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Melanocyte-endothelial melanomas: Plasticity and transdifferentiation in melanomas

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ABSTRACT

Melanomas with extensive metastasis can rarely regress on the appearance of a halo nevus (HN). This study explores the possible factors involved. The component melanocytes of HN, flatten, depigment and line vascular spaces to replace and result in the involution of the nevus. This simulates vasculogenesis in amelanotic melanomas, malignant melanocytes differentiating into endothelial cells. The reverse is seen in pigmented tumors. Nestin positive endothelial cells lining angiogenic tubes, enter the tumor margins, show glial differentiation and form transient tumor vascular complexes (TVC) with stepwise neural differentiation and pigment laden cells, suggesting transdifferentiation of endothelial cells to melanocytes. During development, a range of molecular tools are used by blood vessels as well as nerves, including ephrins/Eph, NP-I and Notch signalling. This is vindicated by morphologic evidence of melanocyte/endothelial transdifferentiation as shown in this study. These observations, suggest a common multipotent stem cell, differentiating into vascular and melanocyte/neural stem cell depending on the surrounding microenviroment. Concurrent tumor regression may involve a rapid transdifferentiation triggered by a molecular switch or shut down of the common stem cell and/or presence of circulating antibodies to melanomas released on the appearance of HN.

Key Words: Halo nevus, Transdifferentiation, Melanocytes, Neural crest, Neural markers, Biogenic amines, Endothelial cell, Molecular tools

1. INTRODUCTION

Recent evidence reveals the existence of common molecular tools during neural/vascular development. Families of proteins and signalling pathways such as Semaphorin III/Neuropilin, Ephrin/Eph and Delta/Notch which had earlier been described in the developing nervous system have also been found to play a role in vasculogenesis.[1] Additionally, vasoactive peptide growth factors such as bFGF, VEGF, PDGF and TGF-β are also known to play a role in blood vessel assembly.[2] Further, the neural stem/progenitor cell marker nestin is expressed in proliferative endothelial cells as well.[3] The present study was taken up to explore the morphologic evidence of a common stem using melanocyte-angiogenic interactions in melanocytic lesions as a model, the melanocytes being neural crest derivatives.[4,5]

The appearance of a halo nevus and/or vitiligo[6–8] can herald a complete regression of melanomas in occasional cases. In a random series of nodular melanomas, the appearance of a halo nevus (HN) along with vitiligo was associated with a complete regression of the co-existing melanoma with extensive metastasis. The involution of the HN is due to replacement of pigment cells by a vascular network as observed in a recent study.[9] The possible role of this process in the regression of melanomas has been explored in the present study.

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2. MATERIAL AND METHODS

All procedures were performed with the informed consent of patients, in full compliance with the Helsinki Declaration. All experimental protocols were approved by relevant ethical committees of National Institute of Pathology, Safdarjung Hospital, New Delhi and the affiliated Pigment Cell Center.

A random series of 137 Nevi (75 HN with associated vitiligo, and a random series of 27 solid nodular melanomas showing tumorigenic vertical growth) were received from the Dermatology and Cancer Surgery Units of the Safdarjung Hospital, New Delhi. The nevi were received as excision biopsies to include the entire lesion and were fixed in 10% formol glutaraldehyde. The pattern of vascularisation was assessed within the nevi and VGP (vertical growth phase) melanomas.

A total of 10 blocks were taken from each VGP melanoma. A series of 5 μm thick frozen and paraffin sections (20-40 from each block) were maintained at 4°C. Sections were processed for routine hematoxylin-eosin (HE) to detect pigment, reticulin and Auro histochemistry. Additionally, enzyme histochemistry for Dopa Oxidase (DO) was performed on sections which were then counterstained with Nuclear Fast Red (NFR) specific for endothelial cells. Sections were also processed for immunohistochemistry and electron microscopy following en bloc staining for Dopa.[10–12]

Immunohistochemical staining was performed to identify a number of markers were identified in sections using the Avidin/Biotin method.[10] Specific antibodies against the melanocyte markers HMB-45 (1: 100; M0634, Dakopatts, Denmark) and S-100 (PU058-UP, BioGenex, CA, USA) were used to identify amelanotic melanocytes in sections. Additionally, the expression of neural markers and multipotent stem cells [Nestin (Nes), Glial Fibrillary Acidic Protein (GFAP; MU020-UC; BioGenex, CA, USA), Neural Filament Protein (NFP, MU073-UC; BioGenex, CA, USA) and Synaptophysin (Syn; MU363-UC; BioGenex, CA, USA)], pituitary hormones [adrenocorticotrophic hormone (ACTH; 1: 500; 02A3; Dakopatts, Denmark), prolactin (PRL; 1: 300; A0569; Dakopatts, Denmark), human Growth hormone (HGH, 1: 400; A0570; Dakopatts, Denmark)]; indoleamines [serotonin (SER; 1: 100; M0758; Dakopatts, Denmark), melatonin (MEL; BioGenex, CA, USA)]; catecholamines [dopamine, (DA) and noradrenalin (NA), Dakopatts, Denmark] and matrix proteins [laminin 5, (LN5, Kappa Zymed, CA, USA); integrin ($\alpha 1 \beta 5$; Dakopatts, Denmark)] have been examined. Further, markers for proliferation PCNA (1: 400; M0879; Dakopatts, Denmark) and Ki67 (1: 250; M7240 Dakopatts, Denmark) were used for identifying mitotic cells.[10–12]

Sets of slides for each of the markers listed above included negative controls wherein one of the serial sections was not incubated in the primary antibody. Additionally, samples from human skin wherein staining was known to be present were used simultaneously as positive controls.

