Multipotent Astrocytic Stem Cells Identified During Angiogenesis in Melanoma

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Abstract

Background: This study is a sequel to a recent finding that melanoma cells revert to the embryonic pattern of differentiation, with radial glial like cells and into neuronal positive cells during tumor-vascular interaction. The present work, studies the sequence of formation of tumor-vascular complex [TVC] composed of an angiogenic vessel and a perivascular mantle zone [PMZ] of 1 to 5 layers of tumor cells at the tumor-stroma interphase using gold impregnation to identify multipotent astrocytic stem cells [MASC] in this 3D system.

Methods: Serial frozen and paraffin sections are stained with HE, for aurophilia and, dopa oxidase; immunostaining for HMB45, nestin [Nes], GFAP, NFP and synaptophysin [Syn]. The presence of MASC is assessed by Nes and GFAP immunopositivity and compared with aurophilia. Positive cells counts in layers L1 to L5 of the PMZ, on serial frozen and paraffin sections have been subjected to Statistical Analysis by ANOVA: Kruskal-Wallis One Way Analysis of Variance; All Pairwise Multiple Comparison Procedures [Tukey Test].

Results: A single tumor cell layer ensheathes the angiogenic vessels entering the tumor margins. These perivascular cells show intense aurophilia, and extend dendritic arbors into the outer layers of the mantle zone. Nes and GFAP positivity is seen in the cell bodies and dendritic processes. As further layers are added on, tumor cells show NFP & Syn positivity in L4 & L5 with a transition zone between L2 to L3.

Discussion: During tumor-vascular interaction the perivascular radial glial like cells exhibit aurophilia and Nes/GFAP+vit. Sequential changes are highlighted by gold impregnation. Nes, a marker of multi-lineage progenitor cells, identifies them as MASC. The 4th and 5th layers show NFP & Syn+ve positive cells. Thus TVC exhibits embryonic microenvironment as during neurogenesis. Melanoma cell/endothelial coculures could be a source of MASC for therapeutic uses. Aurophilia does not fade easily unlike fluorescence and immunostained material thus opening up interesting possibilities for use in the study of developmental and adult neurogenesis in the brain as well as in the study of neurospheres in culture.

Key Words: angiogenesis, aurophilia, nestin, multipotent astrocytic stem cell, tumor-vascular interaction.

Background

Melanocytes are derived from the multipotent neural crest cells [NC] which give rise to peripheral neurons, glial cells, and neuroendocrine cell types during embryogenesis of the neural tube [1]. The NC cells arise from the neural fold which lies at the confluence of the neuroepithelium and the general epidermis and so can differentiate into neuronal, epidermal as well as pigment cells. The melanocytes function as pigmented neurons to actively produce catecholamines as well as indoleamines [2-7]. Melanomas are highly malignant tumors arising from the melanocytes in the basal layer of the epidermis, uveal tract of the eyes, inner ear, mucous membrane, genital organs, anus and leptomeninges [8].

Human cutaneous melanomas show molecular plasticity and often express genes characteristic of neural cell lineages [9]. Neuronal differentiation with expression of neuron-associated markers such as intermediate filament protein peripherin, neuropeptide substance P, muscarinic acetylcholine receptors, and neuron-specific enolase in primary and metastatic melanomas has been shown in some studies [10-11]. In vertical growth phase [VGP] melanomas there is a definite pattern of neural differentiation in relation to angiogenesis on viewing the tumor as a three dimensional system [12].

Although most human cancers persist in situ for months in a prevascular phase, they require efficient vascularisation, for further growth and metastasis [13]. Production of new blood vessels[angiogenesis] and their remodeling are required for the development of the tumor microcirculation [14]. Neoangiogenesis results from the proliferation, sprouting, and migration of endothelial cells within normal tissues adjacent to the tumor. Studies have shown that varying types of vascularisation are evident in melanomas [15-19]. Angiogenesis is predominant in pigmented melanomas, neovascular channels arising from pre-existing stromal vessels at the invasive margins. In the more aggressive amelanotic melanomas, embryonic vasculogenesis predominates [20].
Recent studies demonstrate reciprocal paracrine interactions between astrocytes, endothelial cells and ependymal cells. The present work has been undertaken to investigate whether a similar interaction occurs in melanomas in response to angiogenesis. This study is a sequel to a recent finding that melanoma cells revert to the embryonic pattern of differentiation, with radial glial like cells and neuronal positive cells during tumor-vascular interaction [12]. Cajal’s gold impregnation has been modified and employed to identify glial cells. The present study assesses the development of the tumor vascular complex as a 3D embryonic microenvironment and the efficacy of gold impregnation to identify multipotent astrocytic stem cells [MASC] during tumor cell-vascular interaction in this 3D system.

