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FISH analysis for diagnostic evaluation of challenging melanocytic lesions

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Summary. The differential diagnosis of malignant melanomas and atypical melanocytic nevi is still a diagnostic challenge. The currently accepted morphologic criteria show substantial interobserver variability, likewise immunohistochemical studies are often not able to discriminate these lesions reliably. Techniques that support diagnostic accuracy are of the greatest importance considering the growing incidence of malignant melanomas and their increase in younger patients.

In this study we analyzed the feasibility of fluorescence in situ hybridization (FISH) analysis for the discrimination of malignant and benign melanocytic tumors. A panel of DNA probes was used to detect chromosomal aberrations of chromosomes 6 and 11. On a series of 5 clearly malignant and benign melanocytic tumors we confirmed the applicability of the test. Then we focused on examination of ambiguous melanocytic lesions, where atypical cells are often difficult to relocalize in the 4',6-Diamidino-2-phenylindol (DAPI)-fluorescence stain. FISH analyses were conducted on destained H&E-stained slides. By comparison of the DAPI-image with photos taken from the H&E stain, unambiguous assignment of the FISH results to the conspicuous groups of cells was possible.

The results of FISH analysis were consistent with the conventional diagnosis in 11 of 14 small ambiguous lesions. Of the remaining 3 cases, 2 showed FISH-results close to the cut-off level. Comparison of FISH results on thin and thick sections revealed that the cut-off values have to be adopted for 2 μm destained sections.

In conclusion, FISH analysis is a useful and applicable tool for assessment of even smallest melanocytic neoplasms, although there will remain unclear cases that cannot be solved even after additional FISH evaluation.

Key words: Melanoma, FISH, Nevus, Melanocytic lesion, Diagnostics

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Introduction

The incidence of malignant melanoma in Caucasian populations is still increasing. Being much more aggressive than the more frequent squamous cell carcinoma of the skin, malignant melanoma does not only affect older persons but young people as well. Since successful treatment is missing for advanced stage disease, it is mandatory to diagnose it in its early stages. Histological examination is the "gold standard" to establish the diagnosis. Whereas the diagnosis of clear-cut benign and malignant neoplasms can mostly be posed without major problems, assessment of ambiguous lesions is often difficult, and ancillary immunohisto-chemical investigations, e.g. by use of the monoclonal antibodies to melan A or HMB45, are not always helpful.

Genetic alterations are known to occur early during tumorigenesis. Losses and/or gains of chromosomes are found in a majority of malignant melanomas. In contrast, in most benign melanocytic nevi chromosomal aberrations are absent. Comparative genomic hybridization (CGH)-analyses found significant differences in DNA copy number changes between melanoma and nevi. Those benign melanocytic neoplasms harbouring alterations were mostly classified as Spitz nevi. Their pattern of chromosomal alterations differed significantly from melanomas showing mostly an isolated gain of the short arm of chromosome 11 (Bauer and Bastian, 2006; Bastian et al., 2003). In melanomas common losses and gains were observed on

chromosome 6q (28%) and 6p (28%) (Bastian et al., 1998).

Based on these CGH results fluorescence *in situ* hybridization (FISH) analysis of cutaneous melanocytic neoplasms was established to distinguish between malignant and benign melanocytic tumors. A FISH test is commercially available that comprises a panel of four probes on chromosomes 6p (6p25; RREB1), 6q (6q23; MYB), 11q (11q13; CCDN1) and centromere 6 (Abbott Molecular Laboratories, USA) for the assessment of copy number changes in these chromosomal regions. In recent publications the value of FISH analysis in diagnosis of melanocytic lesions was described (Gerami et al., 2009; Morey et al., 2009; Newman et al., 2009a,b; Pouryazdanparast et al., 2009).

In our study the FISH test discriminated reliably between clear-cut malignant melanomas and benign melanocytic nevi. However, more importantly it proved useful for diagnosis of difficult melanocytic lesions. For these lesions we developed a special protocol applicable to 2 μm destained H&E sections.

Materials and methods

Paraffin-embedded material of invasive malignant melanomas, melanocytic nevi and ambiguous melanocytic neoplasms were retrieved from the archives of the Institute of Pathology Lucerne.

Diagnosis of melanocytic lesions was based on histomorphologic criteria (e.g. junctional activity, cellular atypia, invasion of the surrounding tissue, prominent melanin pigmentation, mitotic activity etc.) and, if needed, immunohistochemical analyses (HMB-45, melan-A etc.).

A tissue microarray was constructed of five clear-cut malignant melanomas and five clear melanocytic nevi. The tissue cores had a diameter of 3mm and were representative for the whole lesion. Furthermore, we analysed four samples of Spitz nevi and 10 other diagnostically difficult samples on gross sections. Melanocytic lesions that posed diagnostic difficulties to one of our board-certified staff pathologists and required internal or external consultation were considered as difficult cases and were analysed (see Table 1). For nine of ten samples a diagnosis could be made based on conventional histomorphologic and immunohistochemical criteria. Five cases were classified as malignant melanomas, three as benign melanocytic nevi, one as a malignant melanoma in situ in a dermal melanocytic nevi and one could not be classified. An overview of all cases is given in Table 1.

