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Expression of the Neural Stem Cell Markers NG2 and L1 in Human Angiomyolipoma: Are Angiomyolipomas Neoplasms of Stem Cells?

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Angiomyolipomas are benign tumors of the kidney which express phenotypes of smooth muscle, fat, and melanocytes. These tumors appear with increased frequency in the autosomal dominant disorder tuberous sclerosis and are the leading cause of morbidity in adults with tuberous sclerosis. While benign, these tumors are capable of provoking life threatening hemorrhage and replacement of the kidney parenchyma, resulting in renal failure. The histogenesis of these tumors is currently unclear, although currently, we believe these tumors arise from "perivascular epithelioid cells" of which no normal counterpart has been convincingly demonstrated. Recently, stem cell precursors have been recognized that can give rise to smooth muscle and melanocytes. These precursors have been shown to express the neural stem cell marker NG2 and L1. In order to determine whether angiomyolipomas, which exhibit smooth muscle and melanocytic phenotypes, express NG2 and L1, we performed immunocytochemistry on a cell line derived from a human angiomyolipoma, and found that these cells are uniformly positive. Immunohistochemistry of human angiomyolipoma specimens revealed uniform staining of tumor cells, while renal cell carcinomas revealed positivity only of angiogenic vessels. These results support a novel histogenesis of angiomyolipoma as a defect in differentiation of stem cell precursors.

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INTRODUCTION

Tuberous sclerosis (TS) is a relatively common autosomal dominant genetic disorder due to defects in hamartin (tsc1) and/or tuberin (tsc2) (1–5). TS is notable for the development of benign neoplasms of the kidney, lungs, brain, and skin, as well as cortical tubers (2,6). Cortical tubers are giant cells that express neuronal and glial markers (7,8). While seizures remain the leading cause of morbidity and mortality in children, improvements in neurological care are leading to an increased population of adults with TS. The leading cause of morbidity and mortality in adults with TS are kidney tumors. Kidney tumors in adults with TS are overwhelmingly angiomyolipomas, a tumor which histologically expresses cells with smooth muscle, fat, and melanocytic markers (9). Previous studies of angiomyolipomas presumed that these were hamartomas, given the mixture of cell types, but more recent studies have shown that these lesions are clonal, thus representing true neoplasms (10,11). Supporting this concept, loss of heterozygosity of tsc1 or tsc2 is commonly observed in angiomyolipomas from patients with TS, and in a lower frequency in sporadic angiomyolipomas (12,13). Currently angiomyolipomas are thought to derive from perivascular epithelioid cells, a cell type of which no normal counterpart is known.

The histogenesis of angiomyolipoma is unknown, and there are no known cells in the normal adult vertebrate that consistently express both smooth muscle and melanocytic genes. However, recent studies have shown that neural crest cells can give rise to smooth muscle cells, as well as melanocytes, and that, in the mouse, multipotent stem cells can be isolated that can differentiate into either melanocytes or smooth muscle cells, depending on the culture condition. These precursor cells express the cell surface markers NG2 and L1 (14–18). In order to determine whether angiomyolipomas may represent the human equivalent of precursor cells to melanocytes/smooth

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muscle, we stained angiomyolipoma cells with NG2 and L1 antisera, and found strong expression in angiomyolipoma in vivo, but not in conventional renal cell carcinoma. These findings suggest that angiomyolipomas may arise from a disturbance in differentiation of stem cells. We also found that angiomyolipoma expresses members of the microphthalmia (MITF/TFE3/TFEB) family of transcription factors, suggesting that angiomyolipoma may share a common neural crest origin with melanoma, pediatric renal cell carcinomas, and alveolar soft part sarcoma, which also express these transcription factors.

MATERIALS AND METHODS

Immunofluorescence

Live SV7tert human angiomyolipoma cells (18,19) were immunostained using a 30 min incubation at room temperature with primary antibodies diluted in DMEM containing 2% fetal calf serum. After three washes with DMEM/FCS, cells were incubated with secondary antibodies for an additional 30 min. Following three more washes, cells were fixed for 1 min with cold 95% ethanol, air dried, and coverslipped in Immumount (Shandon, Pittsburg, PA, USA). Specimens were examined using a Nikon Optiphot microscope equipped for epifluorescence.