Material and Methods

Nodular melanomas in the vertical growth phase [VGP] were received from the Cancer Surgery Unit of Safdarjang Hospital, New Delhi, fixed in 10% formal glutaraldehyde. The formaldehyde-glutaraldehyde cold fixation retains the morphology, gives crisp staining and efficient immunohistochemical staining both in frozen as well as paraffin sections. The same blocks can be subjected to electron microscopy as well. 10 nodules were sampled from each of 27 tumors to make a total of 270 blocks, in the ratio of pigmented to poorly pigmented areas in each tumor [fig.1]. Each block was subjected to: Frozen sections, Paraffin sections. Serial sections 5?m thick [20-40] were cut from each block and maintained under refrigeration at 4°C for:

Histochemistry, [HE, PAS, modified gold impregnation [21];

Enzyme histochemistry: dopa oxidase method;

Immunohistochemistry by the Avidin/Biotin method:

monoclonal antibodies [mAb] used: HMB45, NFP [neuro filament protein], GFAP, Synaptophysin [Syn], [BioGenex] nestin [Nes], [Chemicon] a marker of multi-lineage progenitor cells [22-25].

As negative control all slides included a serial section stained with no mAb. The same mAb were used simultaneously against known positive sections from human skin as positive controls.

Modified Gold impregnation technique

The Cajal’s gold impregnation method was modified, to assess the vascular pattern and the presence of astroglia, which show intense aurophilia [Auro], using 57 thick paraffin sections. The modification involves a very short exposure to silver nitrate and a prolonged exposure to gold chloride.

Method

5µ thick paraffin sections were dewaxed, dehydrated, preoxidized with 1% Potassium permanganate, followed by 2% Potassium metabisulphite to inhibit the argyrophilia of nerve cells and fibers. Ferric Ammonium Sulphate [alum] is used as sensitising agent to increase the affinity, and then immersed in ammoniacal silver solution for 15 to 30 sec. after prolonged wash in tap water. After several changes of distilled water, sections were reduced in 10% formalin and transferred to a freshly prepared gold chloride sublimate [GCS] for 15 to 25 mins for gold impregnation. Reduction with formaldehyde solution results in the deposition of metallic gold from yellow gold chloride solution and metallic silver with a resultant increase in contrast of the stained section. Any superfluous gold chloride not bound to tissue structures was eliminated by thorough rinsing distilled water. They were treated for 2 min with freshly prepared 2% potassium metabisulphite, rinsed distilled water and finally treated with Sodium Thiosulphate for 5 mins. Fixing in sodium thiosulphate removes unreduced gold/silver. Sections were washed in water, dehydrated, cleared and mounted in DPX [Dibutyl Phthalate Xylene] for viewing.

[Caution: Extreme caution in the use of mercuric chloride for preparing GCS is required since it is a cumulative poison [26] [Clarkson, Magos, 2006]. Sensory impairment (vision, hearing, speech), disturbed sensation and a lack of coordination are typical symptoms. The type and degree of symptoms depends upon the dose, and the method and duration of exposure.]

Gold chloride sublimate specifically and exclusively stains astrocytes [confirmed by GFAP and nestin positivity] which stain intensely black [34]. Reticulin is seen as a network of fine fibrils and the vascular basement membrane.

Tumor-vascular complex: TVC [fig.1]:

The marginal interphase between the tumor and stroma is included in 63 blocks from the 135 sampled pigmented nodules. These were selected [fig.1], to study the tumor/vascular interaction during angiogenesis. Interacting angiogenic vessels to a depth of 2HPF from the tumor margin have been included in the study.