FISH was carried out as follows: H&E stained slides were soaked in acetone till the cover film could be detached, rinsed in fresh xylol, and rehydrated in a series

Table 1. Summary of cases.

Case-No.	Diagnosis	TNM	Clark	Breslow	Sex	Age
Melanomas						
1	amelanotic malignant melanoma	pT4b	V	10.0 mm	f	80
2	malignant melanoma	pT2a	III	1.8 mm	m	57
3	malignant melanoma	pT4b	IV	7.0 mm	f	93
4	malignant melanoma	pT2a	III	1.4 mm	f	52
5	nodular malignant melanoma	pT3a	IV	3.0 mm	m	64
Nevi						
6	melanocytic nevus, dermal type				m	39
7	melanocytic nevus, dermal type				m	42
8	melanocytic nevus, dermal type				f	91
9	melanocytic nevus, dermal type				f	31
10	melanocytic nevus, dermal type				f	40
Spitz-Nevi and difficult lesions						
11	melanocytic nevus, compound Spitz type				f	25
12	melanocytic nevus, compound Spitz type				m	6
13	melanocytic nevus, dermal Spitz type				f	24
14	melanocytic nevus, junctional type				f	68
15	lentigo maligna melanoma	pT1b	IV	1.0 mm	m	64
16	melanocytic lesion of uncertain dignity				m	64
17	malignant melanoma	pT1a	III	0.7 mm	m	85
18	melanocytic nevus, dysplastic type				m	60
19	melanocytic nevus, desmoplastic nevus Spitz				m	49
20	malignant melanoma, superficial spreading	pT1a	III	0.4 mm	m	27
21	melanocytic nevus, compound type				f	35
22	malignant melanoma	pT1a	II	0.2 mm	f	46
23 a	melanoma in situ		I		m	70
23 b	melanocytic nevus, dermal type					
24	melanoma in situ		I		m	73

of decreasing concentrations of ethanol (96%, 80%, 70%). For bleaching the H&E slides were incubated for 15 min in 1% HCl/70% EtOH and briefly washed in distilled water. After 20 min incubation in 10 mM citrate buffer in a boiling water bath, the slides cooled down for 20 min at room temperature. Following a further washing step the tissue was digested with pepsin (2.5 mg/ml in 0.01 N HCl, purchased from Fluka, Buchs, Switzerland) for 20 min at room temperature, rinsed with aqua dest., post-fixed with 4% formaldehyde, washed a last time and then air dried. The melanoma probe (Abbott, Baar, Switzerland) was applied to the area of interest, covered with a cover slip, sealed with fixogum (Marabu, Tamm, Germany) and co-denatured by a 10 min 73°C incubation on a heating plate. The hybridization was carried out overnight at 37°C in a humidified chamber. After 5 min post hybridization washing in 1.5x SSX/0.1% Tween20 at 73°C the slides

Table 2. FISH results on histologically clear melanomas and melanocytic nevi.

Sample-No.	6q*	11q*	6q-loss (%)	6p≠2 (%)+	FISH result
1	1.66	1.65	53	77	melanoma
2	1.29	1.60	23	67	melanoma
3	1.41	2.13	46	81	melanoma
4	1.27	2.49	83	74	melanoma
5	1.27	2.21	64	66	melanoma
6	1.52	1.48	21	44	nevus
7	1.59	1.68	12	31	nevus
8	1.43	1.25	9	18	nevus
9	1.58	1.60	8.4	12.6	nevus
10	1.38	1.50	7.8	18.6	nevus

Thickness of slices 4 μm; count of 60 per 100 nuclei each. *: Ratio of signals per nucleus. +: Percentage of nuclei with more or less than 2 signals.