Samples

Fresh frozen tissue sections of two renal AMLs and one renal cell carcinoma were obtained from files at the Department of Pathology of Emory University Hospital. All samples were collected under protocols approved by the institutional review board (IRB)(Emory IRB number: 255-2002). Fresh tissues were immediately embedded in Tissue Tek OCT medium (Thermo Shandon, Pittsburgh, PA, USA), snap-frozen in liquid nitrogen, and stored at –80° C until use. Frozen sections were cut and placed on commercially-provided charged slides (Fisher Scientific, Pittsburgh, PA, USA), at 5 μm with a cryostat and immediately fixed in acetone for 10 min. After complete dehydration by air drying, samples were kept in a –20° C deep freezer until use.

Cell lines

An angiomyolipoma cell line, UMBSVtel, was established from an angiomyolipoma surgically removed from a tuberous sclerosis patient. The cells were sequentially transfected with SV40 large T antigen and then infected with telomerase as previously described (18). The SV7tert cell line was derived from a spontaneous angiomyolipoma. Sequencing for mutations was performed as previously described (19).

Antibodies

Three anti-human NG2 monoclonal antibodies were used for immunohistochemistry. The B5 hybridoma (20) was obtained from American Type Culture Collection. 9.2.27 (purified IgG; Chemicon) and N143.8 (ascites fluid (14)) have been described previously (14). The F84.1 monoclonal antibody (21) and rabbit anti-L1 antibody (RaL1/EC aff) (22) have been described previously. F84.1 is an antibody directed against DM-GRASP, an antigen found on both neural and hematopoetic stem cells (23). Table 1 indicates the summary of known characteristics of the epitopes for those antibodies. The specificity of all antibodies has been confirmed by Western blot.

Immunohistochemistry

After removal from the –20° C deep freezer, the slides were washed in TBS buffer. Then the tissue sections were incubated for 15 min with the following primary antibodies: the B5 hybridoma at the dilution of 1:16; the 9.2.27 at the dilution of 1:4,000; the N143.8 at the dilution of 1:800; the F84.1 at the dilution of 1:16, and rabbit anti-L1 antibody (RaL1/EC aff) at the dilution of 1:800. Table 1 indicates the summary of known characteristics of the epitopes for those antibodies. The specificity of all antibodies has been confirmed by the Western blot method.

After washing the unbound primary antibodies, sections were treated with commercial biotinylated secondary anti-immunoglobulin followed by avidin coupled to biotinylated horseradish peroxidase, according to manufacturer's instructions (LSAB2 kit for mouse and rabbit primary antibodies, DAKO Corp., Carpinteria, CA, USA).

Diaminobenzidine was used as the chromogenic peroxidase substrate for five minutes, and sections were counterstained with hematoxylin for 15 min after immunohistochemistry. These incubations were performed using automated immunostainer (DAKO). Specificity of the procedure was verified by negative control reactions with primary antibody replaced with buffer.

Cell pellets from SV7Tert and UMBSVtel cells were lysed in running buffer (40 mM HEPES, 100 mM KCl, 40% glycerol, 2 mM β-mercaptoethanol, 0.5% NP-40) and sonicated. 20 μg of protein sample was separated on a 10% acrylamide Bis/Tris gel and protein expression was analyzed by Western Blotting (Western Breeze, Invitrogen, Carlsbad, CA. USA) using PAX3 rabbit polyclonal antibody (J Li et al, (1999) Development 126: 2495-2503) (25).

RNA was isolated from SV7Tert cells using Trizol reagent (Sigma, St. Louis, MO, USA). After generation of cDNA (Superscript, Invitrogen) transcripts were amplified by polymerase chain reaction using primers for PAX3 (ATA GTG GAG ATG GCC CAC CA and CTC CTC AGG ATG CGG CTG AT) and GAPDH (AGT GGG TGT CGC TGT TGA AGT and TGC CAA ATA TGA TGA CAT CAA GAA).

