CASE REPORT

Immunohistochemistry of a choroidal melanoma: nestin, CD34 and CD117/c-kit labeling

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Abstract

In a case of choroidal melanoma (CM) in a 70-year-old male patient, was firstly aimed at studying the processes of angiogenesis by use of nestin and CD34 antibodies. Anti-CD117/c-kit antibodies were further considered for their progenitor cells specificity. Choroidal melanoma was histopathologically confirmed. Nestin-positive endothelia were found in the CM and the adjacent retina, but not in endothelia elsewhere in that eye. Nestin-positive non-pigmentary cells were found within the CM. Filopodia-projecting endothelial tip cells (ETCs), nestin- and CD34-positive were found in the CM. CD34-positive ETCs were also found in the iridal stroma. There were found two different immune patterns of the retinal Müller cells (MCs). They were nestin-positive in the retina adjacent to the tumor, but negative in any other part of retina. On the other hand, CD117/c-kit antibodies labeled MCs as follows: (a) discontinuously, or continuously, in the retina adjacent to the CM; (b) only the inner segments of the MCs were labeled in the retina unrelated to the CM. While nestin could be a reliable marker for retinal damage, the CD117/c-kit phenotype of MCs still needs further investigations. Antiangiogenic therapy appears as a good choice for tumor therapy.

Keywords: eye, retina, Müller cell, endothelial tip cells, immunohistochemistry.

Introduction

Angiogenesis represent the formation of new blood vessels from pre-existing ones. It plays an essential role in physiological conditions such as embryonic development [1], healing and reparatory processes [2, 3], but also in pathological situations like diabetes [4] and tumor growth and progression [5]. Two major modes of angiogenesis have been described: sprouting and intussusception [6].

Sprouting angiogenesis is a process with several steps. The endothelial tip cells (ETCs) are specialized cells, located at the edge of the vascular bed, which play a key-role for the migration of the endothelial cells in the environmental matrix. The ETCs protrusions (filopodia) guide the ETCs migration, under the control of the angiogenic factors [7].

A relationship between tumors and the blood supply was identified as early as 1908 by Goldman. At the time, the significance of this observation was not clear [8]. Judah Folkman, the “father” of modern angiogenesis, was the first who concluded that tumor development is angiogenic-dependent [9]. The “angiogenic switch”, the disrupt balance between the pro- and the anti-angiogenic factors, is a pivotal step for the development and progression of solid tumors [10].

The uveal melanoma (UM), arising from melanocytes of the uveal tract, is the most common cause of primary malignant intraocular neoplasm in the western world [11]. The UM can arise from any portion of the uveal tract, but the choroidal melanoma is the most common form [12]. The UM can determine the apparition of secondary new vessels in the retina and in the iris [13]. Choroidal melanoma is a subtype of UM [14].

Certain UMs present “vascular mimicry”, the capacity to form vascular beds in the absence of endothelial cells; these vessels are formed by aggressive cells with an important metastatic capacity [15].

Tumor cell migration and local expansion play important roles in ocular cancer metastasis. For posterior uveal melanoma, tumor expansion can be an invasion in the retina and vitreous cavity or infiltrating the sclera. Aggressive choroidal melanoma can even break the sclera. The eye lacksymphatic vessels; thus, choroidal melanoma spreads by the hematogenous pathway, especially to the liver [16]. Aggressive choroidal melanomas also break Bruch’s membrane and invade retina, with subsequent degenerative alterations of the outer segments of the photoreceptors. Severe detachment of the neurosensory retina is common [12].

Case Report

A 70-year-old male presented with a two months history of left eye decreased visual acuity. On examination, the visual acuity was 6/6 in the right eye and no perception of light in the left eye. Slit lamp examination and intraocular pressure was normal. Indirect ophthalmoscopy revealed a brownish, elevated, and oval-shaped mass nasally. The B-scan ultrasonography of the left eye showed a large dome-shaped mass with acoustic hollowness and orbital shadowing. The A-scan revealed low to medium reflectivity, and negative angle kappa. Ocular examination of the right eye revealed no abnormalities. The chest X-ray was normal. Ultrasonography of the liver did not revealed metastasis. A clinical diagnosis of choroidal malignant melanoma of the left eye was made. Enucleation of the left eye was surgically performed with the informed consent of the patient.

