Virchows Arch (2008) 452:27–32
DOI 10.1007/s00428-007-0524-2

ORIGINAL ARTICLE

C-kit protein expression correlated with activating mutations in KIT gene in oral mucosal melanoma

Rosario S. Rivera · Hitoshi Nagatsuka · Mehmet Gunduz · Beyhan Cengiz · Esra Gunduz · Chong Huat Siar · Hidetsugu Tsujigiwa · Ryo Tamamura · Kok Ng Han · Noriyuki Nagai

Received: 27 June 2007 / Revised: 27 August 2007 / Accepted: 4 October 2007 / Published online: 8 December 2007

Abstract C-kit is a trans-membrane receptor tyrosine kinase (RTK) encoded by the proto-oncogene KIT located at 4q11-12. Gain-of-function mutations arising to c-kit activation independent of its ligand were observed in various tumors related to germ cells, mast cells, and interstitial cells of Cajal. C-kit also participates in melanocyte development; hence, its involvement in oral mucosal melanoma (OMM) tumorigenesis was investigated. Immunohistochemistry and mutation analysis were performed using 18 cases of human primary OMM. Results revealed 16 cases positive to c-kit protein. Atypical melanocytes expressed c-kit. All in situ components expressed c-kit, but only four cases exhibited intense expression in the invasive component. Missense mutations were observed in four cases, and two of those correlated with increased protein expression. C-kit expression in atypical melanocytes suggests the role of c-kit in the early stage of OMM tumorigenesis. C-kit protein expression correlated with activating mutations indicating the pertinent role of the proto-oncogene KIT in the tumorigenesis of OMM.

Keywords Oral mucosal melanoma · C-kit mutation · Immunohistochemistry

R. S. Rivera · H. Nagatsuka (✉) · M. Gunduz · B. Cengiz · E. Gunduz · H. Tsujigiwa · R. Tamamura · N. Nagai
Department of Oral Pathology and Medicine,
Graduate School of Medicine,
Dentistry and Pharmaceutical Sciences, Okayama University,
2-5-1 Shikata-cho,
Okayama 700-8525, Japan
e-mail: jin@md.okayama-u.ac.jp

R. S. Rivera
College of Dentistry, University of the East,
Manila, Philippines

C. H. Siar
Department of Oral Pathology,
Oral Medicine and Periodontology, Faculty of Dentistry,
University of Malaya,
Kuala Lampur, Malaysia

K. N. Han
Unit of Stomatology, Cancer Research Centre,
Institute for Medical Research,
Kuala Lumpur, Malaysia

Introduction

Oral mucosal melanoma (OMM) is a rare malignant soft tissue neoplasm, which comprises 0.5% of oral malignancies [5, 13]. The mechanism involved in its progression and aggressiveness is not clearly understood. Because of the slow progress in the treatment of melanoma, significant advances may require new insights into melanoma biology. Molecular analysis may give better perception and selection of therapy, targeting signaling molecules responsible for OMM progression.

Melanocytes are specialized melanin-producing cells that originate from the neural crest and migrate to the skin, hair follicle, and retina. Melanocytes can also be found in the oral mucosa, but their existence and function are unknown [13]. C-kit is one of the receptor tyrosine kinases (RTK) that influences proliferation, migration, and survival of melanocytes. C-kit, a protein encoded by the proto-oncogene KIT, is activated by its ligand, stem cell factor, during melanogenesis [1]. However, in tumors, c-kit activation independent of its ligand through oncogenic mutations enables the receptor to phosphorylate various substrate proteins. This will then lead to activation of signal transduction cascades that regulate cell proliferation, apoptosis, chemotaxis, and adhesion [21, 23].

Gain-of-function mutations in c-kit were identified in various solid tumors particularly in gastrointestinal stromal tumor (GIST) [4, 7, 9, 10, 14, 15, 18, 19, 23, 26, 32, 37, 38]. The in-frame mutations vary from single base pair substitutions to complex deletions and insertions [7, 38]. The identification of c-kit mutation led to further studies on molecular therapy of GIST and other tumors with imatinib mesylate (STI-571, Gleevec), a specific competitive inhibitor of c-kit [39]. C-kit mutations most commonly involve the juxta-membrane domain (JMD) encoded by exon 11, although mutations in other domains were also found [8, 21, 26, 38].