The formation of the tumor vascular complexes [TVC] around a central angiogenic vessel was assessed by gold impregnation in a total of 882 developing complexes and the sequence of appearance of aurophilia and marker positivity recorded. Of the 882 TVCs 257 show a single layer of tumor cells, 213 show 2 layers, 209 show 3-4 layers while 203 are fully formed with 5 layers.
Cell Counts: The innermost L1 around the vessel has 15 to 20 cells; the outermost L 5 is formed of 75 to 80 cells, the ratio being 1:5. TVC with 5 to 6 layers are composed of 240 to 250 cells; For analyses the layers in the TVC are numbered from L1 to L5 with L1 being closest to the vessel. Percentage of cells positive for Auro, Nes, GFAP, NFP, and Syn in each layer is represented as graphs, to show the positivity of each marker in relation to the angiogenic central vessel. Specific morphological features as well as aurophilia and nestin positivity in the different layers of the spheroids have been studied to correlate with the immunopositivity.

Quantitation: First, the total number of cells in each layer was counted. This was followed by counts of positive cells in each layer. Since the cell numbers vary in different layers, marker positivity has been presented as percentages of positive cells in the total number of TVCs for comparison.

Results

Angiogenesis:
There is proliferation of the adjacent stromal blood vessels, which throw out endothelial buds. As the endothelial cords grow towards the tumor margin and cannelise they enlarge and acquire a silver positive basement membrane before entering the tumor margins. At this stage the endothelial cells lining angiogenic vessels are nestin positive. They show extensive branching into the tumor substance [fig.2a, b].

Tumor cell-neovascular interaction:
The formation of the tumor-vascular complex is best outlined by the gold impregnation technique as given below.

Aurophilia [Auro]:
Endothelial buds arising from the stromal vessels, canalize and acquire a reticulin positive basement membrane before closing in on the tumor margin. A single layer of tumor cells [L1] ensheath these vessels on the abluminal surface [fig.2]. The cells along the BM show aurophilia first in the cell membrane followed by the cytoplasm and by the appearance of a single process extending outwards into the proliferating layers of cells [fig.3a]. Some cells are seen to extend processes along the BM of the vessel [fig.3a,b]. Aurophilic cells extend into L2 as the TVC enlarges and further layers are added on. The aurophilic processes elongate outward along with the increasing concentric layers of tumor cells from L3 to L5 [fig.3c]. These processes branch and arch over to form a complex supporting framework along with reticulin fibres for the proliferating tumor cells [fig.5a].

Nes [fig.4] and GFAP positivity:
Nes positivity is seen in the early stages of the TVC, upto formation of L1 layers there being 56.25% positive cells in L1 around the vessels [fig.4]. Nes positivity is first seen along the cell membrane as single cells [fig.4a]. As the TVC enlarges positive cells increase from 2 to 15 cells and along the length of the vessel [fig.4bc]. The cells extend dendiritic processes in 2-3 layered TVCs, which are positive for nestin and are well outlined [fig.4d].

GFAP: The same features are well brought out with GFAP positivity as well [fig.5b]. GFAP+ve cells are seen in both in L1 and in L2. GFAP +ve cells continue to be present in the fully formed TVC. The cells showing aurophilia and nestin/GFAP positivity in L1 & L2 resemble the radial glia seen during neurogenesis, nestin being a marker of multi-lineage progenitor of neuroepithelial stem cells, [25]. The tumor cells grow out into concentric layers to form a compact spheroid structure, the TVC, composed of 5 to 6 layers of tumor cells demarcated by slender reticulin fibers [fig.5a]. The TVCs are clearly demarcated from the surrounding sheets of tumor cells which are pushed aside by the expansile growth. In the fully formed TVC the perivascular cells are GFAP+ve and aurophilic [fig.5ab].

NFP and Syn positivity [fig.5cd]:
The outer layers, L4 & L5 show NFP and Syn positivity in the fully formed TVC associated with the presence of Nes/GFAP/Auro positive perivascular cells [fig.5cd].