2.1 Tumor-vascular complex

Angiogenic channels were surrounded by a mantle of interacting tumor cells (perivascular mantle zone, PMZ) at the tumor/stroma interphase giving rise to spheroidal structures called the tumor-vascular complex (TVC). A total of 218 fully formed TVCs with 5 to 6 layers were assessed in the present study, which are generally composed of 240 to 250 cells. While the innermost layer (L1) just adjacent to the blood vessel is made up of 15 to 20 cells, the outermost (L5) is composed of 75 to 80 cells.

Quantitation

Cell counts: The total number of cells in individual layers was counted followed by counts of marker positive cells in each layer. Marker positivity has been presented as percentages of positive cells in the total number of TVCs for comparison. The percentage of cells positive for each marker is represented as a graph, to show the relationship of tumor cells to the angiogenic central vessel.

2.2 Morphometry

A semi-automated Zeiss Morphomat, software from Scion Image (NIH) and a Reichert Cytospectrophotometer were used to compare the size and nuclear parameters of tumor cells with normal epidermal melanocytes. The nuclear content was assessed as follows: Area of nucleus $\times$ optical density $\times$ 5 (tissue thickness) = DNA (Q).

The areas of cells and their nuclei as well as the DNA content were measured in 200 cells on random pigmented and amelanotic nodules in each tumor and represented as scatter diagrams for comparison. Melanocytes (n = 100) from the overlying normal epidermis serve as control. The observer was blind to the category of the tissue block (pigmented, amelanotic or mixed) while counts were performed.

Mitotic activity was assessed in pigmented, amelanotic areas and TVCs. Counts of mitotic cells were compared in the different areas in PCNA and Ki67 stained sections.

2.3 Analysis

Cells stained for different markers were counted and their areas were compared across different parts of each tumor (including pigmented, amelanotic areas and TVCs). Further, mitotic activity was assessed by comparing the percentage of PCNA or Ki67-labelled cells in different areas across tumors.

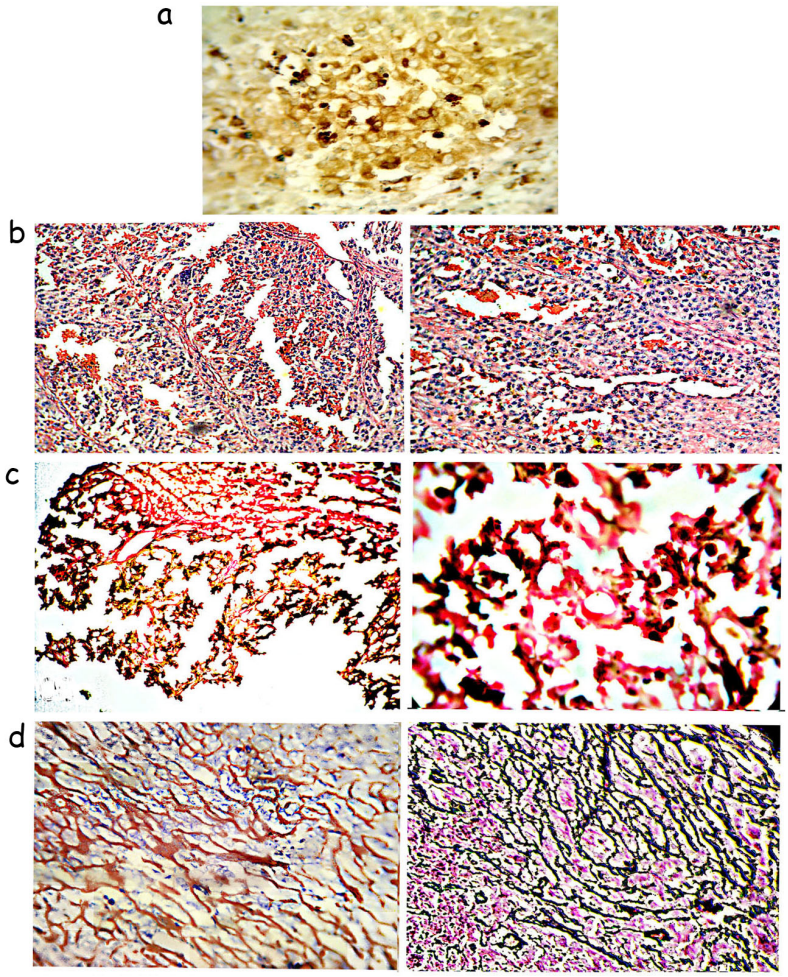

Figure 1. Vasculogenesis in amelanotic melanoma (AM). (a) Spaces form within sheets of uniform amelanotic cells(Synaptophysin × 400); (b) Spaces enlarge to form blood filled spaces lined by flattened tumor cells (HE × 200); (c) DO/NFR staining shows vascular spaces lined by partially DO positive cells being replaced by NFR cells (left: DO/NFR × 200; right: DO/NFR × 400); (d) Thin walled vessels lined by Nestin and Auro positive cells (left: Nestin × 400; right: Auro × 400).

