Chromosomal copy number changes supporting the classification of lentiginous junctional melanoma of the elderly as a subtype of melanoma

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Recently the term lentiginous melanoma of the elderly has been suggested for a pattern of melanocytic neoplasia characterized by frequent occurrence in elderly patients, broad lentiginous growth pattern, with poorly cohesive nesting, suprabasilar extension of melanocytes and moderate cytological atypia. However, there are limited reported cases with follow-up information to confirm the malignant nature of these neoplasms. Using fluorescence *in situ* hybridization (FISH) targeting chromosomal loci that are frequently found to have copy number changes in melanoma, we evaluated cases of lentiginous junctional melanoma of the elderly in order to compare with the frequencies and patterns of chromosomal aberrations identified in other subtypes of melanoma. Previous studies have shown that by using a FISH assay targeting 6p25, 6q23, 11q13 and CEP6 with previously validated criteria, one could discriminate benign nevi from melanoma with high sensitivity and specificity. In this study, 16 of 19 cases (84%) showed sufficient copy number changes in one of the targeted chromosomal loci to meet FISH criteria for melanoma. A total of 17 control cases of lentiginous junctional nevi tested negative for all criteria. These findings support the classification of this pattern of melanocytic neoplasia as a subtype of melanoma.

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Several authors have recently proposed the term lentiginous melanoma to describe a pattern of melanocytic neoplasias characterized by prominent lentiginous single-cell growth with focal, poorly cohesive nesting, suprabasilar migration of melanocytes and preservation of the rete ridges.^{1,2} These cases typically occur in elderly patients, they commonly lack solar elastosis, and have a prolonged *in situ* or radial growth phase.^{2,3} In 2005, King *et al* presented 16 patients aged 45–90 years with such pigmented lesions and showed three index cases in which the histological changes of melanoma were manifested only after complete excision. Previously, cases with this histology were variably classified as atypical lentiginous nevi, melanocytic hyperplasia,

evolving melanoma *in situ* or nevoid lentigo maligna.^{4,5} Definitive classification of these lesions is difficult, as only few lesions with follow-up have been reported in the literature.

Recently, we have used a new fluorescence *in situ* hybridization (FISH) assay for diagnosis in melanoma.⁶ This assay targets four chromosomal loci that frequently show copy number alterations in melanoma, but not in nevi. These loci include *RREB1* (6p25), *MYB* (6q23), *CCND1* (11q13) and CEP6 (centromere of chromosome 6). This assay was performed with a sensitivity of 87% and a specificity of 95% in our original validation study.⁶ To further study the subgroup of melanomas referred to as lentiginous melanoma, we identified 19 such cases from our archives and evaluated them with the same FISH assay. A total of 16 of the 19 cases (84%) tested positive with this test, whereas none of the control cases were positive. This sensitivity is similar to that seen in other subtypes of melanoma.⁷ Our data support the findings of King *et al* and the classification of this pattern of

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melanocytic growth as a subtype of melanoma by molecular standards.

Materials and methods

After obtaining approval from the IRB and the Northwestern University Cancer Center, we reviewed our dermatopathology files at the Northwestern University for cases with a diagnosis of lentiginous melanoma between 2005 and 2009. We identified 19 cases. Table 1 contains demographic information, including the age and sex of the patients, in addition to the site of the melanoma. A total of 17 cases of lentiginous junctional nevi with variable levels of atypia were selected as controls.

Probes targeting four loci were used for FISH evaluation on the basis of the results of our previous study.⁶ These included Ras responsive element-binding protein-1 (Vysis LSI *RREB1*-Spectrum red), myeloblastosis (Vysis LSI *MYB*-Spectrum gold), cyclin D1 chromosome 11q (Vysis LSI *CCND1*-Spectrum green) and centromeric enumeration probe control for chromosome 6 (Vysis LSI CEP6-Spectrum aqua) from Abbott Molecular Inc, Des Plaines, IL, USA.