C-kit is an important receptor for melanocyte growth and maturation and its expression might be inferred in melanoma [19]. Likewise, the detection of c-kit activating mutation in OMM may give an insight to the value of specific RTK inhibitor in a subset of melanoma. Although several studies involving c-kit and melanoma have been reported, there has been no research done on OMM. Thus, we investigated the protein expression and activating mutations in c-kit exons 11 and 13. C-kit protein expression correlated with activating mutations, which might be in part responsible for OMM tumorigenesis.

Materials and methods

Paraffin-embedded tissue samples

Hematoxylin and eosin (HE) sections from 18 cases of formalin-fixed, paraffin-embedded primary OMM were retrospectively analyzed. The cases were histologically classified according to the Western Society of Teachers in Oral Pathology classification [5, 28].

Immunohistochemistry

New sections of tissue samples were cut for immunohistochemistry (IHC). Deparaffinized tissue sections were blocked for endogenous peroxidase activity using 3% hydrogen peroxide in methanol for 30 min, washed with Tris-buffered saline solution, and treated for antigen retrieval in citrate buffer, pH 6.0 (heat pressure for 5 min at 121°C). The slides were covered with serum-free protein block (DakoCytomation, Carpinteria, USA) for 15 min, followed by C-kit antibody (DakoCytomation) with a dilution of 1:100 and incubated overnight at 4°C. Immunoreaction was carried out with Envision™ Detection Reagent Peroxidase (DakoCytomation) and detected with 3-amino-ethylcarbazole substrate chromogen (DakoCytomation). Immunoreaction was defined as negative (−) when no immunoreaction was observed and focal (±) when less than 20% melanoma cells were positive and if the degree of staining was weak. When the degree of staining was strong, it was regarded as moderate (+) when 20–50% melanoma cells were positive and intense (++) when more than 50% melanoma cells were positive. Immunoreactions for in situ and invasive components were recorded separately.

Mutation analysis

Genomic deoxyribonucleic acid (DNA) was extracted from formalin-fixed paraffin blocks with Dexpat Kit (Takara Bio, Shiga, Japan) following the manufacturer's procedure. Although hot spots for c-kit include exons 9, 11, 13, and 17, the study focused on exons 11 and 13 because most mutations in GIST were found in these exons. Exons 11 and 13 were amplified by polymerase chain reaction (PCR) using primers shown in Table 1. PCR was performed in 20-μl reactions containing 2 μl reaction buffer, 1.6 μl dexoyribonucleotide triphosphate, 0.5 μl of primer, and 0.1 μl r*Taq* (Takara Bio) and diluted to volume with sterile water. PCR-cycling conditions were as follows: initial denaturation at 94°C for 3 min, 45 cycles of denaturation at 94°C for 30 s, annealing at 58 (exon 11) and 62°C (exon 13) for 1 min, and extension at 72°C for 1 min. A final extension at 72°C for 7 min was also performed. PCR products were purified with Geneclean III Kit, and others were treated with ExoSAP-IT (USB, Ohio, USA) before sequence-specific PCR. For confirmation purposes, new sets of PCR products were subjected to single-strand confirmation polymorphism (SSCP); aberrant bands were cut and subjected to PCR followed by ExoSAP-IT treatment and sequence-specific PCR.

Direct sequencing was performed using Big Dye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, CA, USA). Sequence reactions were purified with ethanol and ethylenediamine tetraacetic acid precipitation and analyzed on a 3130xl ABI Prism Genetic Analyzer (Applied Biosystems, Hitachi, Japan).

Results

Immunohistochemistry

Figure 1a (in situ) and c (invasive) are representative HE sections of OMM. The expressions observed were mem-

Table 1 Primer sequences for C-kit

Exon	Sequence	
Exon 11	Forward	5′-CTCTCCAGAGTGCTCAATGAC-3′
	Reverse	5′-AGCCCCTGTTTCATACTGACC-3′
Exon 13	Forward	5′-CGGCCATGACTGTCGCTGTAA-3′
	Reverse	5′-CTCCAATGGTGCAGGCTCCAA-3′