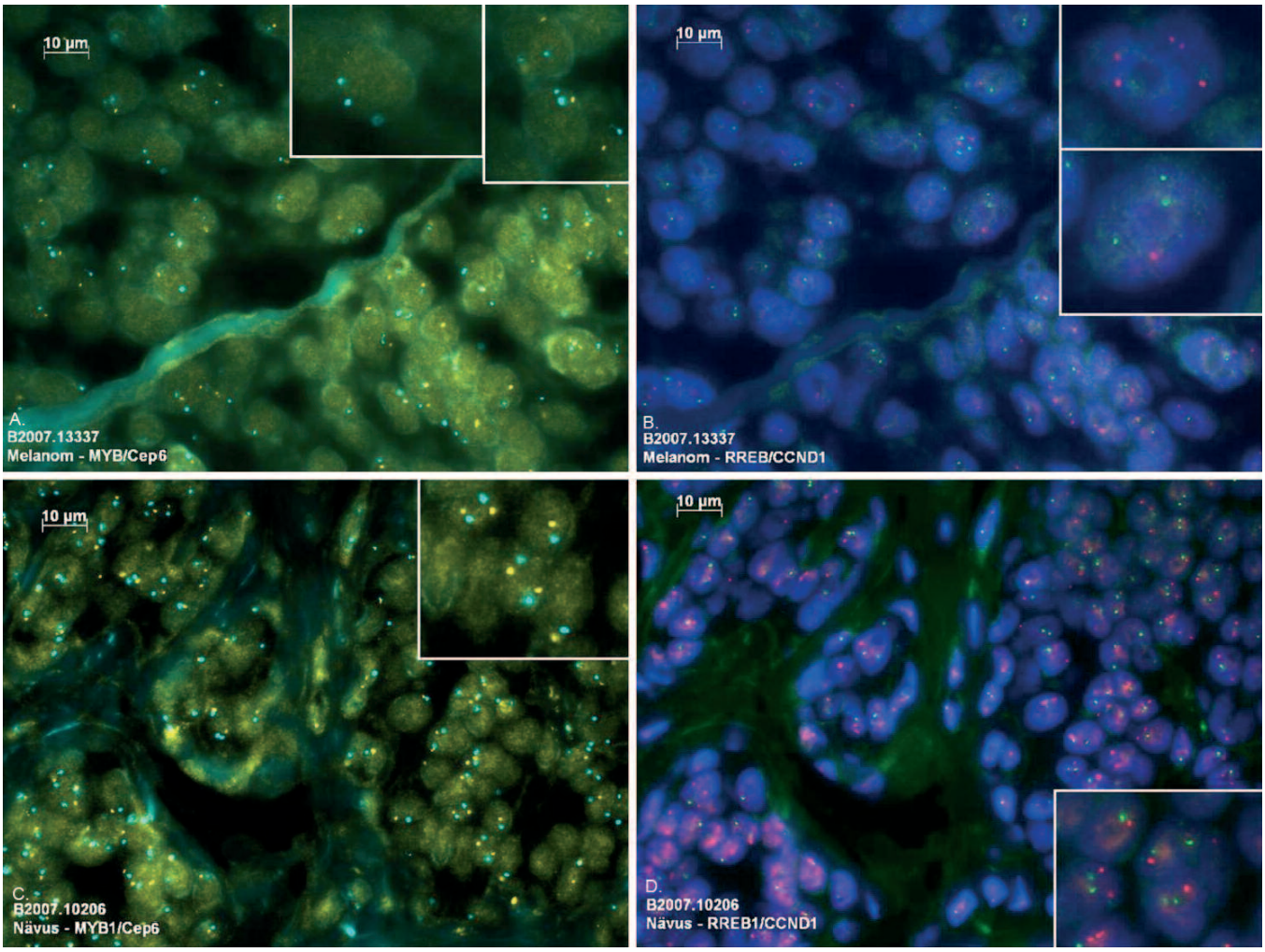

Fig. 1. Example of FISH results of a melanoma (**A, B**) and a melanocytic nevus (**C, D**) showing signals from 6q (Gold) and centromere 6 (Aqua), on the left, and 6p (Red) and 11q (Green), on the right.

were mounted with Vectashield-DAPI (4',6-Diamidino-2-phenylindol; Vector Laboratories, Peterborough, England).

After destaining and performance of the adapted FISH protocol, cells of interest could easily be relocated by comparison of DAPI patterns with H&E images. FISH assessment was performed according to the manual of the manufacturer. If not indicated otherwise thickness of sections was 4-6 μm. Signals were counted in at least 60 nuclei. Standard quality features for the hybridization procedure (signal intensity and signal number in normal cells and tumor nuclei, background, etc) and for evaluation of FISH analyses (non-overlapping nuclei) were always matched. Four criteria were evaluated: gain of 6q (MYB) or 11q (CCND1)-signals to equal or greater than 2.5 per nucleus; loss of 6q (MYB)-signals relative to centromere 6 in equal or more than 31% of nuclei; abnormal 6p (RREB1)-signals in equal or more than 63% of nuclei. Meeting one of the criteria sufficed for the diagnosis of melanoma.

Results

A total of 24 samples of melanocytic neoplasms

Table 3. Difficult cases.

Sample-No.	6q*	11q*	6q-loss (%)	6p≠2 (%)+	FISH result	Histological diagnosis
11	1.58	1.73	31	43	nevus	nevus Spitz
12	2.00	2.05	26	49	nevus	nevus Spitz
13	2.12	2.20	30	47	nevus	nevus Spitz
14	1.21	1.14	22	62	nevus	nevus
15	1.91	1.65	10	63	nevus	lentigo maligna melanoma
16	1.86	1.73	25	67	melanoma	melanocytic lesion of uncertain dignity
17	1.68	1.82	18.3	83.3	melanoma	melanoma
18	1.6	1.82	16.67	41.7	nevus	nevus
19	2.3	2.13	18.3	56.67	nevus	desmopalstic nevus Spitz*
20	2.02	1.97	25	55	nevus	superficial spreading melanoma*
21	1.67	1.83	11.67	43.3	nevus	nevus*
22	1.9	1.72	36.7	63.3	melanoma	malignant melanoma
23 a	2.22	2.52	36.67	83.3	melanoma	melanoma *in situ*
23 b	1.43	1.53	20	56.67	nevus	nevus
24	2.15	3.39	72.73	66.67	melanoma	melanoma *in situ*

Thickness of slices 4 μm. *: Ratio of signals per nucleus. +: Percentage of nuclei with more or less than 2 signals.