RT-PCR for TFE3, TFEB, TFEC

Total RNA was purified from SV7tert, UMBSVtel, or control (501mel, EWS502) cell lines with TRIzol reagent (Invitrogen) as recommended by the manufacturer. Five μg RNA was reverse transcribed after oligo $(dT)_{16}$ priming with Superscript III (Invitrogen) as recommended by the manufacturer. cDNA was amplified by PCR with primers specific for TFE3 (5′-GCAGGCGATTCAACATTAACG-3′,

5′-ATAATCCACAGAGGCCTTCAG-3′), TFEB (5′-GGGAGTTGGATGATGT-CATTG-3′, 5′-GGCATCTGCATTTCA GGATTG-3), TFEC (5′-GGACAACCACA ACCTCATTGA-3′, 5′-CCAGCGCATATC AGGATCATT-3′) or GAPDH (5′-GAAG GTGAAGGTCGGAGT-3′, 5′-GAAGATG GTGATGGGATTTC-3′) mRNA using Taq DNA polymerase repeating 35 cycles of 94 °C for 30 s, 54 °C for 30 s and 72 °C for 30 s. PCR products were separated by agarose gel electrophoresis.

RESULTS

Tables 1 and 2 and Figure 1 summarized the specificities for and the results of immunostaining for these antibodies. Expression of three NG2 antibodies including B5 hybridoma, 9.2.27, and N143.8 was consistently positive in renal AML, but negative in RCC (Figure 2). Blood vessel walls were also positive for these NG2 antibodies. Expression of F84.1 antibody was positive in RCC and AML as well as blood vessels (data not shown). Expression of RaL1/EC aff, which recognizes the neural crest marker L1 was positive in AML but negative in RCC (Figure 3). Endothelial cells were faintly stained for RaL1/EC aff but vascular wall was negative (Figure 3).

Angiomyolipoma cells did not express the pre-migratory neural crest gene pax3 at the level of RNA (data not shown). This is consistent with angiomyolipoma being a postmigratory phenotype. Angiomyolipoma cells express high levels of TFE3 RNA (Figure 4), but angiomyolipomas do not express TFE3 by immunohistochemistry, consistent with a translational block (data not shown). Both SV7tert and UMBSVtel cells stained positively for NG2. We found a deletion mutation in UMBSVtel cells "4083-4087 del AGTCG" in exon 33 of the TSC2 gene. This cell line represents the first cell line derived from an angiomyolipoma cell with a defined mutation in tuberin, making it valuable for studies of tuberous sclerosis. The pattern of TFE family RNA expression in UMBSVtel cells is nearly identical to that of SV7tert (data not shown).

Table 1. Antibodies to Neural Crest Antigens

Antibody	Source	Type	Dilution
B5	American Type Culture Collection	monoclonal	1:16
9.2.27	Chemicon	monoclonal	1:4,000
N143.8	Stallcup	monoclonal	1:800
F84.1	Stallcup	monoclonal	1:16
RaL1/EC aff	Stallcup	polyclonal	1:800

Table 2. Summary of staining results

Antibody	AML	RCC	BVW
B5	positive	negative	positive
9.2.27	positive	negative	positive
N143.8	positive	negative	positive
F84.1	positive	positive	positive
RaL1/EC aff	positive	negative	negative

BVW, blood vessel wall; EC, endothelial cells.

DISCUSSION

Angiomyolipomas are tumors that show histologic evidence of smooth muscle, fat, and melanocytic markers. While these tumors are histologically benign, they are the major cause of morbidity and mortality in adults with tuberous sclerosis. Angiomyolipomas are notoriously susceptible to rapid hemorrhage, and multiple angiomyolipomas may obliterate the renal parenchyma, causing renal failure, and the need for renal transplantation. Currently, the only treatments for angiomyolipomas are excision or embolization.