Quantitation of positivity in TVC [fig.6]:
The pattern of differentiation in the expanding tumor cell layers around the angiogenic vessel, is studied by aurophilia and positivity for Nes, GFAP, NFP and Syn. Quantitation and comparison by positive cell counts has been given below in 882 TVCs. 33 neovascular channels remain quiescent with a single layer of tumor cells outside a thin silver positive BM [fig.2a]. There is no aurophilia or immunopositivity in these cells nor is there any proliferation as seen with positive dendritic cells.

Nes [fig.6]: Nes positivity is seen in L1 upto the formation of L1& L2. Positive cells form 56.25% [3933 of 6992] cells counted in the 437, 1&2-layered TVCs.

Auro [fig.6]: Auro positivity is highest in the L1 and L2. Auro positive cell counts are 68.75% in L1 [4807/6989 cells], and 81.25% in L 2 [11362/13984 cells]. In the outer layers of the TVC there no Auro+ve cell bodies, being replaced by arbors of dendritic processes.

GFAP [fig.6]: Highest GFAP positivity is in the L1 and L2. GFAP positive cell counts are 43.7% in L1 [3056/6995 cells], 57.3% in L 2 [8013/13985 cells]. In the outer layers of the spheroid the GFAP positivity
declines, being 25.5% cells in L3 [5043/19776 cells] and 3.8% cells in L4 [1002/26388 cells]. None of the tumor areas are positive in the L5. Thus the maximum GFAP positive cell counts are in the layers L1 and L2.

**NFP [fig.6]:** NFP positivity is low in the inner layers with a positive cell count of 3.8% in L1 [266/6592 cells] and 7.9% positive cells in L2 [1105/13187 cells]. Maximum NFP positivity is in the L3 and L4 with 38.9% cells [7693/19773 & 13632/26371 cells] respectively; while L5 is with 50.6% positive cells [16679/32963 cells], maximum positivity being from L3 to L5.

**Syn [fig.6]:** Syn positivity is higher in the outer layers of the spheroid as compared to the inner layers. The layers L1 and L2 are composed of 9.7% [678/6595 cells] and 21.9% [3058/13184 cells] positive cells respectively. Positivity increases to 38% positive cells in L3 [7515/19779 cells]. The positivity goes up to 62.3% in the L4 [16427/26365 cells] and 63.5% in L5 [20932/32968 cells]. Thus peak positivity is in the L4 & L5.

On statistical analysis of the pooled data there is a significant difference among the treatment groups: On assessment with ANOVA: L1 & 2: GF/auro/nestin positive cells in layers 1 & 2 are significantly higher than NFP & Syn. GFAP+ve cells in the surrounding tumor away from the angiogenic channels do not show aurophilia or dendricity and are not related in a specific manner to NFP/Syn+ve cells. Thus aurophilia is seen specifically in GFAP+ve dendritic cells abutting on the neovascular angiogenic vessels.

**Discussion**

Vertical growth phase [VGP] melanomas are highly angiogenic and proliferative lesions [27]. Angiogenesis is defined as the sprouting of blood vessels from preexisting ones [28-30]. To meet the demands of the growing population of tumor cells a complex interaction occurs between the advancing tumor and the surrounding blood vessels as observed in this study. Endothelial buds grow out towards the tumor margins to enter the substance, thus initiating tumor angiogenesis from the pre-existing vasculature. Reciprocal paracrine interactions have been demonstrated between astrocytes, endothelial cells and ependymal cells. Vascular endothelial growth factor [VEGF165] can be released from both astrocytes and neurons. This rise in VEGF levels elicits a burst of mitotic angiogenesis, which is followed by the production of brain-derived neurotrophic factor (BDNF) by the stimulated microvascular cells [31-33]. In the adult hippocampus, neuronal progenitor cells are spatially associated with mitotic endothelial cells, in foci of concurrent angiogenesis and neurogenesis [34-36].

