

Human Melanocytic Naevi

II. Depth of Dermal Protrusion: Comparative Measurements of the Basement Membrane Zone and Diameters of Intracellular Microfibrils using a Microcomputer Analysis System

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Thirteen acquired melanocytic naevi and five control skin biopsies were taken and fixed for observation both in the light and electron microscope. The thickness of the basement membrane, the distance of the naevus protrusion into the dermis, the number of anchoring fibrils attached to the lamina densa and the diameter of intracellular filaments in the naevus cells were measured. Comparative measurements were made in the electron microscope, from micrograph negatives and from micrographs. A non dedicated microcomputer digital analysis system developed specifically from this type of analysis, was also used.

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Acquired melanocytic naevi are separated from the dermis by a basement membrane being in continuity with the epidermal-dermal junction (1, 2, 3). This observation led to the formulation of the following questions: What is the actual depth of naevus protrusion into the dermis? What, if any, is the significance of this depth of protrusion? What are the comparative differences between basement membranes abutting keratinocytes, melanocytes and naevus nests?

Intermediate filaments are characteristic of mammalian melanocytes, being vimentin positive and keratin negative (4). The diameter of naevus cell filaments was measured to compare them at various levels of protrusion into the dermis.

MATERIAL AND METHODS

Thirteen melanocytic naevi were randomly selected from forty-three surgically removed human naevi (1, 2, 3). Blue naevi were not included. Material obtained from five normal skin biopsies was used for control measurements. The material was fixed for electron microscopy (3). The biopsies were numbered from 1 to 43. The samples to be examined were selected by writing and executing a microcomputer program which generated thirteen random numbers between 1 and 43. The thirteen sample numbers generated by the computer were then sectioned and examined in the electron microscope.

In each case (A), the depth of keratinocyte and naevus cell protrusion into dermis (B), diameters of naevus cell protrusions, thickness of the lamina lucida and lamina densa, number of anchoring fibrils and diameters of intracellular fibrils were measured, using a non dedicated microcomputer based digital analysis system (5). Measurements (A) and (B) were also made directly in the electron microscope.

For the anchoring fibrils, in order to avoid ambiguity depending on whether the fibrils had one or two attachment sites to the lamina densa, only points of attachment to the lamina densa were counted.

RESULTS

The depth of naevus cells protrusion is summarized in Table I. The deepest protrusion into the dermis was found to be exceeding 730 micrometers, at which point the lower limit of the biopsy, i.e. section, had been reached. The average depth of protrusion was 126 micrometers. Diameters of the protrusions varied considerably, depending on where the section had cut through the protrusion, but averaged to 47 micrometers in height and 25 micrometers in width.

Comparative thickness measurements of epidermal-dermal junctional basement membrane and the basement membrane surrounding the naevus protrusions into the dermis, are summarized in Table II.

The mean of these measurements show that, at the level of the epidermal-dermal junction, the lamina lucida and lamina densa were 33.5 nanometers in thickness adjacent to keratinocytes, but were twice as thick, i.e. 74.3 nanometers when adjacent to naevus nests. Naevus cells protruding from the junction had a total laminal thickness of 73.5 nanometers, i.e. essentially the same thickness. However, when measured around dermal naevus cell islands the combined laminae measured only 54.5 nanometers.

The number of anchoring fibrils, which varied depending on the cells abutting the epidermal-dermal junction, are summarized in Fig. 1. In order to adequately quantify and compare the counts, the results were normalized, i.e. number of anchoring fibrils per 100 nanometers of lamina densa. It was found that there are twice as many attachment sites along the lamina densa in the regions abutting keratinocytes next to naevus cells than in control skin. Significantly, lamina densa abutting naevus cells had virtually no anchoring filaments. In those regions, where the basement membrane had become doubled and or multilayered, the number of fibrils had decreased to approximately half of the control count for keratinocytes next to naevus cells.

Table I. *Depth of naevus protrusion into the dermis, and naevus protrusion diameters measured in micrometers*

Depth of naevus protrusion Epidermal-dermal junction to		Diameters of protrusions	
Top of closest nest	Base of deepest nest	Y-axis	X-axis
6.3	48.7	42.4	14.6
35.6	66.7	31.1	—
9.5	13.9	4.4	—
9.3	70.2	60.9	52
20.8	50	29.2	16.7
16	66.7	50	—
39	108.3	25	17
67	83.3	16.3	—
75	730+	—	—
16.7	—	—	—
0	75	75	—
8.3	150	141.7	—
12.5	50	37.5	—
Average value	126	47	25

The Y-axis denotes that the measurement was taken at right angles to the epidermal-dermal junction. The X-axis denotes that the measurement was taken parallel to the epidermal-dermal junction.

