Controversies in Atypical (Dysplastic) Melanocytic Nevi

Grading and Progression

Salvador J. Diaz-Cano
Ehab A. Husain

Skin Cancer. A Multidisciplinary Symposium
Royal College of Pathologists, 2003

Atypical Melanocytic Nevus

Does AMN Exist?

- Barnhill et al. Arch Dermatol 1990;126:463–5
  - These findings confirm the existence of dysplastic nevi by histopathologic criteria as early as 1950…
- Black and Hunt. Am J Surg Pathol 1990;14:44–52
  - This study supports the validity of the dysplastic nevus as a clinical and pathologic entity
- Cook, Fallowfield. Histopathology 1990;16:29–35
  - The recognition of severely aberrant differentiation in the form of classic dysplastic nevus is worthwhile,… it confers a very high risk of developing melanoma

Significance and Controversy of AMN

- AMN is an important marker of melanoma risk (>8–fold increased risk of malignant melanoma)
- Minimal criteria must be based on both nuclear atypia and architectural disorder
- Subjective definition for grading AMN has resulted in no consensus on that matter

AMN Histopathologic Diagnosis

- Major Criteria
  - Basilar proliferation of atypical melanocytes (three rete ridges beyond the dermal component)
  - Lentiginous or epithelioid growth pattern
- Minor Criteria
  - Fibrosis
  - Neovascularization
  - Inflammatory response
  - Fusion of rete ridges

Diagnosis requires both major + 2 minor criteria

Clemente et al. Hum Pathol 1991;22:313-9

AMN Distribution 1989–1998

Pozo L, Diaz-Cano SJ. Arch Dermatol 2000;136:934-935

AMN. Histologic Criteria

- **Architectural disorder**
  - Lentiginous melanocytic hyperplasia
  - Upward melanocyte migration
  - Nesting variation and bridging
- **Cytological atypia**
  - Nuclear enlargement (basal keratinocyte)
  - Prominent nucleolus
  - Pleomorphism
  - Hyperchromatism

AMN. Histologic Criteria

- Tissue response
  - Lymphocytic infiltrate
  - Fibroplasia (lamellar

Grading Dysplasia

- 60 melanocytic lesions, 10 from each group, including:
  - common MN, Clark nevus, AMN-mild, AMN-moderate, AMN-severe, primary MM
- Concordance for diagnosing dysplastic nevi was 77% (kappa statistic 0.55-0.84)
- Reliable distinction of dysplastic nevi from conventional nevi and MM
- Concordance in grading
  - 35% to 58% (kappa value 0.38-0.47)
  - 16% to 65% (kappa value 0.05-0.24)

Duncan et al. J Invest Dermatol 1993;100:318S-321S

Critical Analysis of AMN

- 123 AMN classified according to standard criteria: 31 AMN-mild, 61 AMN-moderate, 31 AMN-severe
- 40 cases associated with the atypical-mole syndrome
- Systematic evaluation of architectural and cytological features (>30 variables), as well as tissue response & regression
- Multivariate statistical analysis (grading and association with atypical mole syndrome)

Architectural Features in AMN
100
90
80
70
60
50
40
30
20
10
0
Junctional Asymmetry
Suprabasilar Melanocytes
Regression
AMN-mild
AMN-moderate
AMN-severe

Nuclear Features in AMN
120
100
80
60
40
20
0
Nuclear Pleomorph
Coarse Chromatin
Prominent Nucleoli
Anisokaryosis
AMN-mild
AMN-moderate
AMN-severe

AMN-mild

Pozo L, et al. Am J Clin Pathol 2001;115:194-204

AMN-moderate

Pozo L, et al. Am J Clin Pathol 2001;115:194-204

AMN–severe

Pozo L, et al. Am J Clin Pathol 2001;115:194-204

Grading Dysplasia in AMN

Grading Dysplasia in AMN

- Histologic criteria reliably distinguish high-grade AMN (severe dysplasia) from low-grade AMN (mild-moderate dysplasia) only, based on the application of major features (3 nuclear and 2 architectural)
- Low-grade AMN are significantly associated with histologic regression. Its meaning needs further analyses

Pozo L, et al. Am J Clin Pathol 2001;115:194-204

Grading Dysplasia in AMN

The accuracy of clinical diagnosis of moderate dysplasia was low (20%); however, all cases of severe dysplasia with or without in situ melanoma were diagnosed correctly.

Increasing darkness and confluence of pigmentation in these dysplastic melanocytic nevi correlated with increasing severity of dysplasia.

