Mutations and Copy Number Increase of *HRAS* in Spitz Nevi with Distinctive Histopathological Features

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Spitz nevus is a benign melanocytic neoplasm that can be difficult or impossible to histologically distinguish from melanoma. We have recently described copy number increases of chromosome 11p in a subset of Spitz nevi. To study the molecular and histological features of this group, we studied 102 Spitz nevi for 11p copy number increases using fluorescence *in situ* hybridization (FISH) on tissue arrays. Copy number increases of at least threefold were found in 12 cases (11.8%) and involved the HRAS gene on chromosome 11p. Sequence analysis of HRAS showed frequent oncogenic mutations in cases with copy number increase (8/12 or 67%), contrasting with rare HRAS mutations in cases with normal HRAS copy numbers (1/21 or 5%, *P* < 0.0001). Tumors with 11p copy number increases were larger, predominantly intradermal, had marked desmoplasia, characteristic cytological features, and had an infiltrating growth pattern. Proliferation rates in the majority of these cases were low to absent. HRAS activation by either mutation or copy number increase alone could explain several of the histological features that overlap with those of melanoma. We speculate that HRAS activation in the absence of co-operating additional genetic alterations drives the partially transformed melanocytes of these Spitz nevi into senescence or a stable growth arrest. Although there is no data suggesting that Spitz nevi with HRAS activation are at risk for progression to melanoma, future studies are warranted to assess their biological behavior more accurately. (*Am J Pathol 2000, 157:967–972*)

Spitz nevi are benign melanocytic neoplasms that can resemble melanoma lesions on histopathological examination. Sophie Spitz first described them as "juvenile melanoma" in 1948 and initially regarded them as a subset of childhood melanoma, in which there was usually a benign course.[1] Spitz nevi account for about 1% of surgically removed melanocytic nevi.[2] Although current histological criteria reliably distinguish melanoma from Spitz nevus in most cases, there is a significant overlap of criteria in a subset of cases. It is a current matter of debate whether Spitz nevus and melanoma reside at the opposing ends of a biological spectrum[3] or represent two separate entities.[4] The diagnostic uncertainty of cases with overlapping histological criteria is expressed in terms like atypical Spitz nevus, malignant Spitz nevus, and spitzoid melanoma. The lack of diagnostic selectivity of current histological criteria as well as the lack of consensus among pathologists is well documented,[5–7] and misdiagnosis of Spitz nevus as melanoma and *vice versa* is repeatedly reported.[8–10]

Previously we reported that chromosomal changes can assist with distinguishing the two entities.[11] The majority of Spitz nevi has a normal karyotype when analyzed by comparative genomic hybridization, whereas increased copies of chromosome 11p are a recurrent finding in a minority of Spitz nevi.[11,12] The aim of this study was to identify additional examples of Spitz nevi with copy number increase of chromosome 11p to determine the histopathological and molecular features of this subset. Here we provide evidence that a mutated HRAS gene drives 11p copy number increase, and is accompanied by a characteristic histopathological appearance.

Materials and Methods

Selection of Cases

Paraffin blocks of Spitz nevi were retrieved randomly from the archives of the Dermatopathology section of the Departments of Dermatology and Pathology at the University of California, San Francisco. Specifically, we performed a computer search of the database of the Dermatopathology Section with the following criteria: all cases from 1/1/98 to 12/31/98 that were assigned a main diagnosis of one of the 30 different descriptive variants of Spitz nevus that are used in our laboratory. Cases sent in as slide in consultation were excluded to avoid a bias toward unusual Spitz nevi. The request yielded 144 cases from which blocks were available.

In addition to these cases, 22 cases of the Department of Dermatology, University of Würzburg, Germany were included in the study. These cases had originally been retrieved for comparative genomic hybridization and only included Spitz nevi with at least 1 mm thickness.

Assembly of Tissue Arrays

Tissue arrays were constructed according to Kononen et al.[13] In brief, a tissue arraying instrument (Beecher Instruments, Silver Spring, MD) was used to punch 0.8 mm biopsy cores of the most cellular areas of the nevi. The biopsy cores were arrayed in recipient paraffin blocks, according to the manufacturer's instructions. Multiple sections of 6 μm thickness were cut with a microtome using an adhesive-coated tape sectioning system (Instrumedics, Hackensack, NJ). H&E sections were used for the histological examination of the biopsy cores. Only cases with at least one area with a cohesive population of neoplastic melanocytes were included in the analysis.