Fluorescence *in situ* hybridization was performed on formalin-fixed paraffin-embedded tissue for all cases.⁶ Sections of 5 μ m thickness were mounted onto SuperFrost Plus positively charged slides (ThermoShandon, Pittsburgh, PA, USA), baked at 56°C overnight, de-paraffinized, submersed in 1 $\times$ SSC (pH 6.3) at 80°C for 35 min and washed in water for 3 min. After protease digestion (4 mg pepsin per ml of 0.2 N HCl) at 37°C for 15 min, sections were rinsed in water for 3 min, passed through graded ethanol and dried. Hybridizations were carried out at 37°C for 16–18 h in an automated co-denaturation oven (HYBrite or ThermoBrite Denaturation/

Hybridization System, Abbott Molecular Inc) according to the manufacturer's instructions. Sections were placed in washing buffer (2 $\times$ SSC/0.3% NP40, Abbott Molecular Inc) at room temperature for 2–10 min to remove the coverslips and then immersed in the washing buffer for 2 min at 73°C, dried and mounted using DAPI I antifade solution (Abbott Molecular Inc).

In each case, a hematoxylin and eosin-stained section from the block was obtained followed directly by an unstained section for FISH. The slides were analyzed with an epifluorescence microscope equipped with single band-pass filters (Abbott Molecular Inc).

All analyses were performed blinded of the specimens' diagnoses. Tumor-bearing areas were localized using the DAPI filter at low magnification ($\times 10$ objective). The tumor area was then thoroughly inspected for the presence of nuclei harboring abnormal copy numbers of any probe. Areas with the most significant copy number changes were selected for enumeration. Wherever possible, three abnormal areas were selected and within each area, 10 random nuclei were analyzed under high-power ($\times 60$ objective). To qualify, the nuclei had to be non-overlapping and harbor sufficiently bright signals. Nuclei that lacked signals for more than one probe were not analyzed. Thirty cells were enumerated for each case.

Fluorescence *in situ* hybridization enumeration was performed by a single blinded reviewer. The slides were thoroughly scanned and areas with copy number alterations were selected for enumeration. At least 10 adjacent cells were enumerated from each selected site and 30 total cells were enumerated for each specimen. A case was considered as having a positive FISH result, if any of the following criteria were met: (1) gain in *RREB1* relative to CEP6 greater than 55%, or (2) gain in *RREB1* greater than

Table 1 Demographic information and classification of lentiginous junctional melanoma

Subject	Age	Sex	Site	Breslow depth (mm)	Solar elastosis
1	81	M	Nasal fold	0	Present
2	84	M	Chest	0	Absent
3	60	F	Back	0	Absent
4	48	F	Back	0	Absent
5	74	M	Back	0.4	Absent
6	48	F	Back	0	Absent
7	63	M	Chest	0.2	Absent
8	64	F	Back	0	Absent
9	58	F	Scapula	0	Absent
10	75	M	Back	0	Absent
11	66	M	Scapula	0.35	Absent
12	50	F	Back	0	Absent
13	67	F	Upper extremity	0.06	Absent
14	60	M	Upper extremity	0	Present
15	67	M	Back	0	Absent
16	64	F	Cheek	0.15	Present
17	66	M	Back	0	Absent
18	73	M	Back	0	Absent
19	64	M	Upper extremity	0	Absent

29%, or (3) loss in *MYB* relative to CEP6 greater than 40%, or (4) gain in *CCND1* greater than 38%. The sensitivity and specificity for the FISH technique were calculated in comparison with the gold standard of histological diagnosis for melanoma. We then determined the overall percentage of cases meeting each of the four criteria in the malignant areas.

Results

Among the 19 patients with lentiginous junctional melanomas, the average age was 65.0 years with a range from 48 to 84 years (Table 1). The female to male ratio was 8:11. A total of 15 cases occurred on the trunk as compared with 3 cases on the extremities and 1 head and neck case.

All cases were characterized by broad size of approximately 1 cm or larger. Histologically, all lesions were typified by prominent lentiginous single-cell growth of melanocytes along the dermal–epidermal junction. Focal and poorly cohesive nesting was also identified and the rete ridges were well preserved (Figures 1 and 2). Some limited suprabasilar movement of melanocytes was also noted. In 16 of 19 (84%) cases, solar elastosis was not significant, whereas in three cases, prominent solar elastosis was noted. These cases, however,

Figure 1 A representative low-power (H&E, ×100) photograph of a lentiginous melanoma showing preservation of the rete ridges and prominent junctional proliferation of intermediate-sized melanocytes.

Figure 2 A representative high-power (H&E, ×400) photograph shows confluent and lentiginous growth of junctional melanocytes.

were included as other features were more congruent with lentiginous melanoma than typical lentigo maligna.