Kelly et al. J Am Acad Dermatol 1986; 14:1044-52

Linear Progression in Melanocytic Lesions

Molecular Pathways in Cutaneous MM

Gene Abnormalities in Lesions of Patients with Sporadic MM

Gene	*Abnormalities*	*Frequency (%)*
p16^{INK4}	Mutation/deletion/silencing	25–40
Ras	Mutation	5–25
PTEN	Mutation/deletion	6
p53	Mutation	5
β-Catenin	Mutation	3
Fas	Mutation	3
Others		<1
PKC-α	Mutation	
c-myb	Mutation	
CDK4	Mutation	
NF-1	Mutation/deletion	
EWS-ATF	Translocation	

Multistep Tumorigenesis
Cell Kinetics
Cell Survival
Neoangiogenesis
Invasion
A
Component
Acquired Capability
Example of Mechanism
Self-sufficiency in growth signals
Activate H-Ras oncogene
Insensitivity to anti-growth signals
Lose retinoblastoma suppressor
Evading apoptosis
Produce IGF survival factors
Limitless replicative potential
Turn on telomerase
Sustained angiogenesis
Produce VEGF inducer
Tissue invasion & metastasis
Inactivate E-cadherin
Genetic Instability
· Chromosomal
· Microsatellite
B
Cancer

Progression in AMN-MIS
• Atypical (dysplastic) melanocytic nevi (AMN) and melanomas in situ (MIS) are heterogeneous conditions, both biologically and morphologically.
• The concept of progression to invasive malignant melanomas (MM) is still controversial for these lesions.
• No evaluation on cell kinetics (proliferation/ apoptosis) and cell cycle regulators is available by topographic compartments.

Topographic Analysis of AMN
Topographic Compartments
Junctional
Superficial Dermal
Deep Dermal

Topographic Analysis of AMN
Compartments for Blood Vessel Count
Superficial
Deep
Peri-lesional
Extra-lesional

AMN. Junctional Compartment
7
6
5
4
%
3
2
1
0
Low-grade AMN
High-Grade AMN
Ki-67 Index
ISEL Index

AMN. Dermal Compartment
6
5
4
% 3
2
1
0
Low-Grade AMN
High-Grade AMN
Ki-67 Index
ISEL Index

Proliferation in AMN
Low-Grade
High-Grade

Apoptosis in AMN
Low-Grade
High-Grade

Regression in AMN

- Melanocyte dropout with melanophages
- Prominent vascular reaction
- Fibrosis
- Lymphocyte infiltrate

Natural History of AMN

- … the decrease in counts of dysplastic nevi associated with increasing age is only partly explained by the disappearance of nevi over time…

Halpern et al. J Am Acad Dermatol 1993;29:51-7

Telomerase Activity in AMN
PCR-based TRAP in 33 common MN, 8 AMN, 38 MM, and 4 MM metastases
140
120
100
80
60
40
20
0
TRAP
Common MN
AMN
MM
MM Metastases
Miracco et al. Hum Pathol 2000;31:1018-21

Telomerase Activity in AMN
Telomerase activity correlated with Ki-67 and closer with repp86 expression
Discriminate clearly benign from malignant, but not common MN from AMN
TRAP assay, Ki-67 (G1,S,G2,M), repp86 (S, G2,M) in 72 MN and 16 MM

Clonal Evolution in MM

- Data obtained … suggest a clonal derivation of VGP cells from RGP
  - Needle microdissection, DNA extraction, PCR and CGH to determine DNA copy number changes

    Wiltshire et al. Cancer Res 1995;55:3954–7
- In-transit melanoma metastases reveal no significant intratumor heterogeneity suggesting a clonal origin
  - Evaluated by LOH microsatellite markers after laser capture microdissection

    Nakayama et al. Am J Pathol 2001;158:1371–8

DNA Repair and AMN

- Individuals with low DNA repair capacity (DRC) and low tanning ability or dysplastic nevi had a higher risk of cutaneous MM
- Statistically significant interactions between DRC and dysplastic nevus status
  - DRC evaluated in lymphocytes with the host-cell reactivation assay

Landi et al. J Natl Cancer Inst 2002;94:94–101

Genetic Instability in AMN

- LOH at 1 loci detected in 17/54 common MN and 4/6 AMN
- AMN might be defined as a monoclonal and genetically unstable , but limited melanocytic proliferation
  - LOH and microsatellite pattern analysis of 4 polymorphic loci in 54 common MN and 6 AMN

Rubben et al. Recent Results Cancer Res 2002;160:100-10

Genetic Instability in AMN

- No significant differences of the protein expression were found between MSS and MSI-L lesions
  - Analysis of PCR-based microsatellite pattern and hMLH1, hMSH2 and hMSH6 expression in 19 common MN, 22 AMN and 9 cutaneous MM