3. Results

3.1 Amelanotic melanomas

Amelanotic areas show a diffuse pattern of continuous sheets of fairly uniform cells, with relatively little nuclear atypia and scanty pigment. Individual cells have large, vesicular nuclei and prominent nucleoli. The continuous sheets of cells in amelanotic areas become disrupted during vasculogenesis. A loose network of spaces (sinusoids) is formed by aggregations of LN5, integrin and synaptophysin ($\alpha 1 \beta 5$) positive tumor cells (see Figure 1a). Irregular sinusoidal blood-filled spaces appear, showing hemopoeitic activity (see Figure 1b). The lining tumor cells become flattened and demonstrate NFR positivity which resembles that seen in endothelial cells (see Figure 1c). The spaces formed by tumor cells become narrow and form a network of innumerable capillaries well highlighted by nestin (Nes) and aurophilia (Auro) indicating the embryonic nature of lining cells (see Figure 1d). A loss of pigment and dopa is seen in sections stained for dopa-NFR with an increase in NFR in the lining cells which show Weibel-Palade bodies (WPB) in EM sections. Thus, a self-propagating system of spaces reminiscent of embryonic vasculogenesis coalesce and interlink into tubular networks in many areas.[13]

Dopa positivity varies from fully positive, partial to negative cells indicating a gradual change from melanocytes to endothelial cells. These features are almost identical to those seen in HN.

3.2 Melanocytic nevi

In the present series of 137 melanocytic nevi, 75 were halo nevi associated with vitiligo. Junctional nevi are flat and pigmented, showing junctional activity with proliferation of

prominent highly dendritic epidermal melanocytes within the basal layer of the epidermis. The underlying dermis does not show any pigment cells (see Figure 2a).

Intradermal nevi are slightly raised and show increased epidermal pigmentation and prominent epidermal dendritic melanocytes. The nevus is formed by closely packed intradermal sheets of tumor cells which are positive for dopa in the underlying dermis and does not possess definite vascular channels. The nevus is separated from the epidermis by a clear Grenz zone (see Figure 2b).

Compound nevi show a combination of the above with junctional activity, proliferation of epidermal dendritic melanocytes which intermingle with underlying sheets of Schwannian nevus cells. The cells are arranged in sheets with no definite vascular channels. Occasional groups of cells are seen to surround clear spaces in the depth of the lesion (see Figure 2c).[14]

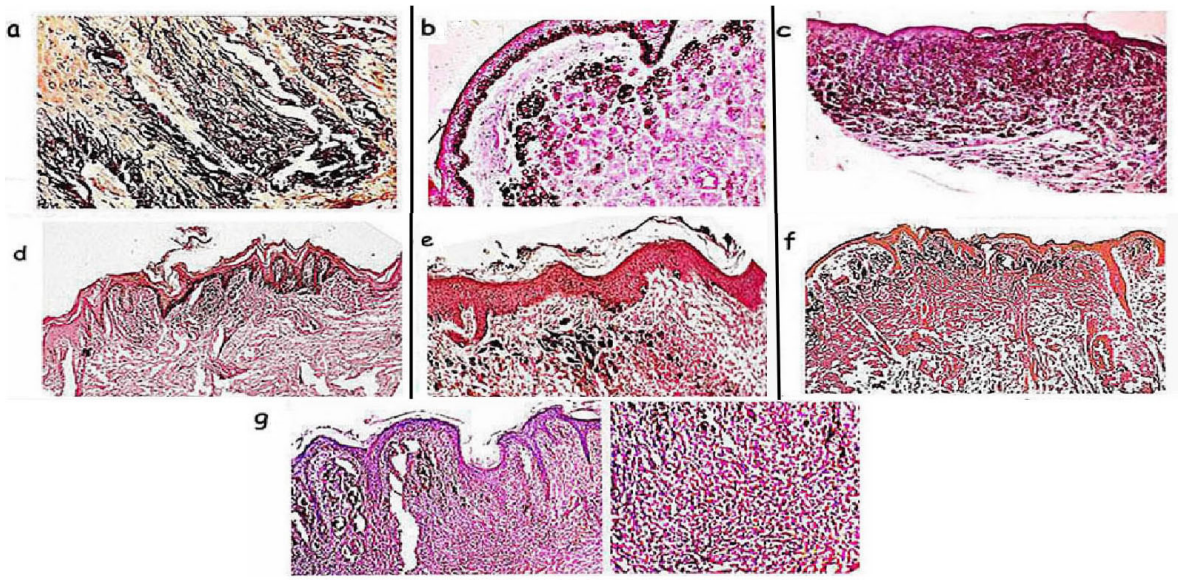

Figure 2. Comparison of pigmented and halo nevi (HN). (a) Junctional nevus: Section showing highly dendritic melanocytes in the epidermis (Sevier-Munger × 400); (b) Intradermal nevus: The underlying dermis shows sheets of nevus cells separated from the epidermis by a clear Grenz zone (DO/NFR × 200); (c) Compound nevus: Combined junctional and intradermal components (DO/NFR × 100); (d) HN, showing partial dissolution of a junctional nevus (DO/NFR × 100); (e) IDN showing almost complete dissolution with few dopa positive cells (DO/NFR × 200); (f) Compound nevus showing extensive dissolution and replacement of nevus cells (DO/NFR × 100); (g) HE stained sections showing complete absence of inflammatory cells in a HN (HE × 100/400).