FISH to Formalin-Fixed Tissue Microarray Sections

Dual-color FISH was carried out on tissue sections of the array as described previously.[11] We used a BAC clone (RMC11B022) that contained HRAS for the detection of copy number increases of chromosome 11p, and a reference P1 clone (RMC11P008) for the q arm of chromosome 11. Probes were labeled with Cy3 (Amersham, Arlington Heights, IL) or with digoxygenin (Boehringer Mannheim, Indianapolis, IN) by nick-translation. Tissue sections were deparaffinized, hydrated, and pretreated for 2 to 4 minutes in 1 mol/L sodium thiocyanate at 80°C, in 4 mg/ml pepsin in 0.2 N HCl at 37°C for 4–8 minutes. After dehydration, sections were denatured in 70% formamide, 2× SSC pH 7.0, for 5 minutes at 72°C, and hybridized over 48 to 72 hours at 37°C in 10 μl of hybridization buffer containing 12.5 to 50 ng of labeled probes (50% formamide, 10% dextran sulfate, and 2× SSC, pH 7.0, 20 μg Cot-1 DNA; Life Technologies, Inc., Gaithersburg, MD). Slides were washed three times in washing solution (50% formamide in 2 × SSC, pH 7.0) at 45°C, once in 2× SSC at 45°C, once in 2× SSC at room temperature (RT), and once in 0.1% Triton X-100 in 4× SSC at RT. Subsequently, sections were incubated with 10% BSA in 4× SSC in a moist chamber at 37°C, and then with a FITC-labeled anti-digoxygenin antibody (Boehringer Mannheim, Indianapolis, IN) diluted in 4× SSC with 10% BSA. Sections were counterstained with 4,6-diamino-2-phenylindole (Sigma, St. Louis, MO) in an anti-fade solution. FISH signals were scored with a fluorescence microscope Zeiss (Jena, Germany) using a 63X objective. Criteria for copy number increase were: at least three times more test probe signals than reference signals in at least 30% of the tumor cells.

DNA Sequence Analysis

DNA was extracted from 30-μm sections from which the tumor-bearing areas were dissected manually with a scalpel under a dissecting microscope. Two to three sections were collected in a 0.5 ml tube and after washing with xylene and ethanol were incubated at 55°C with 0.4 mg/ml proteinase K (Life Technologies, Inc.) in PCR buffer (Perkin Elmer) containing 0.5% Tween 20 for three days. Fresh proteinase K was added every 24 hours to a final concentration of 0.4 mg/ml.

HRAS codon 12 primers were 5′-AGGAGACCCTG-TAGGAGGA-3′ (forward) and 5′-CGCTAGGCTCACCTC-TATAGTG-3′ (reverse) and codon 61 primers were 5′-CTGCAGGATTCCTACCGGA-3′ and 5′-ACTTGGTGTT-GTTGATGGCA-3′. PCR was carried out in a Gene Amp PCR System 9700 Thermal Cycler (Perkin Elmer) in 25 μl reaction volumes. Each PCR reaction contained 3.5 mmol/L MgCl$_2$, 0.2 mmol/L dNTP, 0.625 U *Taq* gold polymerase (Perkin Elmer), 1X PCR Buffer II, 0.5 μmol/L each of forward and reverse primer, and 50 to 300 ng of genomic DNA. PCR cycling conditions were as follows: 95°C for 15 minutes followed by 35 cycles of 95°C for 15 seconds, 55°C for 30 seconds, and 72°C for 60 seconds, and a final hold at 72°C for 10 minutes.

Before sequencing, PCR products were purified using the PCR product pre-sequencing kit (Amersham) to remove excess primers and nucleotides. Fluorescent DNA sequencing was carried out using Big Dye terminator sequencing chemistry (PE Applied Biosystems). Briefly, 30 to 50 ng of purified PCR product and 3.2 pmol of sequencing primer were used for sequencing in a 15 μl reaction according to the manufacturer's instructions. The sequencing products were purified using a Sephadex G50 column, dried in a vacuum concentrator and resuspended in 3 μl of gel loading buffer (83% deionized formamide, 17% gel loading dye) (PE Applied Biosystems). 0.5 μl of the sample was then loaded on a denaturing sequence gel on an ABI automated DNA sequencer. All samples were sequenced in both forward and reverse directions to confirm the presence or absence of mutations. Data were analyzed using the Sequencer software (Gene Codes, Ann Arbor, MI)

Immunohistochemistry

Proliferation was assessed using an antibody against Ki-67 (Mib-1, Beckman Coulter, Fullerton, CA, dilution 1:500) according to the manufacturer's instructions.