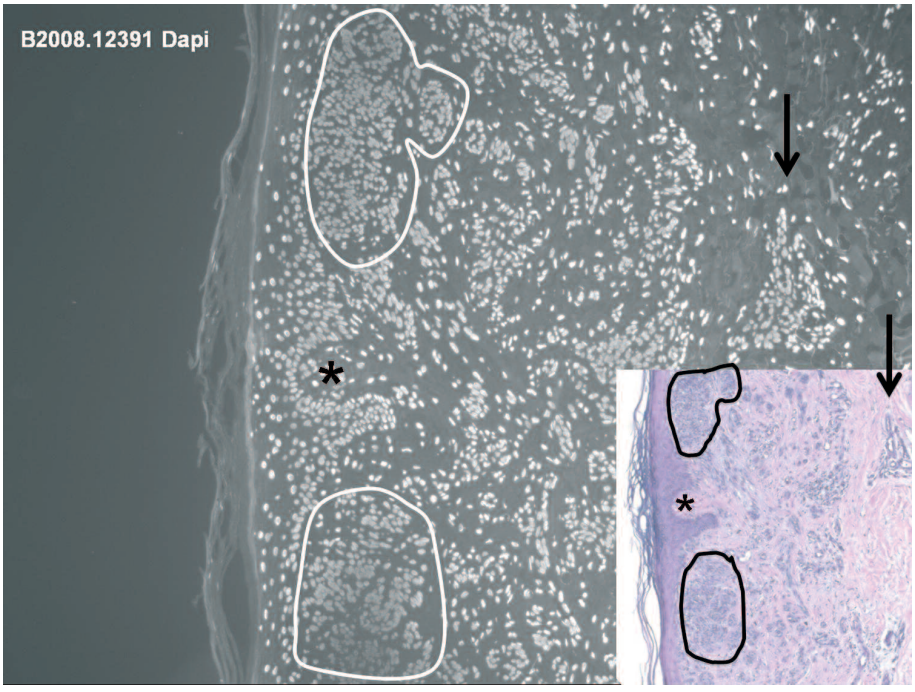

Fig. 2. Relocation of nests of melanocytes by comparing H&E image and DAPI-pattern. Arrows indicate a triangular bunch of vessels and a noticeable epidermal rete ridge is marked with an asterisk. Melanocytic nests are outlined. x 50

were examined by FISH analysis. The panel consisted of five unambiguous malignant melanomas and five melanocytic nevi, as well as four Spitz nevi and ten diagnostically challenging cases. An overview is given in Table 1.

Clear-cut cases

In a first step the reliability of the FISH-assay was confirmed. Five histologically obvious cases of malignant melanomas and nevi each showed FISH results which completely agreed with the histological diagnosis.

In the melanomas arm the criterion of abnormal count of 6p signals in over 63% of melanocytes was achieved by all samples, while 6q-loss relatively to centromere 6 was observed in four of five samples. None of the melanoma samples showed an increase of 6q- and 11q-signals to equal or over 2.5 (see Table 2 and Figure 1).

In contrast, analysis of five histologically certain melanocytic nevi resulted in all cases in the FISH diagnosis of nevus. None of the five samples met any of the four above mentioned criteria (see Table 2 and Figure 1).

Spitz nevi and challenging melanocytic lesions

In a second step of this study we focused on the usefulness and applicability of FISH analysis on

