranial solid tumor that occurs in children. Abnormally elevated levels of PAX3 expression has also been found in some neuroblastoma cell lines and tumors [81]. PAX-3 is overexpressed in glioma tissues compared to normal brain tissues; however, the pathogenic role of PAX-3 in human glioma cells remains to be elucidated. PAX3 was upregulated in the glioma cell lines, while its knockdown resulted in decreased cell proliferation and invasion and induced apoptosis in these cells (Figure 1). Inhibition of PAX3 expression in glioblastoma U-87MG cells suppressed tumorigenicity and upregulation of PAX3 expression in glioma SHG-44 cells promoted tumor formation *in vivo*. These results indicate that PAX3 in glioma is essential for gliomagenesis; thus, targeting PAX3 or its downstream targets may lead to novel therapies for this disease [30]; this data has been represented in Table 1. PAX3 contributes to various cell lineages during embryonic development and is also important in tumourigenesis. Reexpression of PAX3 was found in neuroblastoma cells, and the malignant neuroblastic (N-type) neuroblastoma cells expressed significantly higher PAX3 protein expression in comparison to their benign substrate-adherent (S-type) counterparts. PAX3 knockdown resulted in persistent cell growth inhibition in neuroblastoma cell, through G1 cell cycle arrest, and leads to progressive apoptosis.

Inhibition of PAX3 expression significantly decreased the attachment of S-type SH-EPI cells to extracellular matrix proteins, fibronectin, laminin, and collagen IV. Migration and invasion of both neuroblastoma cell types were markedly reduced after PAX3 downregulation. Microarray analyses revealed that signaling pathways involving cell cycle, apoptosis, cell adhesion, cytoskeletal remodelling, and development were altered in particular, by PAX3 downregulation (Table 1). These novel findings emphasize that PAX3 might contribute to oncogenic characteristics of neuroblastoma cells by regulating a variety of crucial signaling pathways [31].

While PAX3 promotes melanocyte phenotype, it plays an indispensable role in stem cell maintenance and actively promotes lesion survival, while BRAF mutations support tumor proliferation in melanoma [32]. PAX3 also plays a main role, in the pathogenesis of melanoma by influencing pathways through its transcriptional activity [82] and also by inducing p53 loss of function by promoting its ubiquitination [80]. PAX3 is a key transcription factor in regulating the expression of a variety of melanocytic genes [22, 83]. Inhibiting the expression of PAX3 using antisense RNA in melanoma cell lines resulted in dose dependent reduction of proliferation of melanoma cells and induction of apoptosis, suggesting role for PAX3 in melanoma cell survival [78, 79]. Pax3 reduction also induces a loss of melanogenesis in melanoma cells, followed by a sharp decrease in MITF mRNA and

gene promoter activity. Using microarray analyses PAX3 was identified as a regulator of the melanoma susceptibility and progression genes SCF, TGF- β , MUC18, RhoC, and TIMP3 [84–86], and this data has been represented in Figure 1 and Table 1.

PAX3 involved in muscle development is also implicated in tumorigenesis of muscle, rhabdomyosarcoma. Rhabdomyosarcoma is the most frequent soft tissue tumor 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. PAX3 promotes tumorigenesis by influencing downstream MET pathway [87]. Rhabdomyosarcoma cells that undergo apoptosis due to specific antisense treatment is rescued from death by ectopic expression of PAX3, which indicates the prosurvival properties promoted by PAX3 [88]. PAX3 expression was markedly higher in gastric cancer tissues than in adjacent noncancerous tissues. MiR-206 significantly suppressed gastric cancer cell invasion and metastasis both *in vitro* and *in vivo* by downregulating PAX3. *In vivo* metastasis assay demonstrates that overexpression of PAX3 significantly promotes invasiveness and pulmonary metastasis of gastric cancer cells [35] (Table 1).