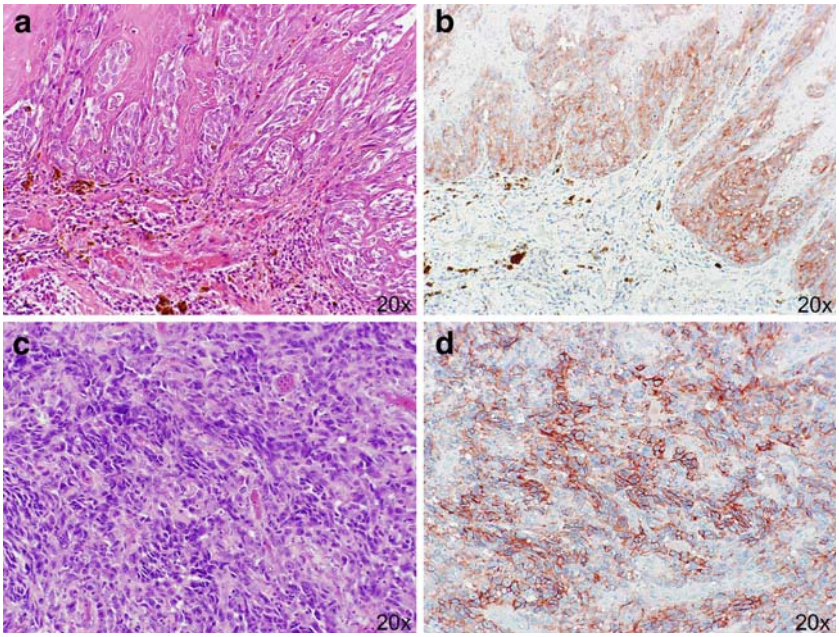

Fig. 1 C-kit expression in OMM. HE sections of the in situ component (**a**) and invasive components (**c**). This figure shows intense expression of c-kit protein in the in situ component (**b**) and invasive component (**d**)

branous and cytoplasmic. C-kit expression was detected in atypical melanocytes. Furthermore, c-kit expression was detected in all in situ components (Fig. 1b). Only one case exhibited focal staining, and the rest were moderate to intense. In invasive components, c-kit expression was observed in 16 out of 18 cases. C-kit expression varied from focal to intense, and only four cases were intensely positive (Fig. 1d).

Mutation analysis

All cases were subjected to mutation analysis including the two IHC-negative cases. Many aberrant bands were observed in SSCP gels for exons 11 and 13 (Fig. 2). When these bands were sequenced, few missense and silent mutations were obtained. Missense mutations were detected in 4 out of 15 cases with DNA amplification (27%), two mutations in exon 11 and two in exon 13. Mutations W557R and K642E have been reported in GIST [4, 9, 14, 15, 18, 19, 23, 26, 37, 38], but K642E was also reported specifically in mucosal melanoma [8]. Our literature search did not reveal any report on the mutation V569G (Fig. 3).

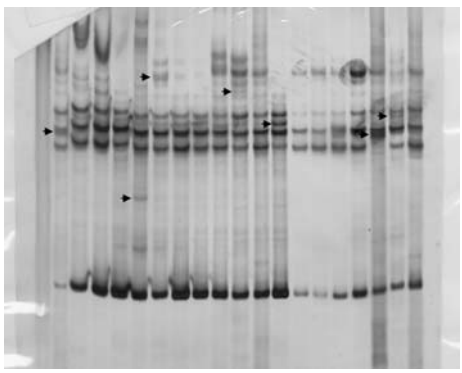

Fig. 2 Single strand confirmation polymorphism. Aberrant bands from PCR products with exon 13 were evident (*arrows*)

Results in Table 2 show that in the four cases with mutations, two showed intense c-kit protein expression (cases 6 and 15). Case 6 exhibited several recurrences, and case 15 was of the amelanotic type. Moreover, two cases with mutations exhibited an increase in protein expression from in situ phase to invasive phase (cases 6 and 9).

Discussion

OMM has a poor prognosis with a 5–20% survival rate [33]. The poor prognosis of OMM has caused some deliberations as to whether OMM has a more aggressive behavior than its cutaneous counterpart or was it due to the delay in diagnosis. Surgical excision, radiation therapy, and targeting specific molecules were employed in the management of melanoma [12, 22]. However, analysis of the molecular biology of the tumor is one important leap toward understanding the nature of the neoplasm and for the provision of ample management.