Statistical Analysis

χ^2 tests were used to calculate the associations of the histological criteria and the mutations of HRAS with copy number increases of chromosome 11p. The Bonferroni adjustment was used to correct for multiple comparisons made and $P < 0.01$ was regarded as statistically significant.
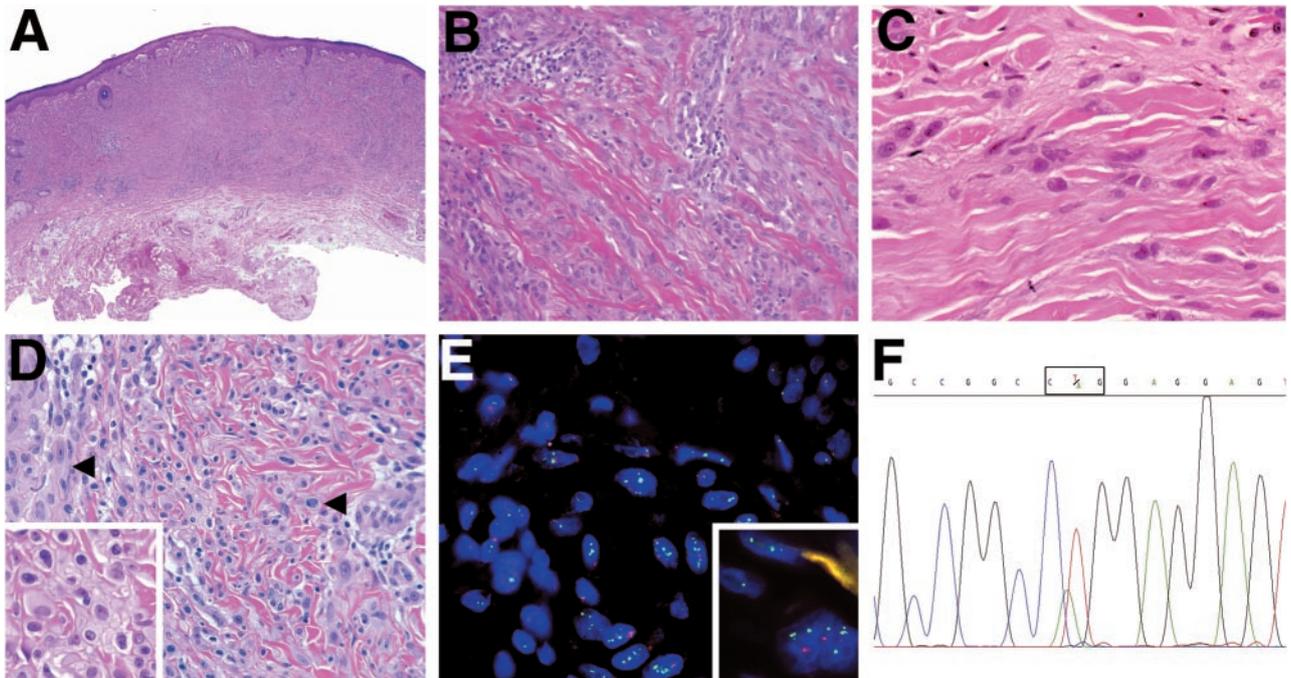
Figure 1. Histological features associated with copy number increase of 11p (**A–D**). **A:** Compound, predominantly intradermal Spitz nevus. **B:** Melanocytes with ample amphophilic cytoplasm and vesicular nuclei with prominent nucleoli between haphazardly arranged collagen fibers. **C:** Melanocytes with pleomorphic nuclei singly and in clusters between thickened collagen fibers. **D:.** Cells are surrounded by a fine eosinophilic rim (**arrowheads** and **inset**). **E:** Dual-color FISH with a probe containing HRAS (green signals) and a reference probe for chromosome 11q (red signals) showing a central nest of melanocytes with multiple green signals surrounded by normal cells. **F:** Sequence profile of a portion of exon 2 of HRAS showing a A→T mutation of codon 61 (**boxed**).