The above-mentioned studies clearly establish the oncogenic role of aberrant expression of PAX3 as a whole molecule in various cancers. Interestingly, PAX3 gene is involved in gene translocations which results in fusion proteins involving PAX3 that offers them oncogenic potential [89]. PAX3-FKHR (forkhead in rhabdomyosarcoma) fusion proteins are involved in promoting different molecular pathogenesis of alveolar rhabdomyosarcoma and embryonal rhabdomyosarcoma by inducing cellular transformation and preventing apoptosis [89, 90]. The tumor promoting effects of PAX3 and PAX3-FKHR in rhabdomyosarcoma tumorigenesis are suggested to be through altering the MET, PTEN, or AP2 signaling pathways (Figure 1, Table 1). PAX3-FKHR mutants through gain of function promote tumor development in myoblasts, which again shows the oncogenic potential of PAX3 transcriptional activity [91]. Interestingly, PAX3-FKHR shows oncogenic effects, predominantly at relatively low levels, while at higher expression levels it induces suppression of growth in 3T3 L1 fibroblasts [92]. This particular study highlighted domain specific function of PAX3 at different levels of activity. At low levels, through its homeo domain and its associated function induced the transformation of 3T3L1 fibroblasts, while at higher levels they inhibited the transformation and growth potential of these cells, by its activity through paired domain. These studies clearly indicate a multifaceted dynamic function of PAX3 depending on the level of activity and cell type.

4. Tumor Suppressing Role of PAX3

The proliferation promoting characteristics of PAX3 have been well established in several cancers as discussed above. However, designating PAX3 as an oncogene has been impaired by contradicting reports available that highlight the growth inhibiting potential of PAX3. The ectopic expression

of PAX3 dramatically inhibited thyroid cancer cell proliferation, colony formation, migration and invasion, induced cell cycle arrest, and apoptosis and retarded tumorigenic potential in nude mice [36]. These reports implicate PAX3 as a novel functional tumor suppressor in thyroid cancer, through inhibiting the activities of PI3K/Akt and MAPK signaling pathways and promoting transcription factor FOXO3a. The study also highlighted that epigenetic alteration such as promoter methylation as a major mechanism by which PAX3 is inactivated in this cancer (Figure 2 and Table 1). Further, PAX3 is a member that belongs to a group of transcription factors that are regulated by posttranslational modification such as acetylation. Pax3 acetylation decreased its transcriptional activity, thereby modulating the activity of downstream effectors Hes1 and increased Neurog2, resulting in sensory neuron differentiation [93]. Overexpression of miR-29 and 206 downregulates the expression of cell cycle genes and induces cell cycle arrest through stabilization of PAX3 in rhabdomyosarcoma, suggesting a tumor suppressor role for PAX3 [6].

5. FOXO-PAX3 Association in Tumor Suppression

PAX3, in addition to its tumor suppressor function, can also combine with other transcription factors and can regulate the expression of a wide variety of proteins that can act as tumor suppressors. FOXO1 transcription factors belong to the FOXO family and are involved in regulating key physiological pathways. In ARMS, gene translocations between PAX3 and FOXO1 resulted in a fusion protein PAX3-FOXO1 which promoted the growth suppressive activity by upregulating the expression of gremlin 1 (GREM1) and death associated protein kinase-1 (DAPK1) tumor suppressor genes [96] (Figure 2). This study also characterized four downstream targets of PAX3-FOXO1 that contribute to the biological activities of growth suppression and myogenic differentiation [96]. PAX3-FOXO1 upregulated Noxa, which in turn promoted the activation of caspases and induced apoptosis. This particular signaling axis is considered as an important aspect of ARMS tumor biology that provides opportunity to create a therapeutic window by induction of apoptosis in ARMS cells [95] (Figure 1 and Table 1).

Similarly, PAX3 can modulate the expression of FOXO3, another type of transcription factor that belongs to the FOXO family that are involved in multiple signaling pathways and plays critical roles in a number of physiologic and pathologic processes [97]. Cancer patients with low FOXO3a expression had poor disease-free survival, cancer-specific survival, and overall survival [94].