C-kit expression has been detected mostly in the in situ components of melanoma [27, 29, 30, 35]. The expression of c-kit in basally growing acral lentiginous melanoma cells suggested that the cells expressing the protein were essential in the initial stage of tumor progression [30]. Atypical melanocytic proliferation may indicate early

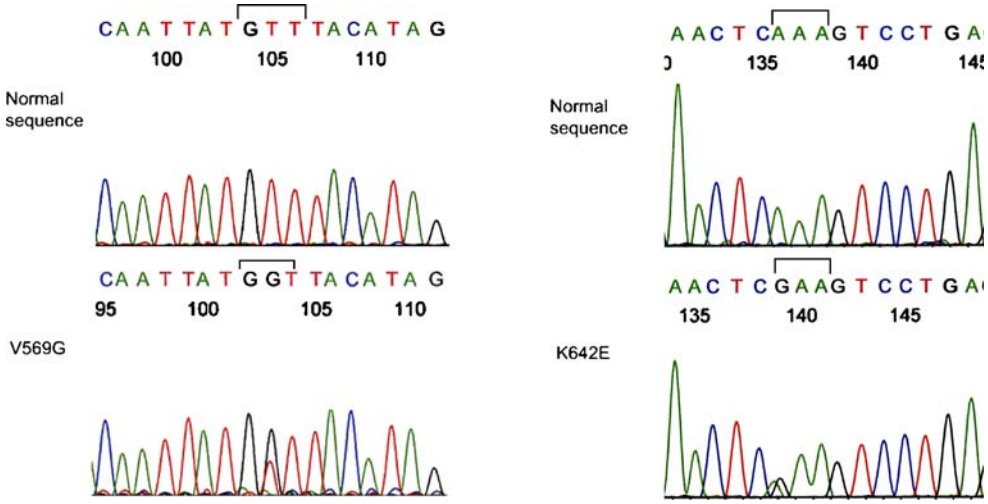

Fig. 3 Direct sequence. The figure shows normal sequence and missense mutations in exon 11 (*V569G*) and exon 13 (*K642E*)

progression of OMM [13, 28]. Therefore, c-kit expression in atypical melanocytes indicates the role of c-kit in the early stage of OMM tumorigenesis.

C-kit mutation in skin melanoma was rarely observed, but several mutations have been found in acral and mucosal melanoma with protein overexpression and increased DNA copy [8]. Although two from our cases failed to express c-kit protein, all cases were subjected to mutation analysis. It is interesting to note that one mutation (W557R) was found in a negative case. The significance of weak and focal c-kit staining is not clear [39]. The negative case might have probably expressed the receptor at a level below the detection limit of IHC assay. Thus, c-kit mutation may also be present, although the immunohistochemical assay gave a negative response.

Table 2 Summary of immunohistochemistry results and mutation analysis

Case	Histological type	IHC		Mutation analysis
		In situ	Invasive	
1	Invasive with in situ	++	+	Wild type
2	Invasive with in situ	+	±	Wild type
3[a]	Invasive	NA	±	Wild type
4	Invasive	NA	±	Wild type
5[a]	Invasive with in situ	++	++	Wild type
6[b]	Invasive with in situ	+	++	K642E[c], P585P
7	Invasive with in situ	+	±	Wild type
8	Invasive	NA	+	Wild type
9	Invasive with in situ	±	+	V569G
10	Invasive	NA	−	W557R[c]
11	Invasive	NA	−	Insufficient DNA amount
12	Invasive with in situ	++	+	Wild type
13	Invasive	NA	±	Insufficient DNA amount
14	Invasive	NA	++	Insufficient DNA amount
15[a]	Invasive	NA	++	K642E[c], S645S
16	Invasive	NA	±	Wild type
17	Invasive with in situ	+	±	Wild type
18	Invasive with in situ	+	+	Wild type

[a] Amelanotic type
[b] With several recurrences
[c] Reported in other tumors

Although a decrease in c-kit protein from in situ to invasive phase was observed, an increased expression was also detected in two cases (cases 6 and 9). Incidentally, these cases conformed to the mutation analysis suggesting the correlation between increased c-kit protein expression and the presence of activating mutation. Furthermore, case 6 exhibited several recurrences suggesting that c-kit protein overexpression and gene mutation may contribute to the high recurrence rate. Moreover, amelanotic melanoma has been associated with a more aggressive type and poor prognosis. Although only three samples from our cases were amelanotic, incidentally, one case (case 15) had intense protein expression and activating mutation. This result suggests that c-kit has a role in the aggressiveness of OMM.