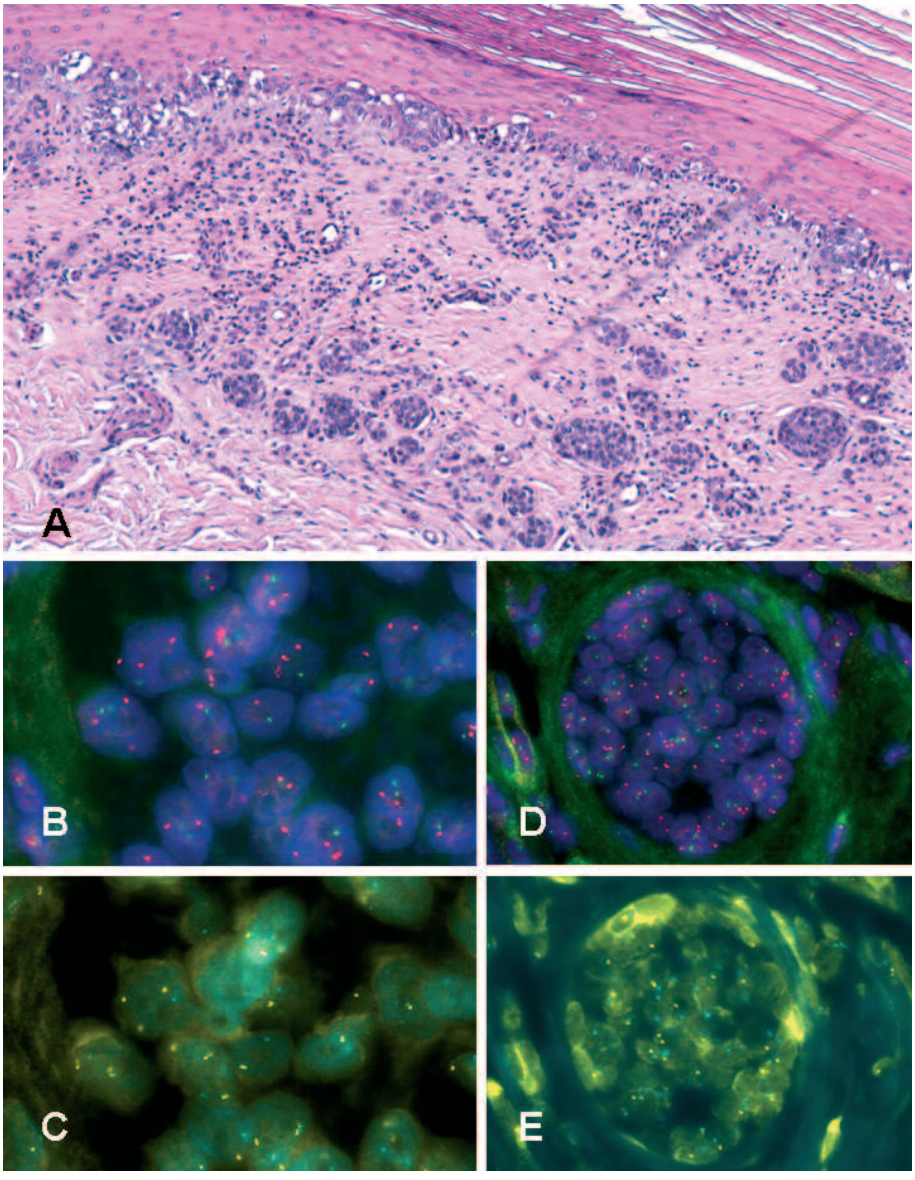

Fig. 3. Case 23: Melanoma in situ associated with a dermal melanocytic nevus in a 70-year old man. Low-power view of an atypical melanocytic lesion with clusters of cells in irregular distribution along the basal epidermis and isolated atypical melanocytes ascending to the upper layers of the epidermis. In the upper dermis several small nests of bland looking nevus cells can be seen **(A)**. FISH analysis of the atypical melanocytes in the epidermis with probes for 6p (Red) and 11q (Green) **(B)** and 6q (Gold) and centromere 6 (Aqua) **(C)** revealed abnormalities characteristic for melanoma, in particular a prominent increase of red signals. In contrast, on the right hand side (**D**: 6p (Red) and 11q (Green); **E**: 6q (Gold) and centromere 6 (Aqua)), FISH analysis of bland looking nevus cells showed no abnormalities. A, x 50; B-E, x 1000.

Table 4. Comparison of number of counted nuclei in melanoma.

Sample-No.	Count of nuclei	6q*	11q*	6q-loss (%)	6p≠2 (%)+	FISH result
1	100	1.66	1.65	53.0	77.0	melanoma
	60	1.67	1.63	48.3	80.0	
	30	1.67	1.73	46.7	90.0	
2	100	1.29	1.60	23.0	67.0	melanoma
	60	1.25	1.63	26.7	73.3	
	30	1.33	1.43	23.3	83.3	
3	100	1.41	2.13	46.0	81.0	melanoma
	60	1.53	2.23	46.7	81.7	
	30	1.30	1.77	50.0	83.3	
4	100	1.27	2.49	83.0	74.0	melanoma
	60	1.38	2.45	81.7	78.3	
	30	1.47	2.30	83.3	70.0	
5	100	1.27	2.21	64.0	66.0	melanoma
	60	1.27	2.33	58.3	63.3	
	30	1.33	1.83	53.3	63.3	

Thickness of slices 4 μm. *: Ratio of signals per nucleus. +: Percentage of nuclei with more or less than 2 signals.