Most of the neoplasms and hamartomas of tuberous sclerosis exhibit multilineage phenotypes. Cortical tubers of

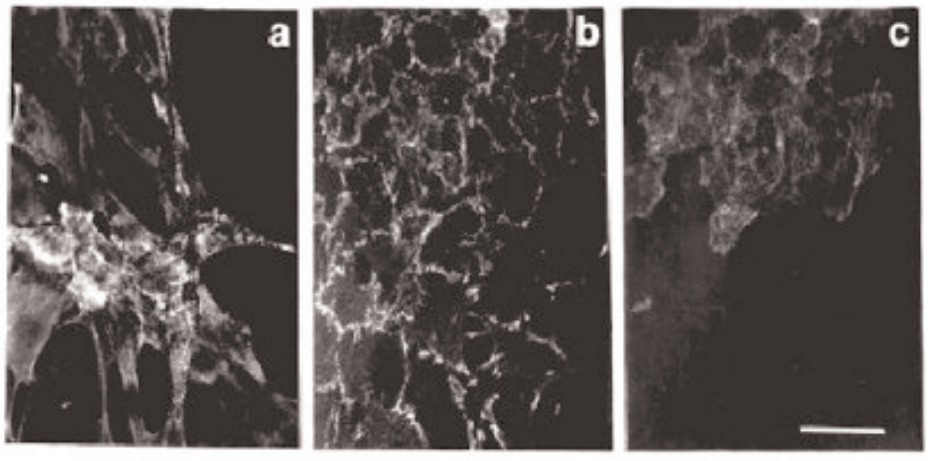

Figure 1. Immunocytochemistry for NG2 and L1 on SV7tert angiomyolipoma cells. A) Living SV7tert cells were labeled with B5 mouse monoclonal antibody against human NG2. All cells were found to be NG2-positive. (B,C). Living SV7tert cells were double-stained for F84.1 (B) and L1 (C). All cells expressed F84.1, a cell adhesion molecule that is widespread during embryonic development. However, only a subpopulation of cells is positive for the L1 cell adhesion molecule, which is expressed by neural crest. Bar in C = 20 μm.