Hussein et al. Am J Dermatopathol 2001;23:308-14

p16INK4a and *TP53* in AMN

- Hemizigous deletions in 9/12 informative cases for *p16INK4a* locus and 6/10 for *TP53* locus
- Mutation analysis:
  - 4 for *p16INK4a*: 3 missense, 1 intronic
  - 2 for *TP53*: 2 missense
- Methods:
  - Microdissected AMN samples, PCR-based LOH, SSCP, and direct DNA sequencing

Lee et al. Biochem Biophys Res Commun 1997;237:667-72

TP53 Mutations in AMN

- *TP53* mutations in 2/11 common MN and 2/5 dysplastic nevi
- PCR-based SSCP from cell cultures

Levin et al. Cancer Res 1995;55:4278-82

p53 Expression in Melanocytic Lesions
45
40
35
30
25
20
15
10
5
0
Junct MN
Comp MN
LG AMN
HG AMN
MIS
VGP MM
Junctional
Superficial
Deep

TP53 Expression in AMN
Low Grade
High Grade

TP53 in Melanoma in situ and Malignant Melanoma RGP
MIS
MM-RGP

p27Kip1 Expression in Melanocytic Lesions
70
60
50
40
30
20
10
0
Junct MN
Comp MN
LG AMN
HG AMN
MIS
RGP MM
Junctional
Superficial
Deep

p27 Expression in AMN
Low Grade
High Grade

p27 in Melanoma in Situ and Malignant Melanoma RGP
MIS
MM-RGP

p21WAF1 Expression in Melanocytic Lesions
30
25
20
15
10
5
0
Junct MN
Comp MN
LG AMN
HG AMN
MIS
RGP MM
VGP MM
Junctional
Superficial
Deep

p21WAF1 in High-Grade AMN

p21^{WAF1} Expression in RGP Malignant Melanoma

Challenging the Linear Progression

AMN-MIS. Conclusions

- High-grade intraepidermal melanocytic lesions are mainly driven by p53 over-expression, which is dissociated from cyclin-dependent kinase inhibitors ($p21^{WAF1}$ and $p27^{Kip1}$) in MIS
- Invasion in malignant melanomas seems to be independent of p53 expression

Alternative Proposal

Dysplasia and Regression in Melanocytic Lesions

- Dysplasia vs. atypia in melanocytic lesions
  - Atypical and non-dysplastic melanocytic lesions
    - Recurrent nevus
    - Nevus with architectural disorder
    - Genital skin nevus
    - Acral type nevus
    - Spitz tumor
- Regression and atypia in melanocytic lesions. What is first?

Melanocyte Dysplasia in the Presence of Regression
Upward Migrat
No Upward Migrat
Asymmetric
Symmetric
AMN-Severe
AMN-Mild-Mod
17
28
68
10
5
14
80
24
6
25
79
13
0%
20%
40%
60%
80%
100%
+Regression
- Regression

Melanocyte Dysplasia in the Presence of Regression
NS
+ Nucleolus
- Nucleolus
Hyperchromatic
Euchromatic
NS
+ Anisokar.
- Anisokar.
+ Pleomorph.
- Pleomorph.
40
27
45
11
17
19
68
19
71
33
14
5
24
30
61
8
0%
20%
40%
60%
80%
100%
+Regression
- Regression

Microvessel Density in Melanocytic Lesions
9
8
7
6
5
4
3
2
1
0
Junct MN
Comp MN
LG AMN
HG AMN
MIS
RGP MM
VGP MM
Peripheral
Superficial
Deep
Extralesional

Intercellular Interactions
Melanocyte
E-cad
Keratinocyte
Cx43
Melanoma Cell
N-cad
Fibroblast
Endothelial Cell

E-Cadherin Expression in Melanocytic Lesions

E-cadherin Expression in AMN

Low-Grade **High-Grade**

AMN in Atypical Mole Syndrome
AMN Grading
AMN -ve
22
43
18
AMS +ve
22
9
9
0%
50%
100%
AMN-mild
AMN-mod.
AMN-severe
Lateral Extension
AMN -ve
21
25
9
28
AMS +ve
8
16
0
16
0%
50%
100%
Lentiginous
Nested
Mixed
N/A
Pozo L, et al. Am J Clin Pathol 2001;115:194-204

AMN in Atypical Mole Syndrome
Junctional Nests
AMN -ve
73
9
1
AMS +ve
27
2
11
0%
50%
100%
Horizontal
Vertical
N/A
Nest Cohesion
AMN -ve
64
18
1
AMS +ve
23
13
4
0%
50%
100%
Yes
No
N/A
Pozo L, et al. Am J Clin Pathol 2001;115:194-204