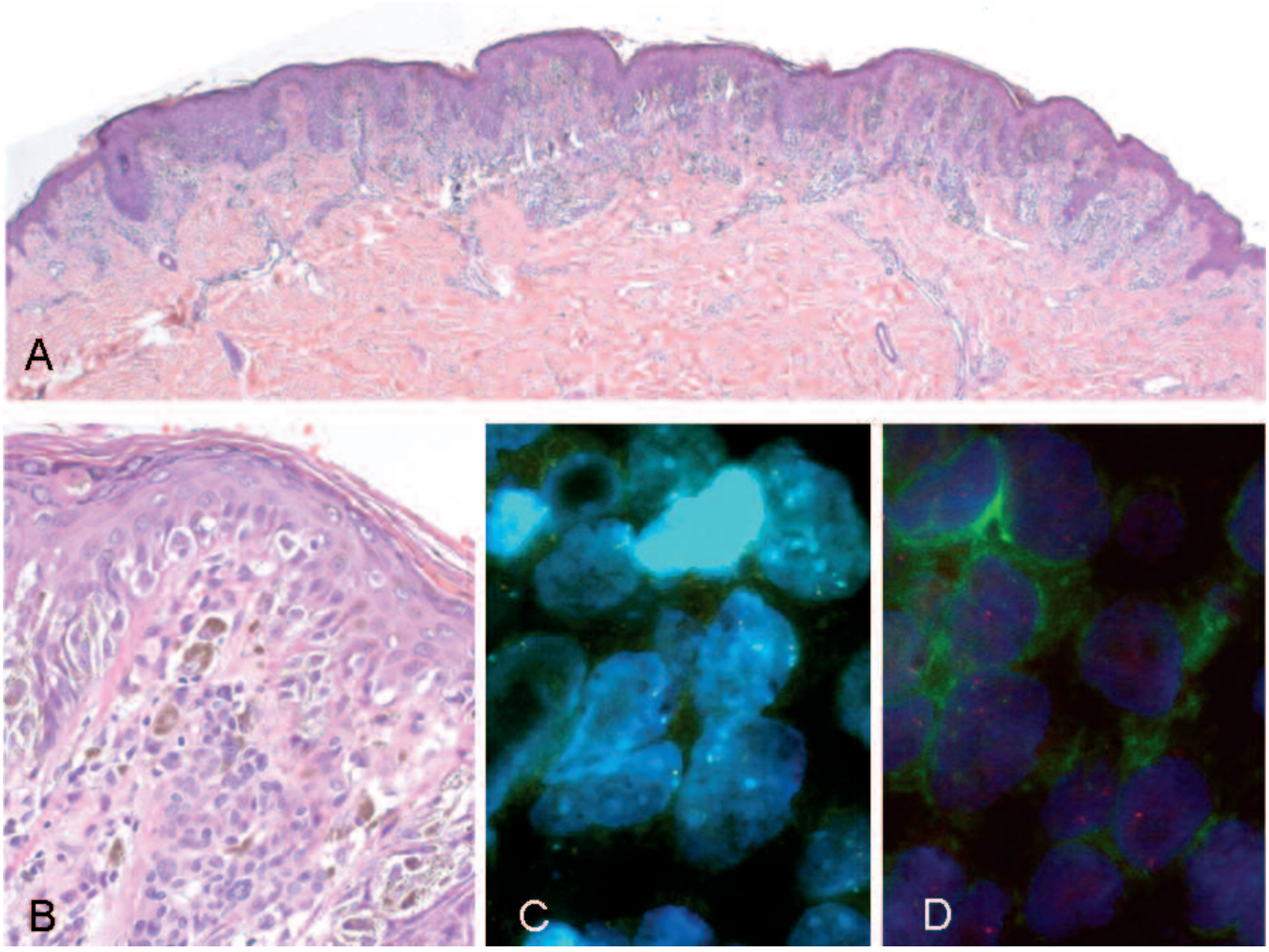

Fig. 4. Case 20: Superficial spreading melanoma of a 27-year old man. Low power-view of a symmetric intraepidermal and junctional lesion of atypical melanocytes **(A)**. High-power view shows highly atypical melanocytes that grow isolated or in small nests are strongly hyperpigmented and tend to ascend to the upper layers of the epidermis (**B**). FISH analysis with probes for 6q (Gold) and centromere 6 (Aqua) (**C**) as well as for 6p (Red) and 11q (Green) (**D**) did not reveal sufficient abnormal signals for the diagnosis of melanoma. A, x 25; B, x 200; C, D, x 1000

ambiguous and/or small melanocytic lesions.

Due to small size and/or confusing architecture (e.g. due to inflammation, cicatrisation, desmoplasia) unclear melanocytic tumors are often difficult to localize during FISH analysis. We solved this problem by identifying the lesion of interest on the H&E slides and photographing it. After destaining the FISH procedure could be performed. By comparing H&E images and DAPI pattern we were able to identify characteristic technical (wrinkles, gaps etc.) and anatomical (glands, hair follicles etc.) structures allowing the mapping of suspicious cells or cell groups. An example is given in Figure 2.