Results

FISH Analysis of Chromosome 11p Copy Number using Tissue Arrays

High-quality hybridizations of cases in which tumor cells could be definitively identified were obtained from 102 cases. This yield of 61.4% is relatively low compared to arrays that we have constructed from melanomas. Most of the cases that could not be analyzed were very small Spitz nevi that consisted only of single cells or small nests of junctional melanocytes so that the neoplastic melanocytes could not be reliably recognized in the array. 39 cases (38.2%) were from male and 61 (59.8%) from female patients, in two cases the gender was not known. The mean age was 30.0 years. 52 (51%) of the cases had features of the pigmented spindle cell nevus variant of Spitz (PSCN).

The hybridization efficiency could be assessed by counting the hybridization signals in normal epidermal keratinocytes, which were present in many of the biopsies. The average copy numbers for test and reference probes in normal keratinocytes were 1.7 and 1.6, respectively. Hybridizations were analyzed of three separate sections of the array, and counts from two or more sections were available for 47 (46.1%) cases. In 45 (95.7%) of these, the result of the separate counts were identical, in one case a definitive copy number increase was seen in one analysis, and was not found in the cells present in the other section. Copy number increases were only scored if more than 30% of the tumor cells had at least threefold increased signals of 11p when compared to the reference probe on 11q. According to these criteria, copy number increase of 11p was found in 12 (11.8%) cases (Figure 1E). The average thickness of cases with copy number increases was significantly greater than the thickness of cases with normal copy number of 11p (1.1 mm *versus* 0.6 mm, $P = 0.01$). The frequency of copy number increases within the randomly retrieved set of cases was 6/84 (7.1%), whereas of the 18 cases that had been selected for thickness, 6 (33.3%) showed copy number increases of chromosome 11p.

Mutations of HRAS

The HRAS gene is located at the tip of chromosome 11p at 11p15.5[14] and is a candidate oncogene targeted by the copy number increase found in Spitz nevi. Oncogenic mutations of HRAS typically involve codons 12 or 13 in exon 1 and codon 61 in exon 2.[15] We obtained genomic sequences of exons 1 and 2 of HRAS of 9 cases in which FISH detected an copy number increase of chromosome 11p, the three remaining cases with 11p copy number increase were not informative. In addition, we obtained sequences of 13 cases in which FISH showed normal copy numbers of chromosome 11p. Five of nine cases (56%) with 11p copy number increase had HRAS mutations, significantly more ($P = 0.002$) than in the cases with normal 11p copy numbers, in which only one (8%) had a mutation. Three mutations were 61Gln→Leu (Figure 1F), two 61Gln→Arg, and one 12Gly→Arg.

Additionally, we sequenced HRAS in 11 Spitz nevi used for our previous analysis using comparative

Table 1. Histological Features Associated with Copy Number Increase of Chromosome 11p in Spitz Nevi

Histological feature	*n*	11p Amp	11p Normal	*P*
Single cells between collagen bundles	17 (22.5%)	5 (41.6%)	12 (13.3%)	0.014305
Desmoplasia	26 (25.5%)	8 (66.7%)	18 (20.0%)	0.000552
Vesicular nuclei	36 (35.3%)	10 (83.3%)	26 (28.9%)	0.000210
Ample eosinophilic cytoplasm	26 (25.5%)	8 (66.7%)	18 (20.0%)	0.000493
Eosinophilic membranes	8 (7.8%)	5 (41.6%)	3 (3.3%)	0.000004
Marked nuclear pleomorphism	30 (29.4%)	10 (83.3%)	20 (22.2%)	0.000015

Percentages in the *n* column refer to the total number of 102 cases. The other percentages refer to the number of cases in the respective column. The *P* value was calculated using the χ^2 test on 2-way tables of the respective histological feature and the copy number increase of 11p.

genomic hybridization (CGH).[11] We found HRAS mutations in all three cases (100%) in which CGH detected increased copies of chromosome 11p. All of these involved codon 61; two cases had a transition of glutamine to arginine, and the other to leucine. The seven cases in which CGH found normal copy numbers of chromosome 11p had wild-type sequences of both exons of HRAS.

Of the total of 33 Spitz nevi in which HRAS was sequenced, 8/12 cases (67%) with amplified 11p had HRAS mutations, significantly more ($P < 0.0001$) than in the cases with normal copies of chromosome 11p (1/21 or 5%).

Based on the height of the peaks of the electropherograms the approximate mutant to wild type allelic ratios were analyzed. Three cases with 11p copy number increase had mutant to wild type allelic ratios of 2 (Figure 1F), three cases had ratios of 1, and in two cases the ratios were 0.7 and 0.5. In the single case with normal copy number of 11p and a mutated HRAS the allelic ratio was 0.3. These findings indicate that in the majority of cases the 11p copy number increase is associated with an increased dosage of mutated HRAS. A ratio smaller than 1 in the two cases with 11p copy number increase could be explained by a considerable contamination of the DNA from normal cells or by concomitant copy number increase of the wild type allele.