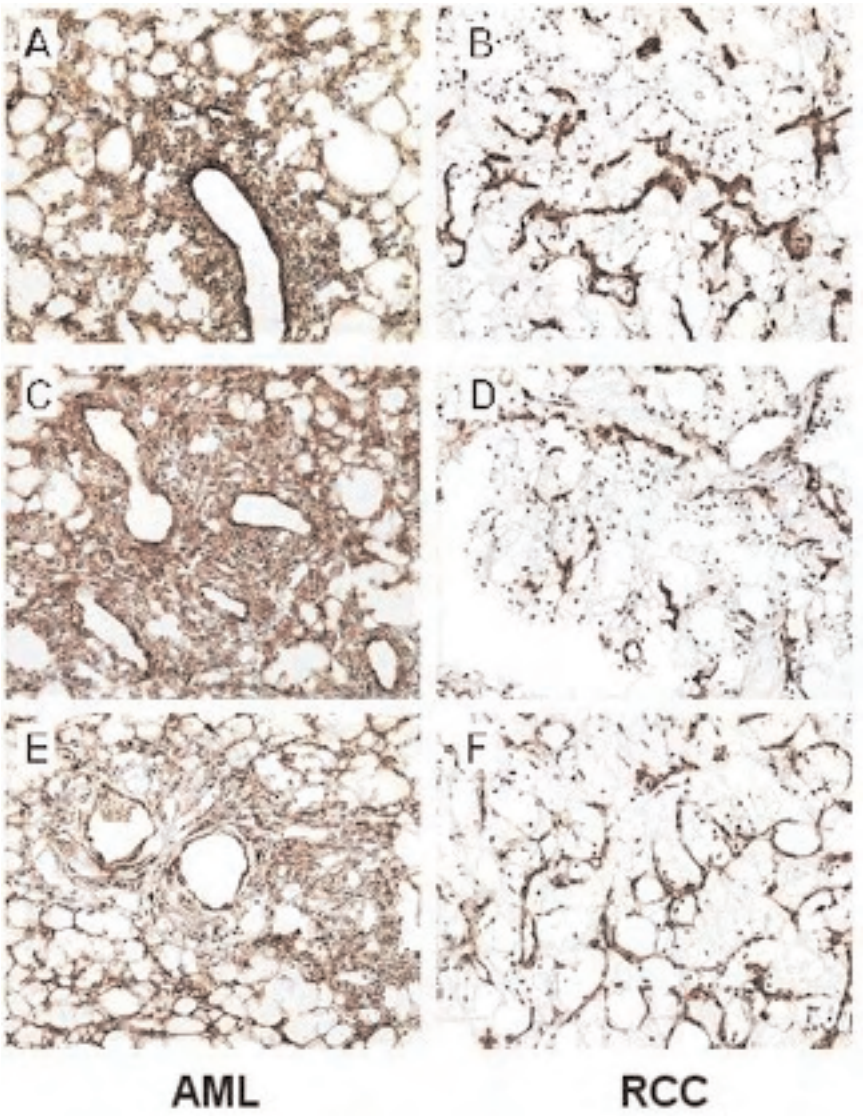

Figure 2. Renal angiomyolipomas, but not renal cell carcinomas, express NG2 in vivo. Renal AML with thick walled blood vessel associated with typical component of smooth muscle cells and normal appearing fat cell was positive for B5 (Figure 2A), 9.2.27 (Figure 2C), and N143.8 (Figure 2E), but RCC tumor cells were negative for those antibodies (Figure 2B, 2D, and 2F). Blood vessels were positive for these antibodies.

tuberous sclerosis, the major cause of seizures in affected children, exhibit a combined neuronal-glial phenotype. Collagenomas exhibit a mixed population of fat, smooth muscle, and collagen deposition. Lymphangiomyomatosis, which may cause respiratory failure, exhibits a similar phenotype of smooth muscle and melanocytic lesions to angiomyolipoma. This clinical finding of multilineage phenotypes suggests that the signaling aberrations in tuberous sclerosis result from a perturbation of stem cell differentiation.

Smooth muscle cells arise from endoderm and neural crest. Specifically, a common neural crest precursor can differentiate into glia, smooth muscle, pericytes, cartilage, myofibroblast, and neurons. Platelet derived growth factor α (PDGFα) and pax family transcription factors are required for initial proliferation of neural crest stem cells, which migrate to peripheral tissues. Pax genes are expressed in neural crest prior to migration to the periphery, and the lack of pax3 expression in SV7tert angiomyolipoma cells is consistent with the known patterns of pax expression (24). Maintenance and differentiation of these stem cell derivatives occurs through locally based trophic factors, such as platelet-derived growth factor α (PDGFα). Angiogenic vascular endothelium produces abundant PDGF-BB, and deficiency of PDGF-BB in knockout mice results in severe kidney defects due to the presence of angiogenic endothelium not being invested by smooth muscle and pericytes (19,25). Of interest, angiomyolipoma cells express high levels of PDGF-BB, and proliferation of angiomyolipoma cells in vitro is inhibited by PDGFRb tyrosine kinase antagonists, including glivec. Activated (phosphorylated) PDGFRb is expressed in angiomyolipoma tumor tissue, indicating pathophysiologic activation of PDGF-BB/PDGFRβ in vivo (26).

Neural crest contributes to the development of the embryonic kidney. Neural crest cells expressing the L1 neural cell adhesion protein are present in the embryonic metanephric kidney rudiment, and their proliferation is enhanced by neurotropin 3 (NT-3) (27,28). Of interest, a population of SV7tert cells and angiomyolipoma tumors express L1 in addition to NG2, supporting a neural crest origin for angiomyolipoma cells. NG2 also may play a role in maintenance of these tumors, as NG2 has been shown to sequester and inactivate angiostatin, thus permitting pathologic angiogenesis (29).