A total of 17 benign lentiginous nevi served as controls. These lesions varied between 3 mm and 1 cm in diameter. They also showed lentiginous growth of melanocytes with preservation of the rete ridges. However, there was less cellular confluence with sparing of the inter-reticular areas and well-defined nesting.

Overall, 16 of 19 (84%) cases of lentiginous melanoma showed sufficient copy number changes to meet one of the four criteria for diagnosis of melanoma, whereas three cases were completely negative (Table 2). There were no distinctive histological or clinical differences in these three cases. A total of 15 of 16 (94%) FISH-positive cases displayed aberrations within chromosome 6 (Figure 3). The remaining positive case from the scapula of a 66-year-old man in an area without chronic sun damage showed prominent gains in 11q13 (*CCND1*). None of the lentiginous junctional nevi, including those with moderate atypia, showed any significant chromosomal aberrations by FISH or met the criteria for melanoma.

Among the 17 cases of lentiginous junctional nevi, the average age was 40.0 years with a range from 12 to 75 years of age (Table 3). The female to male ratio was 11:6. The FISH evaluation was unexceptionally negative in all cases of lentiginous junctional nevi (Table 4). Hence, there was 100% specificity for evaluation of the benign nevi in this category.

Discussion

Treatment for advanced melanoma at this time is limited, and therefore there is an emphasis on early

Table 2 FISH data for lentiginous junctional melanoma

Subject	<i>RREB1</i> /CEP6 gain	<i>RREB1</i> gain	<i>MYB</i> /CEP6 loss	<i>CCND1</i> gain
1	7/30 (0.23)	4/30 (0.13)	7/30 (0.23)	2/30 (0.07)
2	15/30 (0.5)	17/30 (0.57)	5/30 (0.17)	3/30 (0.1)
3	12/30 (0.4)	7/30 (0.23)	14/30 (0.47)	1/30 (0.03)
4	18/30 (0.6)	10/30 (0.33)	10/30 (0.33)	3/30 (0.1)
5	19/30 (0.63)	11/30 (0.37)	8/30 (0.27)	3/30 (0.1)
6	19/30 (0.63)	11/30 (0.37)	9/30 (0.3)	1/30 (0.03)
7	17/30 (0.57)	12/30 (0.4)	8/30 (0.27)	3/30 (0.1)
8	16/30 (0.53)	10/30 (0.33)	7/30 (0.23)	2/30 (0.07)
9	17/30 (0.57)	10/30 (0.33)	7/30 (0.23)	4/30 (0.13)
10	21/30 (0.7)	14/30 (0.47)	8/30 (0.27)	8/30 (0.27)
11	10/30 (0.33)	7/30 (0.23)	10/30 (0.33)	13/30 (0.43)
12	17/30 (0.57)	7/30 (0.23)	9/30 (0.3)	2/30 (0.07)
13	18/30 (0.6)	14/30 (0.47)	6/30 (0.2)	0/30 (0)
14	18/30 (0.6)	10/30 (0.33)	7/30 (0.23)	1/30 (0.03)
15	17/30 (0.57)	12/30 (0.4)	12/30 (0.4)	3/30 (0.1)
16	13/30 (0.43)	8/30 (0.27)	11/30 (0.37)	3/30 (0.1)
17	17/30 (0.57)	10/30 (0.33)	9/30 (0.3)	5/30 (0.17)
18	12/30 (0.4)	7/30 (0.23)	4/30 (0.13)	2/30 (0.07)
19	14/30 (0.47)	10/30 (0.33)	6/30 (0.2)	0/30 (0)

The values that met the criteria are given in bold.

Figure 3 A representative high-power (FISH, $\times 600$) photograph shows multiple cells with 6p25 chromosomal gains.