FOXO3a negatively regulated Twist1 and Y-box-binding protein 1 (YB-1) and positively regulated E-cadherin in KK47 and TCCsup cells that expressed Twist1, but not in T24 cells that did not express Twist1 (Table 1). This suggests that FOXO3a could act as a prognostic factor in urothelial cancer and could represent a promising molecular target for cancer therapeutics [94]. Interestingly, FOXO3 partners with PAX3/7 to coordinately recruit RNA polymerase II

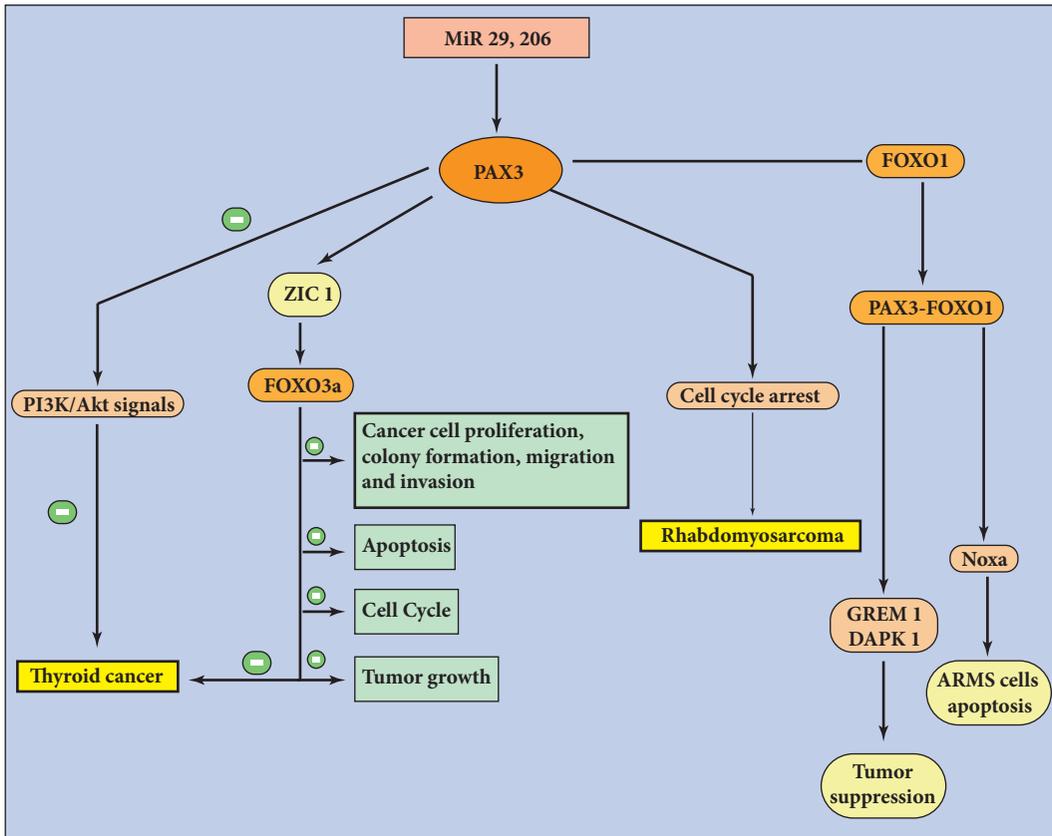


FIGURE 2: Tumor suppressor role of PAX3 in various types of cancer. Ectopic expression of PAX3 inhibits thyroid cancer cell proliferation, colony formation, migration, and invasion, and induced cell cycle arrest and leads to apoptosis through inhibiting the activities of PI3K/Akt and MAPK signaling pathways and promoting transcription factor FOXO3a. MiR 29 and 206 stabilize the PAX3 expression and induce the cell cycle arrest in the rhabdomyosarcoma cell lines. Fusion between the PAX3-FOXO1 induces the tumor cell suppression by inducing the expression of GERM1 and DAPK1 genes which in turn upregulate NOXA (promote activation of caspases and apoptosis) and induce apoptosis in ARMS cells.

and form a pre initiation complex (PIC) to activate MyoD transcription in myoblasts leading to its differentiation [98]. The ectopic expression of PAX3, upregulated the expression of ZIC1, a zinc-finger transcription factor, which in turn increases FOXO3a transcriptional activity in thyroid cancer cells. The regulation of ZIC1 by PAX3 suggests that PAX3 regulates the expression and activity of FOXO3a through multiple mechanisms in thyroid cancer [99]. These studies clearly indicate that, in addition to its own tumor suppressor role, PAX3 can promote tumor suppressor function by interplaying FOXO family of transcription factors to promote the expression of tumor suppressor genes that are controlled by them.