Histopathological Features of Cases with Amplified Chromosome 11p

Copy number increases were most common in compound or predominantly intradermal Spitz nevi (11/47 or 23.4%, Figure 1A), and only rarely occurred in the pigmented spindle cell variant of Spitz nevus (1/52 or 1.9%; $P = 0.0007$). Interestingly, the cases with copy number increase of 11p frequently showed several histological features that occurred infrequently in the cases with normal copy number of chromosome 11p (Table 1). The tumors commonly showed extensive areas with single cells splayed between collagen bundles at the base (Figure 1, B and C) resulting in a pattern of haphazardly arranged collagen and marked desmoplasia. Cells typically had vesicular nuclei with delicate nuclear membranes, and ample amphophilic cytoplasm (Figure 1B). Cells at the base frequently seemed to be surrounded by thin, eosinophilic membranes (Figure 1D, arrowheads), that were only seen in 3 of the 87 cases that had normal copy numbers of 11p. These membranes stained positive with a reticulin stain (not shown). Cases with 11p copy number increases were also notably more pleomorphic

with nuclei varying in sizes and shapes and staining intensity and in some cases intranuclear inclusions (Figure 1C). These features were also present in the three cases that had shown copy number increase of chromosome 11p by CGH in a previous study.[11] The four cases that had a copy number increase of 11p and no detectable HRAS mutation, did not seem to differ from those with copy number increase and mutation. All histological findings listed in Table 1 were found in at least two of these four cases. No association of 11p copy number increase with patient age or sex was found.

Cell proliferation using a Ki-67 antibody was assessed in all 12 cases with copy number increases of 11p and 24 cases with normal copy numbers of chromosome 11p. Immunostains were performed on sections of the original blocks to allow inspection of sufficient numbers of cells. In 11/12 (92%) of the cases with 11p copy number increase and 20/24 (83%) with normal 11p copy numbers the number of labeled nuclei ranged from 0 to at most 1%. A total of five cases had higher labeling rates: Four had normal 11p copy numbers and a labeling index of at most 5%, mostly affecting melanocytes at the dermo-epidermal junction. One case that had an average of 6 copies of chromosome 11p by FISH analysis and a 61G->A mutation had a labeling index of 10%. It was a superficial biopsy, so that the proliferation rate in deeper areas of the nevus could not be assessed.

Discussion

The present study shows that copy number increases of chromosome 11p in Spitz nevi are associated with particular histological features and with mutations of the HRAS gene, which maps to the distal end of chromosome 11p. The frequent mutations of HRAS in cases with 11p copy number increase, together with infrequent mutations in cases with normal copies of chromosome 11p make it likely that mutated HRAS is the target of selection forces of the process leading to increasing copy numbers. Amplification of wild-type HRAS is sufficient to transform sensitive cells.[16] Therefore, the fact that some cases with copy number increase of 11p had no detectable HRAS mutations does not necessarily imply that it is not the gene targeted by the copy number increase. Furthermore, some mutations might have remained undetected because of contamination by normal cells in the microdissected tissue. In keeping with this possibility, we note that all three cases with increased copies of chro-

mosome 11p detected by CGH[11] had detectable mutations. To detect chromosomal copy number changes by CGH, the DNA contributed by normal cells should be below 50%. However, we cannot rule out that additional genes residing on chromosome 11p drive the copy number increase.

HRAS belongs to the family of ras genes, which contains the two additional members KRAS and NRAS.[15] NRAS mutations are found in about 25% of primary cutaneous melanomas, (see[17] for review). Interestingly, HRAS itself is rarely mutated in melanoma.[18,19] Mutations of NRAS are also described in congenital melanocytic nevi,[20] which, depending on their size, are at increased risk to progress to melanoma.[21] In contrast, Spitz nevus is not regarded as a precursor of melanoma. There are no reports of melanomas arising in a pre-existing Spitz nevus. However, this occurrence could be difficult to detect or prove because of the histopathological similarities between Spitz nevus and melanoma. Follow-up over 6 to 8 years of the cases with 11p copy number increase from our initial study[11] that we report here to have HRAS mutations showed no evidence of metastasis. If Spitz nevi with 11p copy number increases or HRAS mutations represented a precursor to melanoma, one would also expect a higher frequency of HRAS mutations or amplifications in melanoma in general, which is not the case. To date we have studied over 150 melanomas by CGH analysis and have only found a single case with an amplification of HRAS.[22] It was an acral melanoma and the change involved only a small fraction of the distal chromosome 11p, different from the findings in Spitz nevi, in which the entire p-arm is gained.[11] These findings indicate that the presence of HRAS mutations and/or copy number increases in a subset of Spitz nevus does not point toward a highly increased risk of progression to melanoma. However, future studies are necessary to assess the behavior of Spitz nevi with HRAS activation more accurately.