AMN in Atypical Mole Syndrome
Melanocyte Maturation
Inflammation Location
AMN -ve
AMS +ve
42
7
34
31
0
9
0%
50%
100%
Yes
No
N/A
AMN -ve
7
2
47
27
AMS +ve
9
2
26
3
0%
50%
100%
NOS
Epidermal
Perivasc.
Interstitial
Pozo L, et al. Am J Clin Pathol 2001;115:194-204

AMN in Atypical Mole Syndrome
Anatomic Site
AMN -ve
AMN +ve
16
19
25
15
19
12
3
6
0%
20%
40%
60%
80%
100%
Arms
Legs
Abdomen
Back
Pozo L, et al. Am J Clin Pathol 2001;115:194-204

Cell-Cycle Regulating Genes
RB
E2F
P
RB
E2F
Expression of genes involved in S-phase entry
G0
M
G1
Cell Cycle
G2
S
CyclinD CDK4/6
CyclinE CDK2
CyclinA CDK2
CyclinA/B CDC2
p16
p27
Myc
p19ARF
p21
p53
MDM2
DNA Damage

AMN-MIS. Methods
• 123 AMN (31 high-grade, 92 low-grade), 10 MIS. Controls included 15 junctional AMN, 20 compound MN, 15 RGP MM, and 27 VGP MM
• Histological evaluation
• Topographic kinetic study
• Immunostaining for Ki-67, p53, p21WAF1, p27Kip1, and E-cadherin
• Apoptosis detection by ISEL
• Statistical study: Student t-test, ANOVA

High-Grade AMN/MIS

E-cadherin Expression in AMN

Cell Kinetics and E-cadherin in AMN

- Maintained proliferation, down-regulated apoptosis, decreased expression of E-cadh are consistent with early tumor progression at the junctional compartment of high-grade AMN
- Low-grade AMN reveals features of a regressive lesion with low progression potential

β-Catenin Pathway

PTEN Pathway
Growth Factor Receptor
Integrin
Cell membrane
SH2
FAK
Shc
Grb2/Sos
PI3Kinase
PtdIns(3,4)P2
PIP3
PTEN
Ras
PKB/AKT
Raf
GSK3
Caspase-9
Bad
MEK1,2
Apoptosis
β-Catenin
MAPK1,2
TCF/SRF/Elk-1
TCF/LEF
Proliferation

HLA Class I and II for Immune Recognition
Growth Factor Receptors for Autocrine and Paracrine Factors
Growth Factors, Cytokines, Inflammatory Proteins, Motility Factors, Hormones for:
Growth
Chemotaxis
Angiogenesis
Stroma Formation
Immune Suppression
Binding (Storage) of Growth Factors and Proteases
Oncogenes Suppressor Genes
Binding-Transport Proteins for:
Calcium
Iron
Copper
Pumps and Channels
Pigmentation-Related Antigens
Intermediate Filaments and Other Cytoskeletal Proteins
Proteinases for:
Invasion
Growth Factor Activation
Proteinase Inhibitors
Extracellular Matrix Proteins for:
Attachment
Motility
Gangliosides for:
Adhesion
Immune Suppression
Growth Regulation
Adhesion Receptors for:
Extracellular Matrix Proteins
Tumor Cells
Lymphocytes
Endothelium

Conclusions

- Both kinetic features (maintained proliferation and down-regulated apoptosis) and decreased expression of E-cadherin at the junctional compartment of high-grade AMN are consistent with tumour cell progression. This expansive pattern is substantiated by increasing the effective microvascular network surrounding the lesion.

Conclusions

- Low-grade AMN reveals features of a regressive lesion showing less proliferation and upregulation of apoptosis with low progression potential.

Conclusions

- High-grade intraepidermal melanocytic lesions are mainly driven by abnormal p53 expression, which is dissociated from cyclin-dependent kinase inhibitors ($p21^{WAF1}$ and $p27^{KIP1}$), especially for MIS. In contrast, invasion in malignant melanomas seems to be p53-independent.

Conclusions

- The inverted pattern for proliferation and apoptosis by topographic compartments and the up-regulation of apoptosis of Spitz tumours are consistent with an overall regressive lesion that shows a superficial expansive compartment.

Conclusions

- Increased vascular proliferation in the deep dermal compartment of Spitz Tumours represents an attempt to maintain cell growth.

Future Work

- Immunostaining for p16^{INK4a} and Rb
- In situ hybridization for (1) telomerase mRNA and telomerase catalytic subunit (2) Vascular endothelial growth factor and Hypoxia inducible factor
- Genetic analysis on microdissected samples for microsatellite pattern