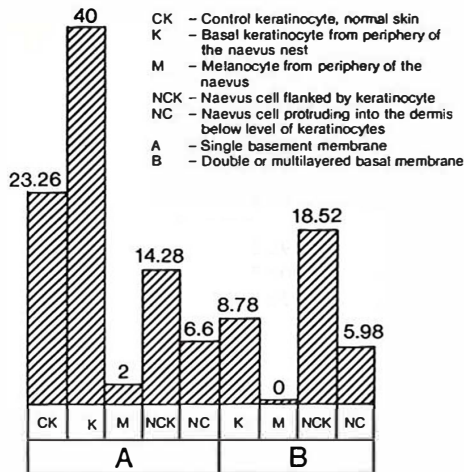


Fig. 1. Anchoring fibrils. Number of attachment points to lamina densa per 100 nanometer length of lamina densa.

Diameters of intracellular filaments were measured in control melanocytes and naevus cells located both above and below the level of the epidermal junction (Table III). The diameters were similar from all locations measured, resulting in an average of 10.7 nanometers, typical for intermediate filaments.

DISCUSSION

Measurements of melanocytic naevus protrusion into the dermis showed that the actual distance by which the basement membrane is stretching, is very short. The deepest protrusion found, was only invaginated to a depth of some 730 micrometers from the plane of the epidermal-dermal junction. An interesting correlation is the fact that naevi, which

Table II. Mean thickness of the basement membrane in nanometers

MC = number of measurements, LL = lamina lucida, LD = lamina densa, S = standard deviation

At the epidermal-dermal junction										Around protruding naevus nests					Around protruding naevus cells				
Keratinocyte					Naevus nests														
MC	LL	S	LD	S	MC	LL	S	LD	S	MC	LL	S	LD	S	MC	LL	S	LD	S
	19±4		23±3			15±4		24±4			52±18		14±2			35±47		18±5	
	12±3		13±1			19±6		29±10			14±5		11±2			47±10		47±10	
						59±9		77±1			9±2		51±11						
60					84					242	40±4		14±2		74				
											12±3		14±2						
											16±3		38±11						
											40±8		57±10						
Mean																			
	15.5		18			31		43.3			26.1		28.4			41		32.5	
Total																			
			33.5					74.3					54.5					73.5	

Table III. *Diameter of intracellular filaments*

D = diameter, S = standard deviation (in nanometers), MC = number of measurements

Control			Naevus nests			Single "dermal"		
	Melanocyte	Keratinocyte		Epidermal	"Dermal"		Naevus cell	
MC	D S	D S	MC	D S	D S	MC	D S	
14	10±1	10±1	14	11±2	10±2	12	9±2	
13	10±1	11±2	30	10±2	9±2	16	10±2	
10	12±2	11±1	19	10±1	10±2	22	12±3	
14	13±1	12±2	7	11±2	12±1			
15	12±1	10±1	13	12±1				
Mean diameter	11.4	10.8		10.8	10.2		10.3	

Mean = 10.7 nm.

have been diagnosed as melanotic, have been found to need excising to a minimal depth of 0.75 mm in order to be completely removed (6, 7, 8, 9).

Thinning of the basement membrane suggesting an imminent break (10), or renovation (11) were not observed.

Variations in thickness for individual measurements of basement membrane, could be due in part to fixation and microtomy artefact, but probably reflects the true state. Variation in thickness of the basement membrane implies several functional possibilities. The thick basement membrane surrounding the naevus nest at the plane of the epidermal-dermal junction may act as a barrier. This barrier could prevent protrusion of the naevus cells into the dermis. A thickened basement membrane could also attempt to accomodate the lack of, or decreased number of anchoring fibrils.

The measurements obtained, were comparable with most reported in the literature (12, 13, 14, 15), but differed from recently published results in which both laminar elements were thinner beneath melanocytes than beneath keratinocytes (16).

Measurements for lamina lucida abutting naevus nests (17) match the results for lamina lucida next to keratinocytes as reported here. Marked differences in thickness of basement membrane, when abutting naevus cells, has not been previously reported.

Anchoring fibrils are generally considered to stabilize the attachment of epithelium to the dermis (18, 19, 20, 21). They are arranged beneath lamina densa abutting keratinocytes, in a series of interconnecting arches (22), being less numerous in areas of melanocyte apposition (12, 23). According to our observations (2), where naevus nests protruded into the dermis, anchoring fibrils virtually disappeared.

Intracellular filament diameters have been previously reported (24, 25), and are similar to the results reported here. The filaments are of the intermediate type, and remain constant with depth of naevus protrusion into the dermis.

As the naevus cells protrude into the dermis, the accompanying basement membrane becomes thinner. This would imply that the thickening at the epidermal-dermal junction plane, is a compensatory mechanism, to provide for needed basement membrane as stretching progresses.

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