Significant amplification (>10 fold) of ras genes has been observed in a variety of human tumors including bladder carcinoma,[23] gastric carcinoma,[24] and ovarian carcinoma.[25,26] However, the overall incidence of ras gene amplification in human neoplasia has been estimated to be less than 1%.[16,27]

It is well established that cancer is not caused by a mutation in a single gene, but requires genetic alterations affecting several pathways.[28] Ras genes are on their own insufficient to induce tumorigenesis but need cooperating oncogenes or inactivation of p53 or p16 to transform normal cells.[15,29] This is illustrated in neoplasms of the colon, where hyperplastic polyps, which only rarely progress to cancer, frequently have mutations of KRAS. In contrast, dysplastic polyps with a high risk of progression initially acquire mutations of APC which are followed by ras mutations.[30] These findings indicate that not only the type of genes whose functions are altered is important, but also the order in which these alterations occur. The HRAS activation in Spitz nevi may be analogous to the KRAS activation in hyperplastic polyps. It could result in incompletely transformed melanocytes that share several features with melanoma cells but have a limited proliferative capacity. Albino and co-workers reported that transduction of normal human melanocytes with a murine retrovirus carrying a mutated HRAS results in anchorage independence and altered morphology, not tumorigenicity.[31] However, when cultured for several months the cells became tumorigenic. This was accompanied by a marked alteration of the chromosomal complement similar to melanoma. The initial phenotype of this model has some similarities to Spitz nevus, however our data together with the view of Spitz nevi as stable lesions indicate that they seem somehow to be protected from acquiring subsequent genetic changes that might make them progress to melanoma. This could be due to senescence induced by exhausted telomeres and a permanent G_1 arrest. This would be consistent with our finding of a very low to absent proliferation rate in the tumors with a mutated or amplified HRAS gene. Expression of activated ras in normal cells induces a senescence-like state by up-regulation of p16 and p53.[32] Spitz nevi have to undergo multiple rounds of cell division to reach their average size, making it likely that control over entry into S-phase is at least temporarily altered. In addition, it is likely that the melanocytes of Spitz nevi are resistant to pro-apoptotic signals that result from signaling imbalances provoked by oncogene activation,[33] since their apoptotic rate is low.[34] In fact, loss of heterozygosity at chromosome 9p21 has been demonstrated in a minority of Spitz nevi,[35] and we have observed two Spitz nevi in which CGH found loss of chromosome 9p occurring with copy number increase of 11p (data not shown). Chromosome 9p21 is the location of INK4A, which encodes the p16 and p14[ARF] proteins that are important components of the G1 checkpoint and p53 dependent apoptotic pathway, respectively.[36] However, in the vast majority of Spitz nevi there seems to be no obvious loss at this locus. Future, more detailed studies are needed to clarify the status of the INK4A locus in Spitz nevus.

The major histological criteria for differentiation of Spitz nevus from melanoma include overall lesional symmetry, predominance of nests of melanocytes over single cells at the dermo-epidermal junction, maturation of cells, absence of significant pagetoid spread or the confinement of that change to the center of the lesion, and the presence of Kamino bodies.[37] Using these criteria and others, the majority of Spitz nevi can be distinguished reliably from melanoma. Diagnostic uncertainty rises if the cases of Spitz nevus in which the histology deviates by one or more criteria from the "stylized depiction" of Spitz nevus.[3] The Spitz nevi with 11p copy number increases were significantly thicker, larger in diameter, and were associated with distinct histological features. Typically, they were predominantly intradermal with a pronounced desmoplastic stroma reaction. The cells were larger and had pleomorphic nuclei. They had in common a pattern of infiltrating growth at their base, characterized by extensive zones of single cells situated between collagen bundles with fine eosinophilic rims surrounding their cytoplasm. Activation of the ras pathway has been shown to cause an infiltrating growth pattern in breast cancer[38] and recently ras has been linked to the regulation of cell size.[39] It is thus conceivable that the histopathological

findings associated with 11p copy number increase are a consequence of the activation of the ras pathway.