Fourteen samples from routine diagnostics were chosen for examination by FISH (see Table 3). All cases were assessed conventionally either in an internal discussion of experienced pathologists or by an external dermatopathologist (B.E.P.). The morphological diagnoses of the series comprised seven benign melanocytic neoplasms (including four Spitz nevi), six melanomas (including one lentigo maligna melanoma and two melanoma in situ cases with one being located in a melanocytic nevus (see Table 3; No. 23a and 23b)) and one melanocytic tumor of uncertain malignant potential (see Table 3; No 16). The last sample was the punch biopsy specimen corresponding to the excision biopsy diagnosed as melanoma (type lentigo maligna melanoma) (see Table 3; No 15). In this sample the FISH result and the histological diagnoses differed. In the excision biopsy (see Table 3; No. 15) the FISH result for 6p signals was just below the cut-off for malignancy, whereas the FISH result of the corresponding punch biopsy specimen led to the diagnosis of melanoma. FISH analysis of the nevus-associated melanoma (see Table 3; No. 23a and b) with benign and malignant parts was able to clearly distinguish between these two lesions (see Fig. 3). Sample number 20, which was conventionally diagnosed as superficial spreading melanoma, showed FISH results of a benign lesion with values well below the cut-off (see Fig. 4).

Table 5. Comparison of FISH results in 2 μm and 4 μm thick slices of benign melanocytic nevi.

Sample-No.	Thickness	6q*	11q*	6q-loss (%)	6p≠2 (%)+	FISH result
6	4	1.52	1.48	21	44	nevus
	2	1.09	1.05	8	65	melanoma
7	4	1.59	1.68	12	31	nevus
	2	0.75	1.03	18.33	73.33	melanoma
8	4	1.43	1.25	25	50	nevus
	2	1.00	0.97	25	68.33	melanoma
9	4	1.58	1.60	23.33	35	nevus
	2	0.77	1.17	18.33	66.67	melanoma

Increases of abnormal 6p-signals leads to a false positive label of benign melanocytic nevi as malignant melanoma. Change of other criteria did not change the diagnosis. Count of 60 or 100 nuclei. *: Ratio of signals per nucleus. +: Percentage of nuclei with more or less than 2 signals.

Table 6. Comparison of FISH results in 2 μm and 4 μm thick slices of melanomas.

Sample-No.	Dicke (mm)	6q*	11q*	6q-loss (%)	6p≠2 (%)+	FISH result
1	4	1.66	1.65	53	77	melanoma
	2	0.86	0.62	48	71	melanoma
2	4	1.29	1.60	23	67	melanoma
	2	0.97	1.30	22	71	melanoma
3	4	1.41	2.13	46	81	melanoma
	2	0.86	1.22	39	75	melanoma
4	4	1.27	2.49	83	74	melanoma
	2	0.54	1.16	63	70	melanoma
5	4	1.27	2.21	64	66	melanoma
	2	0.93	1.19	35	70	melanoma

Redution of slice thickness did not change the FISH result. Count of 100 nuclei each. *: Ratio of signals per nucleus. +: Percentage of nuclei with more or less than 2 signals.

Optimal number of tumor nuclei

The manufacturer of the FISH test suggests the examination of thirty tumour nuclei (ten nuclei from three different locations). Comparison of FISH results based on 30, 60 and 100 counted nuclei showed a decreasing degree of fluctuation around the mean with increasing number and stabilization after counting of 60 and more nuclei. However, the FISH diagnosis of "melanoma" did not change in relation to the number of cells counted (see Table 4).

Slice thickness

In routine diagnostics specimen thickness is about 2 μm. In contrast standard FISH analyses are performed by using 4-6 μm thick tissue sections. As mentioned above difficult cases of melanocytic neoplasms are often of small size. Therefore one is often confronted with the problem that additional FISH analyses cannot be performed due to lack of material after preceding serial sectioning and immunohistochemical staining. An adaptation of the FISH analysis to 2 μm specimens would be therefore highly desirable. Our comparison of the results on 2 μm and 4 μm thick sections of nevi showed an increase of 6p abnormalities due to loss of signals in the thinner specimens. This led to a subsequent change of the FISH diagnosis from "nevus" into "melanoma" in all four cases (see Table 5). The three other criteria did not change the diagnosis. In the five melanomas examined no change of diagnosis occurred after evaluation of 2 μm sections (see Table 6).

Discussion

FISH analysis is established and used in routine

diagnostics in a number of malignant tumours such as carcinomas of the breast (Hicks and Kulkarni, 2008), soft tissue tumors (van de Rijn and Fletcher, 2006), hematologic malignancies (Sreekantaiah, 2007) and lymphomas (Ventura et al., 2006). It provides further diagnostic, prognostic and therapeutic information where morphologic and immunhistochemical approaches are not conclusive.

In some melanocytic neoplasms assessment based on histomorphologic criteria and immunohistochemistry is limited and high interobserver variability has been documented (Lodha et al., 2008). Therefore, further techniques are required that lead to higher diagnostic accuracy.

In this study, we could confirm that FISH analysis is able to distinguish clear-cut benign and malignant melanocytic tumors. This result is supported by other groups that recently published their findings of FISH analysis on melanocytic lesions (Gerami et al., 2009; Morey et al., 2009; Newman et al., 2009a-c; Pouryazdanparast et al., 2009).