Therapies targeting tyrosine kinase activities have been promising in cases of melanoma expressing the c-kit protein [11, 16, 20]. However, lack of response to imatinib has also been reported in melanoma [2, 6, 36, 40]. Phase II studies with imatinib until know failed to show efficacy in melanoma patients [6]. Overexpression of the c-kit protein has been previously observed without the occurrence of mutation, so it is possible that the driving force toward tumorigenesis is not solely related to mutation [3, 31]. Furthermore, it may also be speculated that a specific RTK inhibitor may only be of value in subtypes of melanoma [8]. Although it is unclear whether the presence of mutation or protein overexpression might be of significance for tumor behavior, the use of RTK inhibitors may have a significant outcome in melanoma subtypes with increased protein expression or activating mutation [34]. In addition,

(vascular endothelial growth factor) VEGF expression might be regulated by a c-kit signal transduction cascade. Several RTK inhibitors have antitumor effects by blocking c-kit activation as well as suppressing VEGF transcription and translation [17, 24, 25]. Because VEGF is highly expressed by OMM cells (unpublished data), the use of specific RTK inhibitor may block c-kit activation as well as suppress angiogenesis.

In summary, c-kit expression suggests a role in the early stage of OMM tumorigenesis. Increased c-kit protein expression correlated with activating mutations suggesting pertinent role of the proto-oncogene KIT in the tumorigenesis of OMM. Further studies may be carried out to elucidate the molecular mechanisms that underlie the correlation of activating mutation and protein expression with clinical outcome.

Acknowledgments This work was supported in part by grants-in-aid for scientific research from the Ministry of Education, Culture, Sports, Science and Technology (B17406027, C19592109). The authors would also like to thank Ms. Kazuko Funakoshi for her technical expertise. The experiments complied with the current laws of Japan.