Gold chloride sublimate [GCS] used in Cajal’s stain specifically stains astrocytes [37]. Cajal’s stain, utilizes gold chloride [with no silver] for 4h to 6h on frozen sections [21,24]. Exposure to both silver nitrate and gold chloride is for 1min each in the stains for reticulin fibers, while the silver impregnation time is prolonged in contrast to gold toning which is for a fraction of the time in stains for nerve cells and fibers. In the present modification gold impregnation is for 15-25min while silver impregnation is for a very brief 15-30sec and serves as a counter stain. Any aurophilia of nerve cells and fibers is inhibited by the preoxidation with Potassium permanganate and bleaching in potassium metabisulphite. Sodium thiosulphate removes unreduced silver/gold and develops the specific staining. Thus the important modification is the prolonged time of exposure to gold versus silver. The modified gold impregnation technique used in this study brings out exquisite details of the evolution of radial glia like cells and the TVC. The added advantage of this technique is that it utilizes paraffin sections, these preparations do not fade and can be stored permanently for further studies of archival material.

As described above, during angiogenesis the sprouted endothelial tubes cannelise and acquire a reticulin positive basement membrane before entering the tumor margins. The neovascular channels show extensive branching into the tumor substance near the margin. Initially a single layer of tumor cells ensheath the vessels on the abluminal surface of the basement membrane [figs.1&7]. These cells abutting on the basement membrane acquire aurophilia [figs.1,2]. Nes [figs.4a-d] and GFAP [figs.5b] positivity first in the cytoplasm, followed by the outward extension of single processes. As concentric layers of tumor cells grow out from this layer, aurophilic, Nes and GFAP positive processes extend outward through the layers of tumor cells towards the periphery. The processes form a supporting framework for the extending layers of tumor cells. Occasional processes extend along the circumference of the central vessel [fig.7]. Nes positivity is seen till the formation of 1 to 2 layers, thereafter to be replaced by Auro and GFAP positivity.

Nestin has been the predominant marker used to describe stem and progenitor cells in the mammalian CNS. It is a protein belonging to class VI of intermediate filaments produced in stem/progenitor cells in the mammalian CNS during development, and...
is expressed mainly in neuroepithelial stem cells but not in mature elements. During neuro- and gliogenesis, nestin is replaced by cell type-specific intermediate filaments, e.g. neurofilaments and glial fibrillary acidic protein (GFAP). During embryogenesis, nestin is expressed in migrating and proliferating cells, being restricted to areas of regeneration in adult tissues. Its presence in cells indicates multi-potentiality and regenerative potential.

In neural cell cultures during the first week in culture, approximately 10-30% of the total cell population stained for the glial cell marker GFAP, and nearly all commounostained for nestin. Nestinis expressed in endothelial cells of CNS tumor tissuesand of adult tissues that replenish by angiogenesis and so can be used as a marker protein for neovascularization [25,38-41]. This fact is well brought out in the present study, since the endothelial cells lining the angiogenic vessels are positive for Nes as they enter the tumor. As the TVC forms, the L1 cells show Nes positivity as well as GFAP/Auro positivity and resemble radial glia the stem and progenitor cells.

GFAP, traditionally referred to as a specific marker for astrocytes of the CNS is a 50 kDa intracytoplasmic protein, which constitutes the major cytoskeletal protein in astrocytes [42]. GFAP positivity and glial differentiation is inversely proportional to astrocytic anaplasia. GFAP positivity identifies the radial glial multipotent astrocytic stem cells during embryogenesis as described in several studies [42, 43].

It has been shown that on interacting with the angiogenic vessels, MASC give rise to the full gamut of neuronal differentiation in neurospheres in cultures [43-51]. Neurofilaments, the neuron-specific intermediate filaments localized by NFP positivity for neuronal differentiation form the dynamic axonal cytoskeleton together with other axonal components such as microtubules to maintain and regulate neuronal cytoskeletal plasticity [reviewed by Kesavapany et al, 2003] [52]. During development neuroepithelial cells in the neuronal lineage lose nestin and vimentin [53] to express NF-H when the maturing cells are forming synapses [54]. NFP positivity is seen in differentiated ganglion cells, neoplasms of neuronal or mixed cell origin as well as neuroendocrine tumor cells. Ramirez et al, 1999 [55] found rabbit choroidal melanocytes, perivascular and intervascular fibers positive for NFP.