Furthermore, technical problems of relocalization due to the small size or confusing architecture of some lesions could easily be solved by photo-documentation of the initial H&E stained slide and comparison with structural features in the DAPI stain. With this technique it is possible to evaluate even the smallest lesions and to analyse separately different components. This can be especially important in cases of melanomas arising in benign melanocytic nevi, where a nevoid melanoma has to be excluded and staging is difficult. FISH analysis of sample No 23, which represented the former example, was able to clearly distinguish between the malignant and benign component. Newman et al. recently published a series of melanomas associated with benign nevi and nevoid melanomas, where FISH analysis was found to be a helpful tool to correct differential diagnosis and microstaging (Newman et al., 2009a).

On this basis, the next question was whether FISH analysis is useful in the diagnosis of problematic melanocytic neoplasms. We were able to show that FISH results were congruent with conventional diagnosis in eleven of fourteen cases (78%). Of the three divergent samples, one was a punch biopsy specimen (No 16) that was initially diagnosed as melanocytic neoplasm of uncertain dignity and labelled by FISH analysis as melanoma due to abnormality of 6p-signals in 67% of cells. The subsequent excision specimen of the whole lesion (No 15) confirmed the initial FISH diagnosis of malignancy, resulting in the final diagnosis of a melanoma (type lentigo maligna melanoma). FISH analysis was repeated on this specimen and now gave a result that met exactly the cut-off level (abnormal 6p signals in 63% of nuclei) leading to the incorrect diagnosis of "nevus". These two samples impressively exemplify the strength and weakness of the FISH test as a diagnostic tool when results are close to the cut-off values. Likely there will remain cases where no congruent diagnosis can be posed, as seen in one case of our series (No 20), where after external consultation of an experienced dermatopathologist (B.E.P.) the diagnosis of melanoma (type superficial spreading melanoma) was rendered, but the FISH analysis did not reveal sufficient chromosomal alterations.

In a very recent large study with a total of 497 examined melanocytic lesions the probe panel and cut-off values for FISH analysis were re-established and adapted (Gerami et al., 2009). The new criteria differ slightly from those we used, some being stricter and others being broader: 1) a gain of 11q signals in more than 38% of nuclei corresponds to an average of 2,38 or more signals per nuclei and thus lies under the previous threshold of 2.5 signals per nucleus. 2) The cut-off for gain of 6q relative to centromere 6 was increased from 31% to 40% of cells. 3) The criterion of abnormal 6p signals is modified and split into two criteria. Only a gain of 6p signals per nucleus in more than 29% of cells or relatively to centromere 6 in more than 55% of cells will be included. 4) Gain of 6q signals is not further included. We applied these modified criteria to our cohort which now led to the correct identification of samples 15, 16 and 20 as malignant melanomas (data not shown). However, on the other hand a clear malignant melanoma (No 2) was now not correctly identified and the overdiagnosis of one Spitz nevus (No 13) as malignant melanoma occurred.

Concerning ambiguous and small melanocytic proliferations further questions concern the adequate number of counted nuclei and the thickness of sections. We observed that the degree of variability of the FISH signal values is dependent on the number of counted nuclei becoming smaller with increase of counted nuclei. Though the manufacturer recommends evaluation of thirty nuclei (ten each from three different locations), we encourage to increase the cell count to sixty in order to reduce the impact of outlying values which can crucially influence the diagnosis, especially in difficult cases.

FISH analyses are usually performed on sections which are thicker than those used for routine H&E stains. However, often melanocytic tumors are small and additional material for FISH analysis is not available. We therefore developed a special FISH protocol which can be used on destained routine sections. In our series of cases, using the conventional 4 μm sections for FISH, an increase or loss of 6p-signals in more than 63% of melanocytes was a consistent criterion in all melanoma cases, whereas two other criteria (increase of 6q- and 11q-signals to equal or over 2.5) for diagnosis of malignancy were never met. Switching to the 2 μm slides, loss of 6p signals due to truncation of the nuclei was a frequent observation. This would have resulted in an incorrect diagnosis of melanoma in standard benign nevi. Only the criterion of loss of 6q-signals relative to centromere 6 signals that met the cut-off level for malignancy in four of five cases, remained reliable independent of slide thickness. Though our collective is small and additional studies will be necessary to prove our approach, performing FISH on 2 μm slides – as used

in routine diagnostics – can be discussed when sufficient material is not available and the criteria are adapted.

In summary, FISH analysis is feasible and useful on challenging melanocytic lesions. It is easy to implement our approach in every routine laboratory equipped with a fluorescence microscope. In terms of a "proof of principle" we were able to show that the FISH procedure can be adapted to destained routine H&E sections by minor changes of the normal protocol, allowing a reliable assessment and analysis of even smallest melanocytic lesions. In a number of cases this is helpful for distinguishing between benign and malignant neoplasms, although our data show that even after FISH analysis one will be confronted with ambiguous cases which demonstrate results in a grey area around the cut-off levels. Adaption of criteria as suggested by Gerami et al. (2009) may be one way to approach this problem. However, even so - as seen in our cohort - not all cases are classified correctly.

Disclosure/Conflict of interest

The FISH probes were kindly provided by Abbott.

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