

PAX3

Melanoma/Cancer Association

A translocation fusing the genomic region encoding both the paired box and homeodomain of PAX3 with the region encoding the carboxyl-terminus of the transcription factor FOXO1A results in an oncogenic fusion protein (PAX3-FKHR). This translocation is seen in the majority of cases of alveolar rhabdomyosarcoma, a malignant pediatric cancer derived from developing skeletal muscle (Barr et al., 1993, Galili et al., 1993, Shapiro et al., 1993).

Summary: PAX3 is a potential diagnostic marker for melanoma, given its widespread expression in melanoma and minimal expression in normal melanocytes. As melanoma progresses, proteins that regulate melanocyte development including PAX3 may be downregulated. Reduction of PAX3 expression in melanoma cell lines results in apoptosis.

Analysis of PAX3 expression in tumor cell lines found **moderate to high expression in melanoma and Ewing's sarcoma cell lines, and low to moderate expression in embryonic rhabdomyosarcoma** (Barr et al., 1999).

PAX3 expression was seen in 77% of cultured melanomas by RT-PCR, and in situ hybridization on tissue sections showed that PAX3 was expressed specifically in tumor cells and not in surrounding normal tissue or in benign lesions. In addition, antisense RNA treatment directed at suppressing PAX3 expression resulted in significant apoptosis in primary melanoma cells (Scholl et al., 2001).

RT-PCR analysis showed **PAX3 was expressed in all melanoma lines tested**, but in only three other cancer cell lines: the neuroblastoma line SK-N-MC, the small cell lung carcinoma line H378, and adenovirus-transformed kidney cells 293 (Vachtenheim and Novotna, 1999).

In addition to all melanoma cell lines tested, PAX3 expression was seen in the majority of breast cancer, brain, and lung cancer cell lines, and occasionally in a variety of other cancer cell lines. **PAX3-targeted RNAi treatment of melanoma cell lines results in apoptosis**, suggesting PAX3 functions in promoting survival of transformed cells (Muratovska et al., 2003).

PAX3 was shown to be a useful predictor of melanoma recurrence in patients with early stage melanoma that showed histopathology-negative sentinel lymph nodes. RT-PCR analysis of sentinel lymph nodes in melanoma patients, using the melanoma markers PAX3, MART-1, MAGE-A3, and GalNAc-T, showed that positive expression of one of these 4 markers was a significant predictor of melanoma recurrence (Takeuchi et al., 2004).

Multi-marker quantitative RT-PCR on circulating peripheral blood, using the melanoma markers PAX3, MART-1, MAGE-A3, and GalNAc-T, identified the presence of melanoma cells. The number of markers expressed directly correlated with melanoma stage, suggesting that this PCR technique is useful for diagnosis of systemic melanoma progression (Koyanagi et al., 2005a).

Similarly, RT-PCR of the four markers PAX3, MART-1, MAGE-A3, GalNAc-T in melanoma patients undergoing biochemotherapy for Stage III melanoma (advanced) showed that **detection of marker expression in peripheral blood directly correlated with melanoma relapse and reduced survival**; this correlation was shown to be statistically significant (Koyanagi et al., 2005b).

Treatment of melanoma cell lines with antisense PAX3 (PAX3-AS) oligonucleotides resulted in apoptosis in both wild type and p53 mutant lines, suggesting PAX3-AS can lead to both p53-dependent and p53-independent mechanisms of cell death. PAX3-AS in combination with the chemotherapeutic drug cisplatin

caused an additive increase in melanoma cell death, suggesting PAX3 overexpression contributes to melanoma cell resistance to drug therapy and could be a useful therapeutic target ([He et al., 2005](#)).

Analysis of PAX3 expression in a variety of neuroectodermal tissues showed that **PAX3 upregulation correlates with pre-malignant or malignant tumors, and is rarely seen in relatively benign neoplasms**. Tumors showing upregulation include melanoma, malignant nerve sheath tumor, classic medulloblastoma, and supratentorial primitive neuroectodermal tumor ([Gershon et al., 2005](#)).

SAGE analysis was performed on the SKmel23 pigmented melanoma cell line, and compared to SAGE databases from testis and colon, and to the Soares 2NbHM primary melanocyte library. This **comparative analysis showed preferentially high expression of PAX3 in melanoma/melanocytes, and showed immunoreactivity of the PAX3d isoform in melanomas** ([Matsuzaki et al., 2005](#)).