3.3 Halo nevi

All 75 HN were assessed for the process of loss of pigment cells. The lesions show a range of dissolution from initial stages to almost complete depletion of the component cells in the nevus (see Figure 2, d-f). The periphery shows loss of surface epidermal melanocytes forming the halo. Occasional melanocytic nevi regress completely and are replaced by a vitiliginous patch (see Figure 2e). Contrary to expectation, there is no inflammatory infiltrate at all stages, in any of the samples studied (see Figure 2g).

Initially, spaces lined by flattened dopa positive nevus cells appear at the periphery and depth of the lesion. In sections stained with dopa-NFR, a graded loss of dopa is associated with increase in NFR in the flattened lining cells. The nevus is replaced by spaces lined with NFR positive flattened cells which become continuous with the endothelial lining of the underlying vasculature to finally merge, and blend with the stromal vessels. A similar process is evident in marginal melanocytes. The nevus cells do not show evidence of necrosis, the NFR positive cells showing viable nuclei (see Figure 3B, a-d). The epidermal melanocytes surrounding the nevus are gradually lost to form a halo. Involution of the nevus results from transdifferentiation of the nevus cells and the epidermal melanocytes into endothelial cells. This process simulates vasculogenesis seen in amelanotic melanomas (see Figure 3A, a-e)[9] . Thus the rapidly growing amelanotic melanoma cells and cells of the halo nevus can transdifferentiate into endothelial cells (see Figure 3C).

Is the reverse differentiation, from endothelial cells to melanocytes possible? Pigmented melanomas suggest an answer.

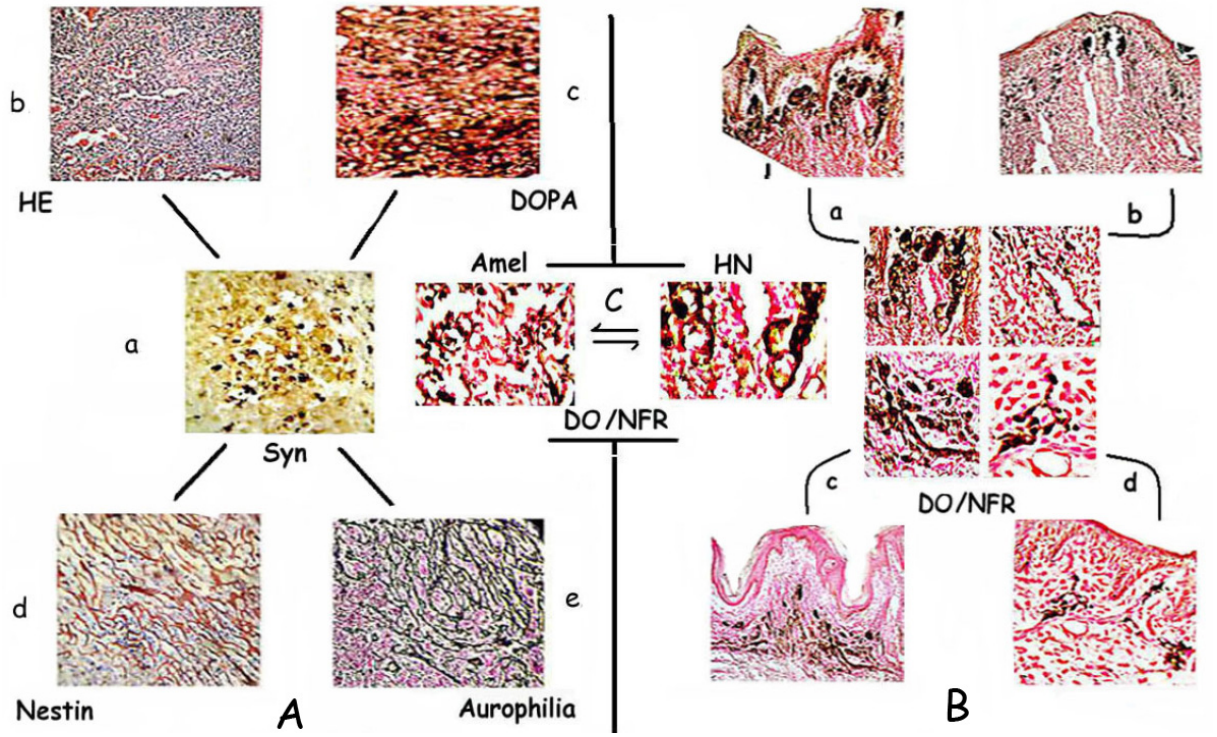

Figure 3. Melanocyte transdifferentiation in amelanotic melanoma (AM) and halo nevi (HN). A) AM: (a) Synaptophysin positive cells lining spaces within amelanotic cells (Syn × 400); (b) Vasculogenic spaces within AM (HE × 100); (c) Irregular loss of dopa staining in cells lining sinusoids (DO/NFR × 200); (d,e) Nestin/Auro positivity of multipotent stem cells (MASC) (Nes/Auro × 200). B) HN: (a) Spaces in depths of IDN, with loss of dopa and increase in NFR; (b) Vascular spaces lined by flattened NFR and partial dopa positive cells; (c) Dopa positive endothelial tubes, with NFR positive nuclei; (d) Vascular channels with occasional dopa positive cells (DO/NFR × 400). C) Comparison of transdifferentiation of melanocytes in HN and AM (DO/NFR × 400).