Our data further strengthens the notion that Spitz nevus shares several of the hallmarks of cancer[28] with melanoma. We believe that the major difference that separates Spitz nevus from melanoma is genomic stability.[11,12] In contrast to the limited chromosomal alterations found in Spitz nevi, CGH profiles of the majority of primary melanomas indicate pronounced genomic instability.[12,22] Evidence is emerging that genomic instability is genetically controlled.[40] If the control over genomic stability is not altered in time, the probability of acquiring the necessary number of genetic events for full transformation is minimal.[41] The partially transformed cell that results would then exhaust its proliferative capacity and enter a stage of arrest that would make subsequent changes unlikely. As genomic instability in melanoma is expressed on the chromosomal level, the determination of chromosomal aberrations could represent a helpful diagnostic tool in Spitz nevi that share overlapping features with melanoma.

Acknowledgment

References

1. Spitz S: Melanoma of childhood. Am J Pathol 1948, 24:591–609
2. Casso EM, Grin-Jorgensen CM, Grant-Kels JM: Spitz nevi. J Am Acad Dermatol 1992, 27:901–913
3. Piepkorn M: On the nature of histologic observations: the case of the Spitz nevus. J Am Acad Dermatol 1995, 32:248–254
4. Shapiro PE: Spitz nevi. J Am Acad Dermatol 1993, 29:667–668
5. Barnhill RL, Argenyi ZB, From L, Glass LF, Maize JC, Mihm MC, Jr., Rabkin MS, Ronan SG, White WL, Piepkorn M: Atypical Spitz nevi/tumors: lack of consensus for diagnosis, discrimination from melanoma, and prediction of outcome. Hum Pathol 1999, 30:513–520
6. Spatz A, Ruiter D, Hardmeier T, Renard N, Wechsler J, Bailly C, Avril MF, Kwee H, Bastian BC, Hill C, De Potter C, Prade M: Melanoma in childhood: an EORTC-MCG multicenter study on the clinico-pathological aspects. Int J Cancer 1996, 68:317–324
7. Farmer ER, Gonin R, Hanna MP: Discordance in the histopathologic diagnosis of melanoma and melanocytic nevi between expert pathologists. Hum Pathol 1996, 27:528–531
8. Peters MS, Goellner JR: Spitz nevi and malignant melanomas of childhood and adolescence. Histopathology 1986, 10:1289–1302
9. Okun MR: Melanoma resembling spindle and epithelioid nevus. Arch Dermatol 1979, 115:1416–1420
10. Goldes J, Holmes S, Satz M, Cich J, Dehner L: Melanoma masquerading as Spitz nevus following acute lymphoblastic leukemia. Pediatr Dermatol 1984, 1:295–298
11. Bastian BC, Wesselmann U, Pinkel D, LeBoit PE: Molecular cytogenetic analysis of Spitz nevi show clear differences to melanoma. J Invest Dermatol 1999, 113:1065–1069
12. Bastian BC, LeBoit PE, Hamm H, Bröcker EB, Pinkel D: Chromosomal gains and losses in primary cutaneous melanomas detected by comparative genomic hybridization. Cancer Res 1998, 58:2170–2175
13. Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Schraml P, Leighton S, Torhorst J, Mihatsch MJ, Sauter G, Kallioniemi OP: Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med 1998, 4:844–847
14. Chang EH, Gonda MA, Ellis RW, Scolnick EM, Lowy DR: Human genome contains four genes homologous to transforming genes of Harvey and Kirsten murine sarcoma viruses. Proc Natl Acad Sci USA 1982, 79:4848–4852
15. Barbacid M: ras genes. Annu Rev Biochem 1987, 56:779–827
16. Pulciani S, Santos E, Long LK, Sorrentino V, Barbacid M: ras gene amplification and malignant transformation. Mol Cell Biol 1985, 5:2836–2841
17. Herlyn M, Satyamoorthy K: Activated ras: yet another player in melanoma? Am J Pathol 1996, 149:739–744
18. Jiveskog S, Ragnarsson-Olding B, Platz A, Ringborg U: N-ras mutations are common in melanomas from sun-exposed skin of humans but rare in mucosal membranes or unexposed skin. J Invest Dermatol 1998, 111:757–761