6. Conclusion

PAX3 is a pivotal gene involved in organ development and is also known to play a very important role in development of cancer. PAX3 regulates cell differentiation, proliferation, migration, and the survival of different cell types through activating several target genes. Majority of studies on PAX3

clearly indicate a crucial role for it in the oncogenesis of several human tumors. Interestingly, the studies involving ectopic expression of PAX3 in thyroid cancers show that it can act as a tumor suppressor. Further, PAX3 in association with FOXO3a transcription factor modulates several pathways that lead to cellular differentiation and growth inhibition in cancer. These facts clearly prevent us from designating PAX3 as an oncogene, as there are results pointing in other direction (Figure 3). The possibility of PAX3 having dual functions as oncogenic and tumor suppressor based on the cell type, context, and physiologic stimuli cannot be ignored. While the oncogenic role has been well established, more studies are required that can strengthen its tumor suppressor role. Studies reporting their antiproliferative effect should not be ignored and should be considered seriously. Due to the existing discrepancies, it is important to understand the exact role of PAX3 in diseases, where PAX3 expression is altered. Future studies should focus on determining the tissues specific functions of PAX3, where it is either mutated or aberrantly expressed. This vital information is important and will be very useful in developing therapeutic strategies targeting PAX3.

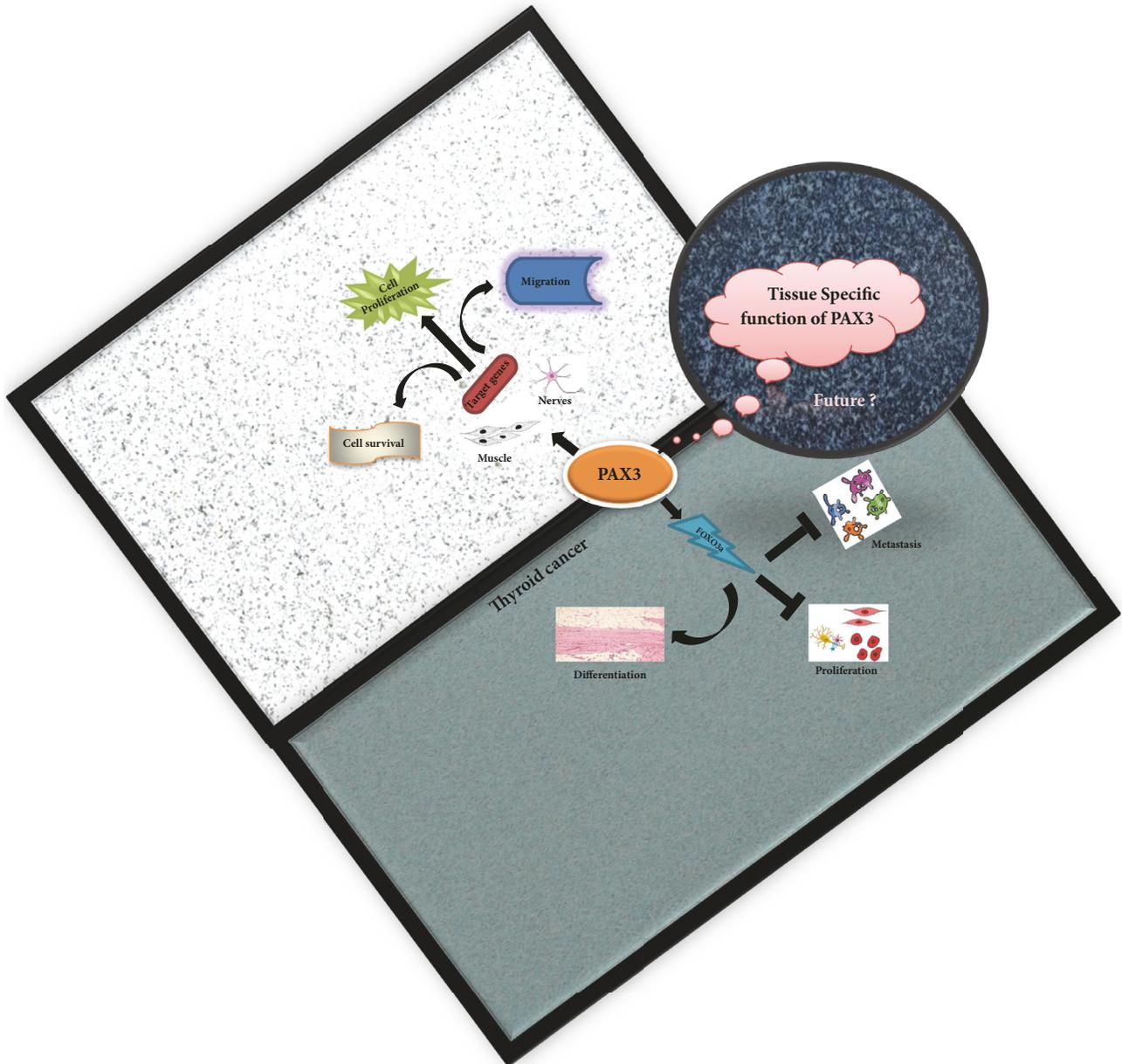


FIGURE 3: Schematic model explaining various tissue-specific cellular functions of PAX3 in normal and disease conditions. PAX3 regulates cell differentiation, proliferation, migration, and the survival of different cell types through activating several target genes. Ectopic expression of PAX3 in thyroid cancers shows that it can act as a tumor suppressor. PAX3 in association with FOXO3a leads to cellular differentiation and growth inhibition in cancer. Determining tissue-specific effects of PAX3 is necessary to understand its role in disease pathogenesis.

Abbreviations

PAX3:	Paired box gene3
FKHR (FOXO1a):	Forkhead transcription factor
MTIF:	Microphthalmia-associated transcription factor
SCF:	Stem cell factor
TGF- β :	Transforming growth factor beta 1
MUC 18:	Melanoma cell adhesion molecule
RhoC:	Ras Homolog Family Member C
TIMP3:	Tissue inhibitor of metalloproteinase 3

YB-1:	Y-box Binding Protein-1
FOXO3a:	Forkhead box O3
PI3K:	Phosphoinositide 3-kinase
Akt:	Protein Kinase B
MAPK:	Mitogen-activated protein kinase
ZIC1:	Zinc-finger transcription factor.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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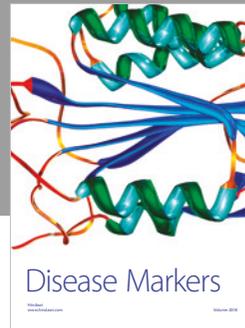
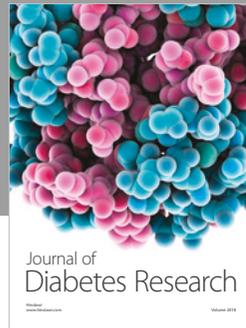
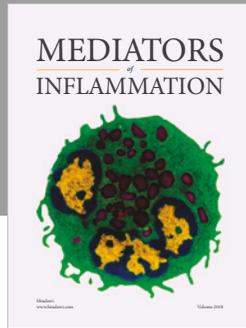
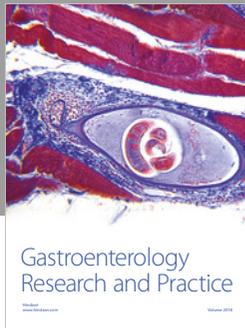
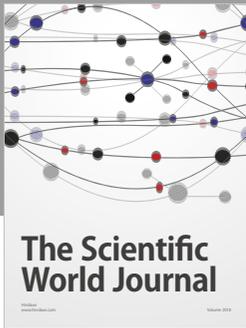
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