3.4 Pigmented melanomas

Angiogenesis is elicited by pigmented tumor cells through the release of VEGF. The adjacent stromal blood vessels extend nestin and Auro positive endothelial buds which cannelise at the tumor margins. These do not have pericytes or a definite basement membrane, as seen on reticulin stain (see Figure 4A, a&b). Transient TVCs are formed by the interaction of angiogenic vessels with tumor cells at the interphase between the tumor stroma, with a perivascular mantle zone (PMZ) consisting of 5 to 6 cell layers (see Figure 4B, a-c).[15]

The periluminal cells, continuous with the endothelial tubes, show nestin positivity, aurophilia (93%), and GFAP positivity (91.5%) to form the innermost layer of the TVC.[16] Nes positivity is seen in 94.5% of cells in the LI layer around the lumen. Processes which are positive for Nes, Auro and GFAP extend towards the periphery, leading to the formation of a frame-work to support the new layers of tumor cells (see Figure 4B, a-c). Here the endothelial lining cells function as radial glial cells which are known to proliferate and differentiate into cells positive for neuronal markers and thus function as multipotent astrocytic stem cells (MASC) with neuronal differentiation as seen in neurosphere cultures.[3,16]

There is a regimented differentiation in the TVCs, beginning with glial differentiation of the innermost layer of cells followed by expression of indoleamines [serotonin (L2: 71.8%; L3: 62.8%); melatonin (L2: 60.9%; L3: 64.1%)], hormones [PRL (L2: 42.5%; L3: 39.8%); HGH (L2/L3: 48.6%)] in the middle layers, associated with mitotic activity.[17–20]

The outer layers express the neural markers NFP (L4: 52.2%; L5: 50.6%) and Syn (L4: 63%; L5: 64.1%); catecholamines: DA, (L3/4: 75%); NA (L3: 70%; L4: 80%; L5: 60%) enzyme DO (L4: 84.9%; L5: 96.9%), Pigment (L5: 60%). and ACTH (L4: 64.2%; L5: 61.6%) (see Figure 4C, a-c)[17–19] and are associated with very few mitotic figures.[20]

3.5 Mitotic counts in TVCs

Interestingly, the TVCs show normal mitosis within the middle layers in contrast to the abnormal figures in the surrounding tumor. Very occasional mitosis is present in L1, L4, and L5. The highest rates of mitosis are seen in layers L2 (87.7% TVCs) and L3 (99. 2% TVCs). Normal mitosis is seen in 43.75% of cells in L2 and 49.3% in L3. No abnormal mitosis

is seen within TVCs.

Results from morphometry demonstrate the ranges of cell/nuclear area and nuclear quantum, in TVCs (cell: 225.8 μ^2; nuclei: 51.6 μ^2) and amelanotic tumor areas (cell: 354.8 μ^2; nuclei: 167.7 μ^2) show uniformity and are comparable with normal epidermal melanocytes (cell: 207 μ^2; nuclei: 40.3 μ^2) as opposed to the large variations seen in melanotic tumors (cell: 1,419.1 μ^2; nuclei: 967.7 μ^2) (see Figure 4D).[20]