Table 3 Demographic information and classification for lentiginous junctional nevi

Subject	Age	Sex	Site
1	12	M	Scrotum
2	44	M	Back
3	75	M	Lower extremity
4	55	F	Neck
5	29	M	Lower extremity
6	43	F	Lower extremity
7	44	F	Back
8	68	F	Chest
9	73	F	Lower extremity
10	65	F	Chest
11	21	F	Lower extremity
12	61	F	Perianal
13	37	F	Back
14	48	M	Lower extremity
15	26	F	Back
16	35	M	Back
17	63	F	Back

Table 4 FISH data for lentiginous junctional nevi

Subject	<i>RREB1</i> /CEP6 gain	<i>RREB1</i> gain	<i>MYB</i> /CEP6 loss	<i>CCND1</i> gain
1	1/30 (0.03)	0/30 (0)	3/30 (0.1)	0/30 (0)
2	4/30 (0.13)	1/30 (0.03)	3/30 (0.1)	0/30 (0)
3	3/30 (0.1)	0/30 (0)	3/30 (0.1)	0/30 (0)
4	3/30 (0.1)	0/30 (0)	3/30 (0.1)	0/30 (0)
5	2/30 (0.07)	0/30 (0)	4/30 (0.13)	0/30 (0)
6	4/30 (0.13)	0/30 (0)	4/30 (0.13)	0/30 (0)
7	5/30 (0.17)	3/30 (0.1)	11/30 (0.37)	0/30 (0)
8	6/30 (0.20)	2/30 (0.07)	11/30 (0.37)	0/30 (0)
9	5/30 (0.17)	1/30 (0.03)	3/30 (0.1)	0/30 (0)
10	3/30 (0.1)	0/30 (0)	7/30 (0.23)	0/30 (0)
11	5/30 (0.17)	1/30 (0.03)	3/30 (0.1)	0/30 (0)
12	4/30 (0.13)	1/30 (0.03)	5/30 (0.17)	0/30 (0)
13	6/30 (0.2)	1/30 (0.03)	4/30 (0.13)	0/30 (0)
14	2/30 (0.07)	2/30 (0.07)	10/30 (0.33)	0/30 (0)
15	4/30 (0.13)	0/30 (0)	7/30 (0.23)	0/30 (0)
16	4/30 (0.13)	0/30 (0)	3/30 (0.1)	0/30 (0)
17	7/30 (0.23)	8/30 (0.27)	6/30 (0.20)	0/30 (0)

detection of melanoma before the tumor cells are capable of metastatic disease. Recently, the term lentiginous melanoma has been used to refer to a pattern of melanoma characterized by occurrence in elderly patients, lentiginous growth pattern preserving the rete ridges, poorly cohesive nesting, moderate cytological atypia, lack of significant solar elastosis and a prolonged radial growth phase.¹ Some of these cases have been shown to recur with more obvious atypia or signs of malignancy on re-excision specimens.^{1–3} However, there are very few series regarding this entity and minimal follow-up from cases in the literature, thus providing little information regarding the natural history of these cases. Hence, there is a need for further study of these lesions to substantiate their classification as malignant melanoma.

In this study, we used FISH to determine whether these neoplasms have copy number changes in 6p25, 6q23, 11q13 or CEP6 as seen in other conventional forms of melanoma. Interestingly, our findings show aberrations involving chromosome 6 within these lesions at a similar frequency (79%) as that seen in other forms of melanoma.^{6,7} Overall 16 of 19 (84%) cases met one of the four FISH criteria for melanoma, and the abnormality in the majority of cases consisted of increased copy numbers of *RREB1* (6p25). These findings support the classification of these lesions as melanoma at the molecular level. Furthermore, none of the lentiginous nevi that we evaluated showed significant copy number changes or met any of the criteria for melanoma.

The performance of the assay within this case series with high sensitivity and specificity suggest that this probe set and assay can be a helpful adjunctive tool when dealing with this differential diagnosis. This is of particular significance as, although these lesions do have nests, the nests are less well formed and there is prominent lentiginous growth. Hence, an experienced reviewer should be able to use the assay successfully in lesions with this morphology. Interestingly, one case exclusively showed gains in 11q13 and lacked any evidence of abnormality in chromosome 6. Thus, although the FISH results do support classification of this pattern of melanocytic proliferation as a form of melanoma, it is unclear whether they are truly a homogeneous subtype of melanoma. Further study combining molecular data and clinical outcome of these patients is necessary.

Conflict of interest

Dr Gerami has served as a consultant to Abbott Molecular Labs and has received honoraria from Abbott Molecular Labs. This work was performed with support from the Irene D Pritzker (IDP) Foundation and the Dermatology Foundation. All other authors report no relevant conflicts of interest or funding sources to declare. This work is original and has not had prior presentation.

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