Angiomyolipomas are the most common kidney neoplasms that express melanocytic markers. In melanocytes, MITF is the major transcriptional switch for the distinct metabolic pathways for melanin formation, but MITF appears to have antiapoptotic and oncogenic functions independent of this. MITF shares biochemical similarity to the TFE3/TFEB transcription factors, and this extends to protection against apoptosis and oncoge-

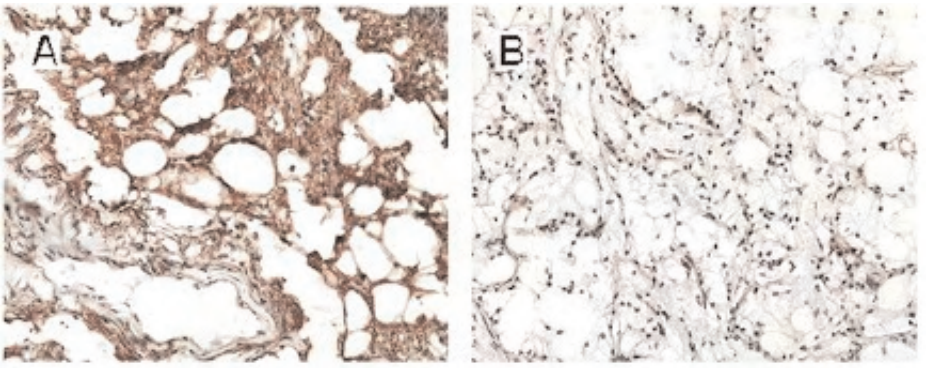

Figure 3. Renal angiomyolipomas, but not renal cell carcinomas, express L1 in vivo. RaL1/EC aff was positive in renal AML (Figure 3A) and negative in RCC (Figure 3B). Endothelial cells were faintly positive for RaL1/EC aff, but vascular wall was negative.

nesis (30,31). Previous mechanisms for activation of these transcription factors include translocation, which occurs in a subset of pediatric renal cell carcinomas and alveolar soft part sarcomas (19,32). In addition, these factors are expressed in Ewing's sarcoma through transcriptional activation. Our data suggests that angiomyolipoma, which is far more common than pediatric renal cell carcinoma, alveolar soft part sarcoma, and Ewing's sarcoma, may arise from a similar neural crest precursor. Angiomyolipoma cells are sensitive to blockade of PDGFRβ, and pharmacologic blockade of PDGFRβ activation with drugs such as glivec, may be useful in the treatment of angiomyolipoma (36). In addition, angiogenesis inhibition may serve to deprive angiomyolipoma cells of trophic PDGF-BB production, and avastin, a VEGF trap, has been shown to be efficacious in renal cell carcinoma. Finally, NG2 binding peptides have been made which target tumor vessels (34). The presence of NG2 on angiomyolipoma cells may enhance tumor killing by peptides which bind NG2. Thus, an understanding of the embryonic basis of angiomyolipoma may be beneficial in therapy of this common tumor.

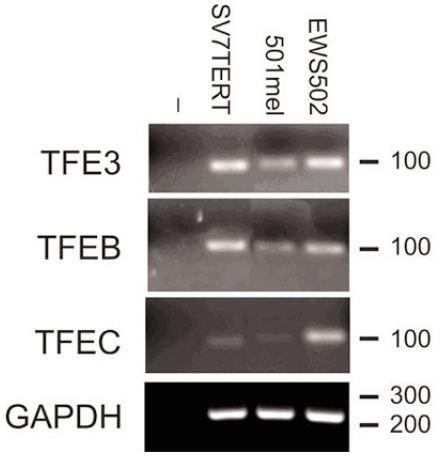

Figure 4. Expression of TFE transcription factors in angiomyolipoma cells by RT-PCR. Lane 1 represents RNA from SV7tert cells, which lane two represents RNA from the 501mel melanoma cell line, and lane three represents RNA from the EWS502 Ewing sarcoma cell lines.