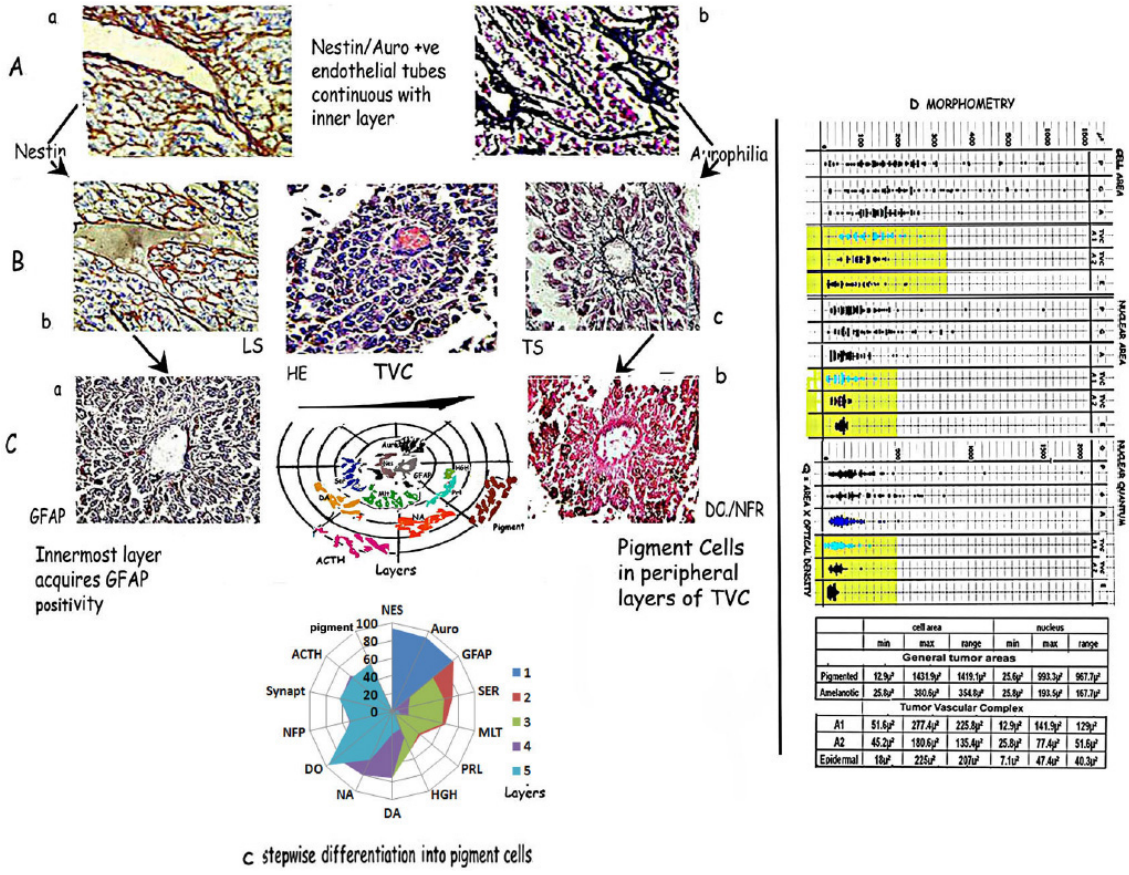

Figure 4. Tumor vascular complex (TVC). A) Nestin (a) and Auro (b) positive angiogenic vessels enter tumor margins (Nes/Auro × 400). B) (a) Well formed TVC (HE × 400); (b) LS: Nestin positive abluminal layer continuous with endothelial tube. Dendritic processes extend outward from these cells (Nes × 400); (c) CS showing Auro positive inner layer and processes (Auro × 400). No basement membrane or separate endothelial layer seen. C) (a) Nes/Auro cells show GFAP positivity (GFAP × 400); (b) NFR positive cells differentiate into outer pigmented layer (DO/NFR × 400); (c) Diagram and graph showing stepwise neurogenesis. D) Scatter diagram & Table comparing cell/nuclear areas and nuclear quantum TVC, parameters are uniform comparable to epidermal cells.

3.6 Mixed tumors

Melanocyte/endothelial transdifferentiation and plasticity is best illustrated in nodules with moderate to low pigment. Vasculogenesis coexists with well-formed TVCs[21] showing trans-differentiaion of melanocytes to endothelial and endothelial to pigment cells. Amongst the sheets and cords of tumor cells are interspersed cells which are strongly positive for LN5 and integrin ($\alpha 1 \beta 5$). In amelanotic areas of the tumors, the lining tumor cells flatten to form capillary channels which are interconnected with the vascular network.[21] TVCs can be seen in the same field (see Figure 5D).

4. Discussion

The present study highlights an interesting feature of melanocyte interactions with endothelial cells. The melanocytes and endothelial cells transdifferentiate from one type into the other, under different circumstances. In HN melanocytes convert into endothelial cells, as in amelanotic melanomas nodules where amelanotic melanocytes show a similar change during vasculogenenesis. In the former, the vascular channels gradually replace the entire nevus to be merged and remodelled within the normal dermis.[9] In comparison, vasculogenenesis in amelanotic melanomas serves to enhance tumor growth. The above findings show

transdifferentiation of melanocytes into endothelial cells.

Is the reverse, the transdifferentiation of endothelium to melanocytes, possible?

The most intriguing is the formation of TVCs. The presence of near normal mitosis, normal sequential neural differentiation as well as morphometric parameters (see Figure 4D) suggest that normal cells are involved.[17–20] It is difficult to accept that angiogenic endothelial tubes can normalize the highly pleomorphic melanoma cells so drastically on contact. On scrutiny, it is seen that the angiogenic endothelial tubes do not show a BM on PAS and reticulin staining. The proliferative endothelial cells are aurophilic and express neural stem/progenitor cell marker nestin.[3] They form the inner most layer of the TVCs. These Nes and Auro positive abluminal cells, acquire GFAP positivity. No separate endothelial layer can be identified there being a single nestin positive layer continuous with the endothelial tube entering the tumor margin during angiogenesis.