References

1. Alexeev V, Yoon K (2006) Distinctive role of the cKit receptor tyrosine kinase signaling in mammalian melanocytes. J Invest Dermatol 126:1102–1110
2. Alexis JB, Martinez AE, Lutzky J (2005) An immunohistochemical evaluation of c-kit (CD-117) expression in malignant melanoma, and results of imatinib mesylate (Gleevec) therapy in three patients. Melanoma Res 15:283–285
3. All-Ericsson C, Girnita L, Muller-Brunotte A, Brodin B, Seregard S, Ostman A, Larsson O (2004) c-Kit-dependent growth of uveal melanoma cells: a potential therapeutic target? Invest Ophthalmol Vis Sci 45:2075–2082
4. Andersson J, Bumming P, Meis-Kindblom JM, Sihto H, Nupponen N, Joensuu H, Oden A, Gustavsson B, Kindblom LG, Nilsson B (2006) Gastrointestinal stromal tumors with KIT exon 11 deletions are associated with poor prognosis. Gastroenterology 130:1573–1581
5. Barker BF, Carpenter WM, Daniels TE, Kahn MA, Leider AS, Lozada-Nur F, Lynch DP, Melrose R, Merrell P, Morton T, Peters E, Regezi JA, Richards SD, Rick GM, Rohrer MD, Slater L, Stewart JC, Tomich CE, Vickers RA, Wood NK, Young SK (1997) Oral mucosal melanomas: the WESTOP Banff workshop proceedings. Western Society of Teachers of Oral Pathology. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 83:672–679
6. Becker JC, Brocker EB, Schadendorf D, Ugurel S (2007) Imatinib in melanoma: a selective treatment option based on KIT mutation status? J Clin Oncol 25:e9
7. Corless CL, McGreevey L, Haley A, Town A, Heinrich MC (2002) KIT mutations are common in incidental gastrointestinal stromal tumors one centimeter or less in size. Am J Pathol 160:1567–1572
8. Curtin JA, Busam K, Pinkel D, Bastian BC (2006) Somatic activation of KIT in distinct subtypes of melanoma. J Clin Oncol 24:4340–4346
9. Daum O, Grossmann P, Vanecek T, Sima R, Mukensnabl P, Michal M (2007) Diagnostic morphological features of PDGFRA-mutated gastrointestinal stromal tumors: molecular genetic and histologic analysis of 60 cases of gastric gastrointestinal stromal tumors. Ann Diagn Pathol 11:27–33
10. Do I, Araujo ES, Kalil RK, Bacchini P, Bertoni F, Unni KK, Park YK (2007) Protein expression of KIT and gene mutation of c-kit and PDGFRs in Ewing sarcomas. Pathol Res Pract 203:127–134
11. Fiorentini G, Rossi S, Lanzanova G, Biancalani M, Palomba A, Bernardeschi P, Dentico P, De Giorgi U (2003) Tyrosine kinase inhibitor imatinib mesylate as anticancer agent for advanced ocular melanoma expressing immunohistochemical C-KIT (CD 117): preliminary results of a compassionate use clinical trial. J Exp Clin Cancer Res 22:17–20
12. Flaherty KT (2006) Chemotherapy and targeted therapy combinations in advanced melanoma. Clin Cancer Res 12:2366s–2370s
13. Hicks MJ, Flaitz CM (2000) Oral mucosal melanoma: epidemiology and pathobiology. Oral Oncol 36:152–169
14. Hirota S, Ohashi A, Nishida T, Isozaki K, Kinoshita K, Shinomura Y, Kitamura Y (2003) Gain-of-function mutations of platelet-derived growth factor receptor alpha gene in gastrointestinal stromal tumors. Gastroenterology 125:660–667
15. Isozaki K, Terris B, Belghiti J, Schiffmann S, Hirota S, Vanderwinden JM (2000) Germline-activating mutation in the kinase domain of KIT gene in familial gastrointestinal stromal tumors. Am J Pathol 157:1581–1585
16. Ivan D, Niveiro M, Diwan AH, Eton O, Kim KB, Lacey C, Gonzalez C, Prieto VG (2006) Analysis of protein tyrosine kinases expression in the melanoma metastases of patients treated with Imatinib Mesylate (STI571, Gleevec). J Cutan Pathol 33:280–285
17. Jin T, Nakatani H, Taguchi T, Nakano T, Okabayashi T, Sugimoto T, Kobayashi M, Araki K (2006) STI571 (Glivec) suppresses the expression of vascular endothelial growth factor in the gastrointestinal stromal tumor cell line, GIST-T1. World J Gastroenterol 12:703–708
18. Kang DY, Park CK, Choi JS, Jin SY, Kim HJ, Joo M, Kang MS, Moon WS, Yun KJ, Yu ES, Kang H, Kim KM (2007) Multiple gastrointestinal stromal tumors: clinicopathologic and genetic analysis of 12 patients. Am J Surg Pathol 31:224–232
19. Kinoshita K, Isozaki K, Hirota S, Nishida T, Chen H, Nakahara M, Nagasawa Y, Ohashi A, Shinomura Y, Kitamura Y, Matsuzawa Y (2003) C-kit gene mutation at exon 17 or 13 is very rare in sporadic gastrointestinal stromal tumors. J Gastroenterol Hepatol 18:147–151
20. Lefevre G, Glotin AL, Calipel A, Mouriaux F, Tran T, Kherrouche Z, Maurage CA, Auclair C, Mascarelli F (2004) Roles of stem cell factor/c-Kit and effects of Glivec/STI571 in human uveal melanoma cell tumorigenesis. J Biol Chem 279:31769–31779
21. Lennartsson J, Blume-Jensen P, Hermanson M, Ponten E, Carlberg M, Ronnstrand L (1999) Phosphorylation of Shc by Src family kinases is necessary for stem cell factor receptor/c-kit mediated activation of the Ras/MAP kinase pathway and c-fos induction. Oncogene 18:5546–5553
22. Leslie MC, Bar-Eli M (2005) Regulation of gene expression in melanoma: new approaches for treatment. J Cell Biochem 94:25–38
23. Lin SC, Liu CL, Wang TI, Chang WS, Tzen CY, Huang MJ (2006) Clinical implications of C-kit gene mutation in patients with large gastrointestinal stromal tumors. J Gastroenterol Hepatol 21:1604–1608
24. Litz J, Krystal GW (2006) Imatinib inhibits c-Kit-induced hypoxia-inducible factor-1alpha activity and vascular endothelial growth factor expression in small cell lung cancer cells. Mol Cancer Ther 5:1415–1422
25. Litz J, Sakuntala Warshamana-Greene G, Sulanke G, Lipson KE, Krystal GW (2004) The multi-targeted kinase inhibitor SU5416 inhibits small cell lung cancer growth and angiogenesis, in part by blocking Kit-mediated VEGF expression. Lung Cancer 46:283–91