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REFERENCES

1. Oesterling JE, Fishman EK, Goldman SM, Marshall FF. (1986) The management of renal angiomyolipoma. *J. Urol*. 135:1121–4.
2. Shepherd CW, Gomez MR, Lie JT, Crowson CS. (1991) Causes of death in patients with tuberous sclerosis. *Mayo Clin. Proc*. 66:792–6.
3. The European Chromosome 16 Tuberous Sclerosis Consortium. (1993) Identification and characterization of the tuberous sclerosis gene on chromosome 16. *Cell*. 75:1305–15.
4. Sampson JR, Harris PC. (1994) The molecular genetics of tuberous sclerosis. *Hum. Mol. Genet*. 3 Spec No:1477–80.
5. Plank TL, Yeung RS, Henske EP. (1998) Hamartin, the product of the tuberous sclerosis 1 (TSC1) gene, interacts with tuberin and appears to be localized to cytoplasmic vesicles. *Cancer Res*. 58:4766–70.
6. Henske EP et al. (1997) Loss of tuberin in both subependymal giant cell astrocytomas and angiomyolipomas supports a two-hit model for the pathogenesis of tuberous sclerosis tumors. *Am. J. Pathol*. 151:1639–47.
7. Kyin R et al. (2001) Differential cellular expression of neurotrophins in cortical tubers of the tuberous sclerosis complex. *Am. J. Pathol*. 159:1541–54.
8. Maldonado M et al. (2003) Expression of ICAM-1, TNF-alpha, NF kappa B, and MAP kinase in tubers of the tuberous sclerosis complex. *Neurobiol. Dis*. 14:279–90.
9. Eble JN. (1998) Angiomyolipoma of kidney. *Semin. Diagn. Pathol*. 15:21–40.
10. Kattar MM et al. (1999) Chromosomal analysis of renal angiomyolipoma by comparative genomic hybridization: evidence for clonal origin. *Hum. Pathol*. 30:295–9.
11. Smolarek TA et al. (1998) Evidence that lymphangiomyomatosis is caused by TSC2 mutations: chromosome 16p13 loss of heterozygosity in angiomyolipomas and lymph nodes from women with lymphangiomyomatosis. *Am. J. Hum. Genet*. 62:810–5.
12. Henske EP et al. (1996) Allelic loss is frequent in tuberous sclerosis kidney lesions but rare in brain lesions. *Am. J. Hum. Genet*. 59:400–6.
13. Stallcup WB. (1981) The Ng2 antigen, a putative lineage marker: immunofluorescent localization in primary cultures of rat-brain. *Dev. Biol*. 83:154–65.
14. Nishiyama A, Lin XH, Giese N, Heldin CH, Stallcup WB. (1996) Co-localization of NG2 proteoglycan and PDGF alpha-receptor on O2A progenitor cells in the developing rat brain. *J. Neurosci. Res*. 43:299–314.
15. Ozerdem U, Grako KA, Dahlin-Huppe K, Monosov E, Stallcup WB. (2001) NG2 proteoglycan is expressed exclusively by mural cells during vascular morphogenesis. *Dev. Dyn*. 222:218–27.
16. Grako KA, Ochiya T, Barritt D, Nishiyama A, Stallcup WB. (1999) PDGF alpha-receptor is unresponsive to PDGF-AA in aortic smooth muscle cells from the NG2 knockout mouse. *J. Cell Sci*. 112:905–15.
17. Chekenya M, Hjelstuen M, Enger PO, Thorsen F, Jacob AL, Probst B, Haraldseth O, Pilkington G, Butt A, Levine JM, Bjerkvig R. (2002) The NG2 proteoglycan promotes angiogenesis-dependent tumor growth in CNS by sequestering angiostatin. *FASEB J*. 2002, 6:586-588.
18. Arbiser JL et al. (2001) The generation and char-