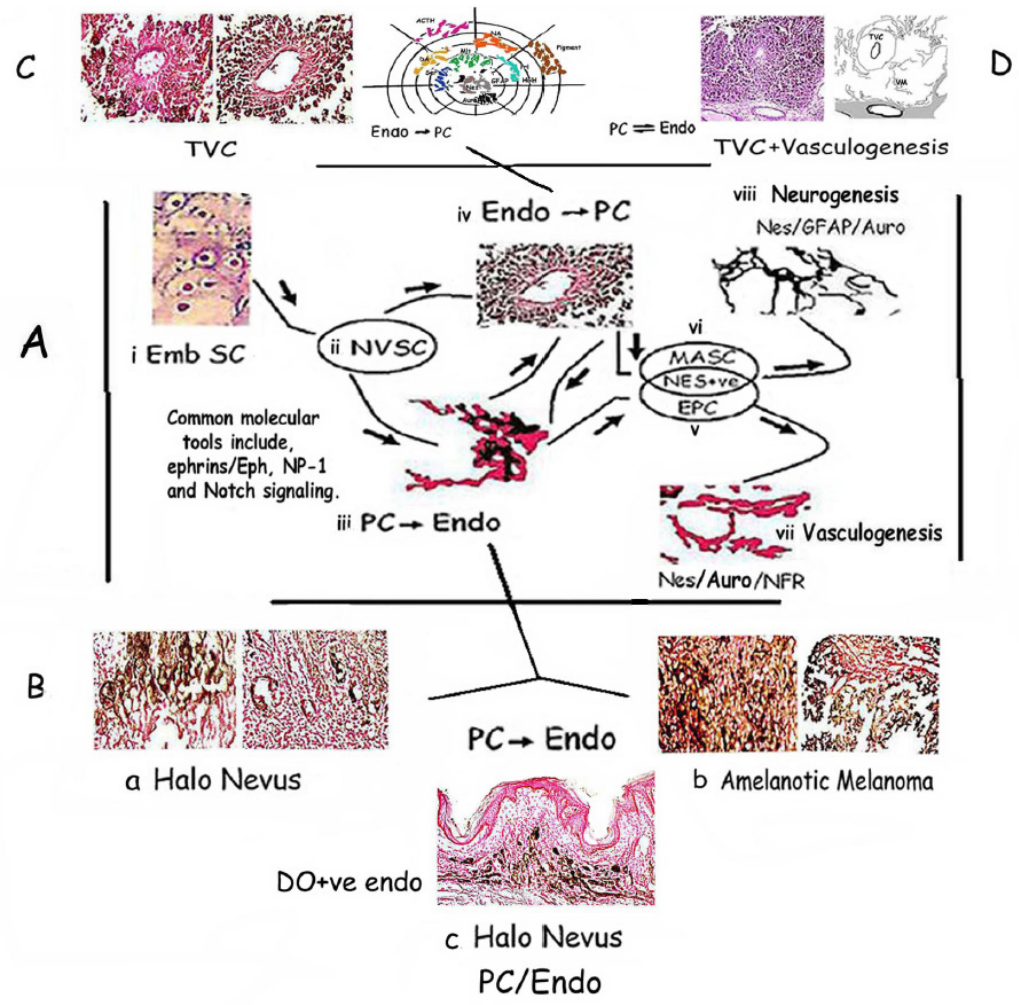

Figure 5. A) Melanocyte/endothelial plasticity: Common pathway for neural: NC/Endothelial:vascular differentiation (i) Embryonic stem cells (Emb SC) differentiate into (ii) common multipotent neuro-vascular stem cell (NVSC) giving rise to (iii) vasculogenic and (iv) neurogenic stem cells which can differentiate to and from neural to endothelial cells. Nes/Auro positive cells differentiate into (v) Endothelial precursor cell (EPC) with (vii) angiogenesis (vi) Multipotent astrocytic stem cells (MASC) giving rise to (viii) neurgenesis. B) Melanocyte to Endothelium: Halo nevus (a) and amelanotic melanoma (b): Dopa positivity replaced by NFR positivity; (c) Halo nevus showing vascular channels lined by DO positive endothelial cells highlighting the common origin (DO/NFR × 400). C) Endothelial to melanocytes in TVCs: Endothelial cells transdifferentiate into pigmented cells by stepwise neurogenesis. D) A mixed nodule, with vasculogenesis and TVC in the same field (HE × 100).

The presence of normal mitosis in the TVCs, in contrast to the abnormal figures in the surrounding tumor, the regimented neuronal differentiation with genesis of indolaminergic, catecholaminergic and pigment cells, suggests that normal endothelial cells arising from stromal vasculature during angiogenesis transdifferentiate into neuronal stem cells under the influence of the surrounding melanoma cells. Reciprocal paracrine interactions include, vascular endothelial growth factor (VEGF) released from astrocytes and neurons which stimulates angiogenesis, which in turn leads to the release of brain-derived neurotrophic factor (BDNF) stimulated microvascular cells[1,22,23] resulting in neurogenesis. Neuronal progenitor cells lie in close proximity to mitotic endothelial cells. This is replicated in the formation of TVCs. Angiogenesis is elicited by VEGF released by pigmented melanocytes. Aurophilia/GFAP in periluminal cells indicates BDNF production by the microvessels.

Several studies have highlighted the plasticity of endothelial and neuronal stem cells.[23,24] For example, Wurmser *et al.*[24] found that six per cent of the neuronal stem cell (NSC) population in co-cultures converted to cells which showed the stable expression of multiple endothelial markers and were also capable of forming capillary networks. These findings demonstrate that NSCs are highly plastic and appear to have a wider differentiation potential including early hematopoietic cells, than previously thought.[25] Taken together with results in the present study, vasculogenesis in HN and AM reflect the plasticity of the neural crest derived melanocytes.