Synaptophysin which is a vesicular integral membrane protein labels small synaptic like microvesicles (SLMV) present in neuroendocrine cells such as the pituitary and adrenal medulla. [55]. Synaptophysin and synaptobrevin are abundant membrane proteins of neuronal small synaptic vesicles. These vesicles characterized by Syn contain considerable amounts of the biogenic amines [56,57]. Earlier studies have identified the presence of biogenic amines, catechol amines as well as indole amines in melanocytes [2-7]. TVCs show organized expression of biogenic amines in relation to the angiogenic vessel in the L2L3 [indoleamines] and in L4L5 [catecholamines] coinciding with areas of Syn positivity [71].

From the present data it is seen that there is a regular pattern of differentiation initiated by the tumor cell-vascular interaction, appearance of Nes positivity followed by GFAP positivity and aurophilia being the first step. Where this positivity is absent there is no further proliferation. As the new layers of tumor cells grow out there is a zone where all three markers are transit between L2 to L3 followed by NFP and Syn positivity in L4&L5 [fig.5cd].

On comparing the levels of positivity in the total number of TVC, GFAP positivity is seen in the layers adjacent to the vessel wall [L1-3]. NFP is positive in the peripheral layers [L3-5]. Both GFAP and NFP are transitional in L3 equivalent to the ‘asterons’ described by Steindler DA, 2006 [64]. Syn positivity is highest in L4-5 coordinated with NFP positivity [fig.6]. Presynaptic Syn/ synaptobrevin complex [Syp/Syb-complex] appear during neuronal development [58, 59]. Thus Syn expression, initiated in the inner layers of the spheroid, peaks as the cells differentiate into NFP positive cells.

The sequence of progression from radial glia-like to neuronal positive cells in the TVC simulates the differentiating patterns in in vitro neurospheres and early embryogenesis of the neural tube. The astrocyte-like stem cells have been shown to have the ability to generate neurons [47,60]. Work of others has shown that in differentiating from primary floating neurospheres newly-generated neurons can assume or revert to an astrocytic phenotype. In differentiating primary floating neurospheres neurons can shift into cells with astrocyte characteristics by transiting through an “asteron” (neuron/astrocyte hybrid) morphotype which coexpress a variety of neuron and astrocyte proteins and genes [50].

Melanocytes, derived from the multipotent neural crest cells [NC], express characteristics of astroglia, including features of radial glial cells, to simulate the multipotent astrocytic stem cell [31-36] on interaction with the endothelial tubes the tumor cells, in melanoma. Thus melanoma cells replicate glia-vascular interaction, to form structures resembling neurospheres in situ.

Studies have recently shown that signals potentially derived from the embryonic microenvironment can influence embryonic stem cells [61,62], multipotent
tumor cells [63,64], and adult cell fate and plasticity [65,66]. The signals between the multipotent tumor or embryonic cell and its microenvironment are thought to be complex and manifested in a combination of intrinsic and extrinsic cell-cell and cell-matrix interactions.

Aggressive tumor cells of melanoma share many characteristics with their multipotent, invasive embryonic progenitors of the neural crest, contributing to the paradigm of tumour cell plasticity [67-70]. The present study suggests that an embryonic microenvironment is created around the interacting neovascular channels during tumor-vascular interaction in melanomas, resulting in the development of multipotent astrocytic stem cell [MASC] as during neurogenesis. The sequence of TVC formation indicates that there is a reversal to the stepwise progression seen during neurogenesis, along a sleeve around the central angiogenic vessel. This finding opens up interesting possibilities for utilizing endothelial/tumor cell cocultures for harvesting stem cells and for the study of developmental and adult neurogenesis in the brain as well as in the study of neurospheres in culture. It could also serve as a basis for research on the reversal of the malignant potential of these highly aggressive tumors by creating a suitable embryonic microenvironment by extensive, controlled angiogenesis.