acterization of a cell line derived from a sporadic renal angiomyolipoma: use of telomerase to obtain stable populations of cells from benign neoplasms. *Am. J. Pathol.* 159:483–91.
19. Arbiser JL et al. (2002) Functional tyrosine kinase inhibitor profiling: a generally applicable method points to a novel role of platelet-derived growth factor receptor-beta in tuberous sclerosis. *Am. J. Pathol* 161:781–6.
20. Houghton AN, Eisinger M, Albino AP, Cairncross JG, Old LJ. (1982) Surface antigens of melanocytes and melanomas. Markers of melanocyte differentiation and melanoma subsets. *J Exp Med*. 156(6):1755-1766.
21. Prince JT, Nishiyama A, Healy PA, Beasley L, Stallcup WB. (1992) Expression of the F84.1 glycoprotein in the spinal cord and cranial nerves of the developing rat. *Brain. Res. Dev. Brain Res.* 68:193–201.
22. Nayeem N et al. (1999) A potential role for the plasmin(ogen) system in the posttranslational cleavage of the neural cell adhesion molecule L1. *J. Cell Sci*. 112:4739–49.
23. Burns FR, Vonkannen S, Guy L, Raper JA, Kamholz J, Chang S. (1991) DM-GRASP, a novel immunoglobulin superfamily axonal surface protein that supports neurite extension. *Neuron*. 7:209–20.
24. Lang D, Chen F, Milewski R, Li J, Lu MM, Epstein JA. (2000) Pax3 is required for enteric ganglia formation and functions with Sox10 to modulate expression of c-ret. *J. Clin. Invest.* 106:963–71.
25. Li J, Jin F, Lu MM, Epstein JA. (1999) Transgenic Rescue of Congenital Heart Disease and Spina Bifida in Splotch Mice. *Development*, 1999, 126:2495-503.
26. Leveen P, Pekny M, Gebre-Medhin S, Swolin B, Larsson E, Betsholtz C. (1994) Mice deficient for PDGF B show renal, cardiovascular, and hematological abnormalities. *Genes Dev*. 8:1875–87.
27. Soriano P. (1994) Abnormal kidney development and hematological disorders in PDGF beta- receptor mutant mice. *Genes Dev*. 8:1888–96.
28. Sariola H et al. (1991) Dependence of Kidney Morphogenesis on the Expression of Nerve Growth-Factor Receptor. *Science*. 254:571–3.
29. Karavanov A, Sainio K, Palgi J, Saarma M, Saxen L, Sariola H. (1995) Neurotrophin-3 rescues neuronal precursors from apoptosis and promotes neuronal differentiation in the embryonic metanephric kidney. *Proc. Natl Acad Sci U. S. A*. 92:11279–83.
30. Goretzki L, Lombardo CR, Stallcup WB. (2000) Binding of the NG2 proteoglycan to kringle domains modulates the functional properties of angiostatin and plasmin(ogen). *J. Biol. Chem.* 275:28625–33.
31. Burg MA, Pasqualini R, Arap W, Ruoslahti E, Stallcup WB. (1999) NG2 proteoglycan-binding peptides target tumor neovasculature. *Cancer Res*. 59:2869–74.
32. Garraway LA et al. (2005) Integrative genomic analyses identify MITF as a lineage survival oncogene amplified in malignant melanoma. *Nature*. 436:117– 22.
33. Nijman SM, Hijmans EM, El MS, van Dongen MM, Sardet C, Bernards R. (2006) A functional genetic screen identifies TFE3 as a gene that confers resistance to the anti-proliferative effects of the retinoblastoma protein and transforming growth factor-beta. *J. Biol. Chem*. 281:21582–7.
34. Ladanyi M et al. (2001) The der(17)t(X;17)(p11;q25) of human alveolar soft part sarcoma fuses the TFE3 transcription factor gene to ASPL, a novel gene at 17q25. *Oncogene*. 20:48–57.
35. Weterman MA, Wilbrink M, Geurts van KA. (1996) Fusion of the transcription factor TFE3 gene to a novel gene, PRCC, in t(X;1)(p11;q21)-positive papillary renal cell carcinomas. *Proc. Natl. Acad. Sci. U. S. A*. 93:15294–8.
36. Burg MA, Pasqualini R, Arap W, Ruoslahti E, Stallcup WB. (1999(NG2 proteoglycan-binding peptides target tumor neovasculature. *Cancer Res*. 59:2869–74.