19. van Elsas A, Zerp SF, van der Flier S, Krüse KM, Aarnoudse C, Hayward NK, Ruiter DJ, Schrier PI: Relevance of ultraviolet-induced N-ras oncogene point mutations in development of primary human cutaneous melanoma. Am J Pathol 1996, 149:883–893
20. Carr J, Mackie RM: Point mutations in the N-ras oncogene in malignant melanoma and congenital naevi. Br J Dermatol 1994, 131:72–77
21. Egan CL, Oliveria SA, Elenitsas R, Hanson J, Halpern AC: Cutaneous melanoma risk and phenotypic changes in large congenital nevi: a follow-up study of 46 patients. J Am Acad Dermatol 1998, 39:923–932
22. Bastian BC, Kashani-Sabet M, Hamm H, Godfrey T, Moore II DH, Bröcker EB, LeBoit PE, Pinkel D: Gene amplifications characterize acral melanoma and permit the detection of occult cells in the surrounding skin. Cancer Res 2000, 1973, 60:1968
23. Fujita J, Srivastava SK, Kraus MH, Rhim JS, Tronick SR, Aaronson SA: Frequency of molecular alterations affecting ras protooncogenes in human urinary tract tumors. Proc Natl Acad Sci USA 1985, 82:3849–3853
24. Bos JL, Verlaan-de Vries M, Marshall CJ, Veeneman GH, van Boom JH, van der Eb AJ: A human gastric carcinoma contains a single mutated and an amplified normal allele of the Ki-ras oncogene. Nucleic Acids Res 1986, 14:1209–1217
25. Filmus JE, Buick RN: Stability of c-K-ras amplification during progression in a patient with adenocarcinoma of the ovary. Cancer Res 1985, 45:4468–4472
26. Yang-Feng TL, Li SB, Leung WY, Carcangiu ML, Schwartz PE: Trisomy 12 and K-ras-2 amplification in human ovarian tumors. Int J Cancer 1991, 48:678–681
27. Yokota J, Tsunetsugu-Yokota Y, Battifora H, Le Fevre C, Cline MJ: Alterations of myc, myb, and rasHa proto-oncogenes in cancers are frequent and show clinical correlation. Science 1986, 231:261–265
28. Hanahan D, Weinberg RA: The hallmarks of cancer. Cell 2000, 100: 57–70
29. Weinberg RA: Oncogenes, antioncogenes, and the molecular bases of multistep carcinogenesis. Cancer Res 1989, 49:3713–3721
30. Jen J, Powell SM, Papadopoulos N, Smith KJ, Hamilton SR, Vogelstein B, Kinzler KW: Molecular determinants of dysplasia in colorectal lesions. Cancer Res 1994, 54:5523–5526
31. Albino AP, Sozzi G, Nanus DM, Jhanwar SC, Houghton AN: Malignant transformation of human melanocytes: induction of a complete melanoma phenotype and genotype. Oncogene 1992, 7:2315–2321
32. Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW: Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. Cell 1997, 88:593–602
33. Evan G, Littlewood T: A matter of life and cell death. Science 1998, 281:1317–1322
34. Wesselmann U, Becker LR, Brocker EB, LeBoit PE, Bastian BC: Eosinophilic globules in spitz nevi: no evidence for apoptosis. Am J Dermatopathol 1998, 20:551–554
35. Healy E, Belgaid CE, Takata M, Vahlquist A, Rehman I, Rigby H, Rees JL: Allelotypes of primary cutaneous melanoma and benign melanocytic nevi. Cancer Res 1996, 56:589–593
36. Chin L, Pomerantz J, DePinho RA: The INK4a/ARF tumor suppressor: one gene–two products–two pathways. Trends Biochem Sci 1998, 23:291–296
37. Weedon D: Skin Pathology. Edinburgh, Churchill Livingstone, 1997
38. Moon A, Kim MS, Kim TG, Kim SH, Kim HE, Chen YQ, Kim HR: H-ras, but not N-ras, induces an invasive phenotype in human breast epithelial cells: A role for MMP-2 in the h-ras-induced invasive phenotype. Int J Cancer 2000, 85:176–181
39. Prober DA, Edgar BA: Ras1 promotes cellular growth in the Drosophila wing. Cell 2000, 100:435–446
40. Lengauer C, Kinzler KW, Vogelstein G: Genetic instabilities in human cancers. Nature 1998, 396:643–649
41. Loeb LA: Mutator phenotype may be required for multistage carcinogenesis. Cancer Res 1991, 51:3075–3079