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References

52. Kesavapany S, Li BS, Pant HC. Cyclin-dependent
Illustrations

Illustration 1

Fig 1

The blocks taken from each case is arranged in a grid graded according to the intensity of pigmentation, the maximum pigment being on the top and left. Cases 1 to 4 are heavily pigmented with no amelanotic nodules. Cases 24 to 27 are poorly pigmented and show no pigmented nodules. Cases 5 to 23 show varying proportion of pigmented and amelanotic nodules. 63 nodules labelled ‘a’ in the grid include the tumor/stroma interphase with angiogenesis. The column on the right shows the number of nodules sampled from these cases.
Illustration 2

Fig 2

Composite diagram with camera lucida representations to show tumor-vascular interaction during angiogenesis [stained by the modified gold impregnation method]: [a] Thin walled stromal vessels show reticulin positive basement membranes. Endothelial sprouts are seen within the vascular walls [>). Within the tumor margins a single layer of tumor cells surround the BM [>). Two vessels are quiescent and show a single layer of tumor cells with no aurophilia or proliferation [X] [AuX100] [b]. The vessels branch and further layers of tumor cells grow out to form tumor-vascular-complexes [TVCs]. The cells abutting onto the BM show aurophilia and appear dendritic around the enlarging vessels [AuX400].
Illustration 3

Fig 3

Composite diagram to show the morphological features of tumor-vascular interaction. [a] A branching vessel with two layers of tumor cells, showing the alignment of the aurophilic cells and the dendritic processes. Dendrites extend outward through the layers, as well as along the basement membrane [AuX400] [b] Serial section to show morphology [HEX400]. [c] A 3-4 layered TVC with well defined aurophilic radial glia like cells forming a network of processes. At places there are two layers of aurophilic cells adjacent to the vessel wall [AuX400].
Illustration 4

Fig 4

Composite diagram showing evolution of Nes positive cells. [a] A single Nes+ve cell enclosing a vascular space [NesX1000]. [b] Newly formed angiogenic tubes with a single layer of Nes+ve cells [NesX200]. [c] Longitudinal section of angiogenic vessel with endothelial cells and a perivascular sleeve of Nes+ve cells in L1 [NesX200]. [d] Vessel surrounded by 2-3 layers of tumor cells with Nes+ve cells in L1 showing prominent dendritic processes [NesX1000].
Illustration 5

Fig 5

Sections showing fully formed 5 to 6 layered TVC with CL diagrams [a] Gold impregnation showing two layers of aurophilic cells extending dendritic processes outward. Fine reticulin fibers define five to six layers forming a mantle around the vessel [AuX400]. [b] Serial section showing GFAP positive cells in the same layers [mAbGFAPX400]. The peripheral layers showing NFP positivity [c] [mAbNFPX400] and Syn positivity [d] [mAbSynX400]. In [d] the Syn+ve cells show dendritic processes.
Graph with accompanying table comparing Nes/GF/auro, NFP and Syn positive cells around the central vessel. Layers L1/L2 show high Nes/GF/auro+ve cells, while L4/L5 show high NFP/Syn. Nes positivity is seen in L1, while GF/auro +ve cells are seen in L1/L2 maximum in L2 associated with high NFP/Syn +ve cells in the peripheral layers L4L5. [ANOVA: L1&2: Nes/ GF/auro positive cells are significantly higher than NFP & Syn [F=13.885; p<0.030]. L4&5: NF & Syn positive cells are significantly higher than GF/auro [F=59.878; p<0.004].]
Composite CL diagram summarising the evolution of the tumor cell-vascular interaction. Angiogenic vessels acquire a reticulin positive basement membrane [BM] [a]. On entering the tumor margin, a single layer of tumor cells ensheath the vessels on the abluminal surface of the BM [b]. The cells along the BM become Nes/GFAP positive and aurophilic first in the cytoplasm followed by extension of single processes. [c] 2-layered TVC: Processes form a complex supporting framework for the proliferating tumor cells. [d] 3-4 layered TVC: with inner aurophilic and GFAP+ve cells extending dendrites outwards through the tumor cell layers. [e] A fully developed TVC with inner aurophilic and GFAP+ve cells extending dendrites outwards. A thin reticulin framework demarcates 5 to 6 layers forming the perivascular mantle zone [PMZ] around central vessel.
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