Several anatomical parallels exist during embryogenesis between the vascular and nervous systems. Both are comprised of two associated units: neurons and glia in nerves, mural and endothelial cells in blood vessels. Additional analogies include the ramification of blood vessels and nerves through most domains of the body and the directionality of information in the motor and sensory pathways of nervous system which is similar to arterial and venous blood flow. Nerves and blood vessels have a clear physical relationship, as seen with the vasa nervorum and the perivascular autonomic nerve fibers which control vascular tone.[26,27] Further, both nerves and blood vessels follow similar paths and modes of migration during embryogenesis.[28,29]

Another point of intersection between the nervous system and vasculature is the transmembrane receptor Neuropilin-1 (NP-I), and its soluble ligand Semaphorin III (ScmIII). While these are implicated in growth cone guidance in the developing nervous system, they are also know to modulate vascular morphogenesis,[30] since NP-I is a receptor for a heparin-binding form of VEGF[31] which a potent inducer of blood vessel growth and vasculogenesis and angiogenesis.[32] The vesicular growth factor exists as 5 isoforms in humans, of which the predominant forms are VEGFl21, VEGFl65, and VEGFl89. Klagsbrun and coworkers[31] have shown that NP-1 (which plays a co-receptor role during axon guidance) acts as a co-receptor for VEGF165.[32] Neurons and blood vessels demonstrated functional competition between SemIII and VEGFl65 as shown in in vitro assays.[33] Taken together, these findings suggest that NP-1 could represent an important link to facilitate communication between the developing vasculature, growing axons and their microenvironment, besides providing an additional facet to VEGF signalling.[34]

Not only does the ephrin/Eph signaling pathway provide guidance cues for growing axons and migrating neural crest cells during embryonic development,[35] it is also involved in signalling cell-cell and cell-substrate adhesion and defining spatial boundaries in the developing embryo.[35–37] A Notch ligand, Delta-like 4, is expressed in early axial arterial endothelium of the mouse,[38,39] and directs the patterning of the adjacent nerve. Both Notch and Delta -Notch signalling are important for the guidance of axonal growth cones in vertebrate neurons.[40] Interestingly, this signalling pathway also helps the pattern of the emerging vascular network, analogous to the formation of axonal connections. These findings suggest that during development, the formation of networks of blood vessels and nerves utilizes similar molecular tools, which includes ephrins/Eph, NP-1 and Notch signalling. Further, earlier studies have shown that the neural stem/progenitor cell marker nestin is expressed in proliferative endothelial cells.[3] Both in vivo and in vitro results show that during angiogenesis, nestin expression is downregulated when proliferating endothelial precursor cell (EPC) make the transition to mature, post mitotic endothelial cells (EC).[41–43]

In the TVCs, GFAP positivity in Nes/Auro positive endothelial cells indicates radial glia induction, that is, endothelial cells are transinduced/switched to MASC by pigment positive melanoma cells. Since the added layers show normal mitosis and neural differentiation it is suggested that the normal endothelial cells arising from stromal vasculature during angiogenesis transdifferentiate under the influence of the surrounding melanoma cells into neuronal stem cells (see Figure 4).

Angiogenesis and the number of TVCs are directly proportionate to pigmentation suggesting that the production of molecular angio/neuro-genic tools relate to differentiation in surrounding tumor cells. As the endothelial tubes enter the tumor they react to the tumor microenvironment to extend dendritic processes and express aurophilia and GFAP

and act as MASC or endothelial-stem-cells/neural-stem-cells (ESC/NSC). As further layers are added stepwise neural differentiation takes place indicating transduction of ESC to NSC. From these observations, it is suggested that a common multipotent stem cell, showing high plasticity, differentiates into vascular and neural stem cell types. These can transdifferentiate from one to the other depending on the surrounding microenviroment (see Figure 5). This feature is highlighted in HN by the presence of vascular channels lined entirely by dopa positive endothelial cells with NFR positive nuclei (in transit) (see Figure 5B, c); and in melanomas by the presence of both vasculogenesis (melanocyte to endothelial) and TVCs (endothelial to melanocyte) (see Figure 5D).

The association of angio-melanocyte plasticity, as seen in this study, vindicates the presence of common molecular tools of neuro/vascular stem cells. A common multipotential, Nes/Auro positive SC, the precursor of the MASC-EPC further differentiates into neuronal/neural crest and angio/vascular components (as outlined in the figure) thus allowing the trans-differentiation of the two cell types (see Figure 5A).

Melanocyte/endothelial plasticity (see Figure 5A): The following schema is suggested by the above observations: A common pathway for neural/neural crest and endothelial cells begins with the embryonic stem cells (Emb SC) which differentiate into a common multipotent neuro-vascular stem cell (NVSC) in response to a gamut of molecular tools common to angio- and neurogenesis. NVSC give rise to a common Nes/Auro positive vasculogenic and neurogenic stem cells which can differentiate to and from neural to endothelial cells. Nes/Auro positive cells differentiate into EPC with angiogenesis and MASC giving rise to neurogenesis.

The replacement of melanocytes by endothelium in HN results in involution of the lesion and remodelling of the vascular channels which merge with the pre-existing connective tissue stroma. It is difficult to explain the extremely rare cases of concurrent regression of melanoma on this basis alone. Factors likely to be involved are either one or a combination of the following: A rapid replacement of tumor cells by extensive transdifferentiation due to a molecular switch, a down regulation of the common SC at step ii or iii (see Figure 5); and/or the presence of circulating antibodies against cytoplasmic antigens in melanoma cells in patients with halo nevi. These antibodies disappear upon excision or spontaneous resolution of the central lesion.[44–46] The above factors could be utilized therapeutically for initiating spontaneous regression of melanomas. Of greater importance is the fact that the above results raise queries on the role of anti-angiogenic therapy which could in some cases block spontaneous regression through melanocyte to endothelial transdifferentiation.[47]

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CONFLICTS OF INTEREST DISCLOSURE

The author declares that there is no conflict of interest statement.

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