An antigenic PAX3 peptide was shown to be presented by melanoma cell lines as well as other cancers. A single amino acid alteration of this peptide strongly induced naive cytotoxic T-cells to recognize cancers expressing PAX3, suggesting this peptide has potential as a cancer vaccine molecule to induce T-cell-mediated tumor regression ([Rodeberg et al., 2006](#)).

Clustering analysis of gene expression profiles of melanoma cell lines representing distinct stages of melanoma progression identified classes of genes that distinguished aggressive melanomas from non-metastatic tumors. Specifically, cluster analysis of melanoma lines relative to normal primary melanocyte lines showed that **the most aggressive melanomas shared similar expression profiles with primary melanocytes, with the notable exception of downregulation of genes that regulate melanocyte development, differentiation, and cellular adhesion. PAX3 was one of these genes with reduced expression in aggressive metastatic melanomas**, along with *c-KIT*, *TYR*, *MELANA*, *MC1R*, *EDNRB*, and *OCA2*. These results suggest that a defect in the intrinsic developmental program of melanocytes may occur in melanoma progression ([Ryu et al., 2007](#)).

PAX3 expression was noted in all melanoma cell lines tested, and significantly **more frequent PAX3 expression was seen in primary melanoma samples than in benign nevi. These PAX3-positive primary melanomas were more commonly melanomas that showed little to no evidence of solar elastosis** (chronic sun damage). This class of melanomas is often linked to *BRAF* mutations, and is more common in younger individuals ([Plummer et al., 2008](#)).

PAX3 expression was seen in 50% of nevi, primary melanomas, and metastatic melanomas. PAX3 expression was more common in acral melanomas than those located at sun-exposed or non-sun-exposed sites, and *PAX3* expression was more frequent in younger patients. In addition, knockdown of *PAX3* expression inhibited melanoma cell proliferation and induced cell cycle arrest. Overexpression of *Pax3* in mouse melanomas rescued the cell-cycle arrest that is induced by TGF-beta ([Yang et al., 2008](#)).

Multi-marker quantitative-PCR analysis of pathology-negative sentinel lymph nodes from melanoma patients found no association between marker expression and melanoma recurrence. However, **alternative PAX3 transcripts were identified whose differential expression distinguished between melanoma and negative lymph nodes**, suggesting *PAX3* mRNA isoform expression has potential use as a marker of melanoma progression to lymph nodes ([Hilari et al., 2009](#)).

Using immunohistochemistry and RT-PCR analysis, PAX3 expression was examined in normal adult melanocytes, nevi, primary melanomas, and metastatic melanomas. PAX3 is expressed in all 3 of these cell types. Normal melanocytes show PAX3 coexpression with BCL2L1 while nevi and melanomas show PAX3 coexpression with MCAM, suggesting PAX3 may play roles in normal melanocyte cell survival and melanoma migration. The highest numbers of PAX3-expressing cells were seen in nevi and primary melanomas. Metastatic melanomas showed PAX3 expression at the periphery of the cancerous lesions ([Medic and Ziman, 2010](#)).

Using immunohistochemistry, PAX3 expression was examined in normal and cancerous human tissues. **Widespread PAX3 expression was seen in normal melanocytes, nevi, and in both primary and metastatic melanomas** (He et al., 2010).

PAX3 was expressed in 35% of primary malignant melanomas, and 100% of melanoma cell lines tested. **Statistically significant coexpression of PAX3, SOX10, and MET was seen in melanoma cell lines and primary malignant melanomas. In melanoma cell lines, PAX3 was shown to directly bind and activate expression of the tyrosine kinase receptor *MET*.** While PAX3 and MITF directly bind and activate the *MET* promoter, SOX10 does not bind directly but was shown to act synergistically with PAX3 and MITF. Experiments using various siRNA constructs in the SK-MEL23 melanoma line suggest that PAX3 also regulates *MET* through a pathway that does not include MITF (Mascarenhas et al., 2010).

To identify different PAX3-regulated pathways in normal skin melanocytes and melanoma cells, **PAX3 downstream targets were compared in HEM1455 cells (human primary melanocytes) and A2058 cells (metastatic melanoma) using ChIP and quantitative RT-PCR. Melanoma-specific PAX3 targets were identified, including NES, TPD52, BCL2L1, and PTEN. In addition, PAX3 binding to target genes utilized in both HEM1455 and A2058 was stronger in A2058 (melanoma cells) as compared to HEM1455, with significantly higher PAX3 binding seen at SOX9, MCAM, and CSPG4 in A2058 cells** (Medic et al., 2011).