26. Lux ML, Rubin BP, Biase TL, Chen CJ, Maclure T, Demetri G, Xiao S, Singer S, Fletcher CD, Fletcher JA (2000) KIT extracellular and kinase domain mutations in gastrointestinal stromal tumors. Am J Pathol 156:791–795
27. Montone KT, van Belle P, Elenitsas R, Elder DE (1997) Proto-oncogene c-kit expression in malignant melanoma: protein loss with tumor progression. Mod Pathol 10:939–944
28. Nagatsuka H, Rivera RS, Gunduz M, Lee YJ, Tamamura R, Gunduz E, Naito I, Sado Y, Nagai N (2005) Immunolocalization and distribution patterns of type IV collagen alpha chains in oral mucosal melanoma. Virchows Arch 447:710–716
29. Natali PG, Nicotra MR, Winkler AB, Cavaliere R, Bigotti A, Ullrich A (1992) Progression of human cutaneous melanoma is associated with loss of expression of c-kit proto-oncogene receptor. Int J Cancer 52:197–201
30. Ohashi A, Funasaka Y, Ueda M, Ichihashi M (1996) c-KIT receptor expression in cutaneous malignant melanoma and benign melanotic naevi. Melanoma Res 6:25–30
31. Pache M, Glatz K, Bosch D, Dirnhofer S, Mirlacher M, Simon R, Schraml P, Rufle A, Flammer J, Sauter G, Meyer P (2003) Sequence analysis and high-throughput immunohistochemical profiling of KIT (CD 117) expression in uveal melanoma using tissue microarrays. Virchows Arch 443:741–744
32. Puputti M, Tynninen O, Sihto H, Blom T, Maenpaa H, Isola J, Paetau A, Joensuu H, Nupponen NN (2006) Amplification of KIT, PDGFRA, VEGFR2, and EGFR in gliomas. Mol Cancer Res 4:927–934
33. Rapidis AD, Apostolidis C, Vilos G, Valsamis S (2003) Primary malignant melanoma of the oral mucosa. J Oral Maxillofac Surg 61:1132–1139
34. Shen SS, Zhang PS, Eton O, Prieto VG (2003) Analysis of protein tyrosine kinase expression in melanocytic lesions by tissue array. J Cutan Pathol 30:539–547
35. Stefanou D, Batistatou A, Zioga A, Arkoumani E, Papachristou DJ, Agnantis NJ (2004) Immunohistochemical expression of vascular endothelial growth factor (VEGF) and C-KIT in cutaneous melanocytic lesions. Int J Surg Pathol 12:133–138
36. Ugurel S, Hildenbrand R, Zimpfer A, La Rosee P, Paschka P, Sucker A, Keikavoussi P, Becker JC, Rittgen W, Hochhaus A, Schadendorf D (2005) Lack of clinical efficacy of imatinib in metastatic melanoma. Br J Cancer 92:1398–1405
37. Wardelmann E, Losen I, Hans V, Neidt I, Speidel N, Bierhoff E, Heinicke T, Pietsch T, Buttner R, Merkelbach-Bruse S (2003) Deletion of Trp-557 and Lys-558 in the juxtamembrane domain of the c-kit protooncogene is associated with metastatic behavior of gastrointestinal stromal tumors. Int J Cancer 106:887–895
38. Willmore C, Holden JA, Zhou L, Tripp S, Wittwer CT, Layfield LJ (2004) Detection of c-kit-activating mutations in gastrointestinal stromal tumors by high-resolution amplicon melting analysis. Am J Clin Pathol 122:206–216
39. Willmore-Payne C, Holden JA, Tripp S, Layfield LJ (2005) Human malignant melanoma: detection of BRAF- and c-kit-activating mutations by high-resolution amplicon melting analysis. Hum Pathol 36:486–493
40. Wyman K, Atkins MB, Prieto V, Eton O, McDermott DF, Hubbard F, Byrnes C, Sanders K, Sosman JA (2006) Multicenter phase II trial of high-dose imatinib mesylate in metastatic melanoma: significant toxicity with no clinical efficacy. Cancer 106:2005–2011