Eye samples were obtained by sagittal cuts and were
fixed for 24 hours in buffered formalin (8%), then were processed with an automatic histoprocessor (DiaPath, Martinengo, BG, Italy) with paraffin embedding. Sections were cut manually at 3 μm, and were mounted on SuperFrost® electrostatic slides for immunohistochemistry (Thermo Scientific, Menzel-Glaser, Braunschweig, Germany). Histological evaluations used 3 μm thick sections stained with Hematoxylin and Eosin. Primary antibodies were used as follows: (a) anti-CD34 (clone QBEnd 10, Dako, Glostrup, Denmark, 1:50); (b) anti-vimentin (clone V9, Dako, Glostrup, Denmark, 1:50); (c) anti-CD105 (polyclonal, Thermo Scientific, Pierce Biotechnology, Rockford, USA, 1:50), anti-nestin; (d) anti-CD117 (c-kit) (clone T595, Novocastra-Leica, Leica Biosystems Newcastle Ltd., Newcastle Upon Tyne, UK, 1:20). Sections were deparaffinized, rehydrated and rinsed in phosphate-buffered solution (PBS) at pH 7.4. Retrieval by cooking in specific buffer was completed: 0.01 M citrate retrieval solution (pH 6.0, 20 minutes, for CD117, CD105 and nestin) or EDTA (pH 9.0, 20 minutes, for CD34). Appropriate endogenous blocking peroxidase was completed before immune labeling (0.1% BSA in PBS). Sections incubated with non-immune serum served as negative controls. Sections were counterstained with Hematoxylin. The microscopic slides were analyzed and micrographs were taken and scaled using a Zeiss working station which was described elsewhere [17].

The eyeball had a 22/22/24 mm. size. At the histopathological exam, a 10 mm diameter dark choroidal tumor was found. The choroidal melanoma presented wide areas of necrosis. In focal areas, the tumor was infiltrating the sclera. There was not found evidence of tumoral spread along the optic nerve. The epithelioid tumoral cells were prevailing.

Nestin immune labeling (Figure 1) was positive for microvascular endothelia within the melanoma and the adjacent retina, but not in ocular tissues unrelated to melanoma, such as the opposite retina or the sclera. Within the melanoma nestin positive filopodia-projecting microvascular endothelial cells were found. There were also found nestin positive non-pigmentary melanoma cells. Retinal Müller cells were nestin-positive in the retina adjacent to melanoma, but were nestin-negative in any other parts of retina. The CD34 antibodies labeled only microvascular endothelia, within the tumoral and non-tumoral tissues (Figure 2). Filopodia-projecting endothelial tip cells were accurately identified within the melanoma, but also in the iridial stroma (Figures 2 and 3). Retinal Müller cells were positively labeled by CD117/c-kit antibodies (Figure 4) labeling was discontinuous in the central part of the retinal area facing the tumor, but continuous in the periphery of this part of retina, where also a paucicellular aspect of the cellular layers of retina was noted. Interestingly, in those parts of retina unrelated to melanoma, the CD117/c-kit antibodies labeled only the inner parts of the retinal Müller cells, the outer halves of these cells being unlabeled by these antibodies.

**Discussion**

Nestin is a type VI-intermediate filament protein [18, 19] known as a marker of progenitor and mitotically active stem cells [20], being expressed in a variety of proliferating tissues, such as heart [21] and skeletal muscle [22], teeth [23], hair follicle [24] and developing retina [25, 26]. In adult retina, Müller cells, the prevalent retinal glial cells [27], with stem proprieties [28], express nestin in pathological conditions such as glaucoma [29], retinal detachment [30] and laser injury [31]. The retinal Müller cells, which have a peculiar and easy identifiable morphology, are a veritable scaffold of the retinal tissue, especially in injuries [32, 33].

It was demonstrated that nestin expression in Müller cells is a reliable and specific marker for retinal injuries of different etiologies [34]. These findings are reinforced by evidence brought here of nestin-positive Müller cells in the retina adjacent to the melanoma, but not elsewhere in retina. Nestin immune reactivity may indeed correlate with the capacity of retina to maintain the structural and functional standards.

In a study on choroidal melanoma was found nestin immune reactivity in the invading tumor edge, in endothelial cells and tumoral non-pigmented cells, and it was concluded that nestin expression in melanoma associates poor prognosis [35]. The bordering areas of the tumor concentrate cells with high aggressive potential [36]. Similar labeling was found for nestin in the present case.

We found nestin-positive endothelia in the melanoma and the adjacent retina and nestin-positive filopodia-projecting endothelial cells in the choroidal melanoma. This seemed justified, as nestin is expressed by endothelial cells of newly formed endothelia, being so a reliable marker for sprouting angiogenesis [37] and tumoral angiogenesis [38]. CD34 is commonly used as an endothelial cell marker of tumor vessels [39] and ETCs [40, 41]. However, this marker detects not only newly formed, but also pre-existing large blood vessels [42]. Although we found endothelia CD34 positive in all eye structures, we identified CD34-positive ETCs only within the melanoma, as well as within the iris stroma. So, both nestin and CD34 antibodies were found labeling ETCs. On other hand, nestin labeling can identify from a CD34-positive microvascular bed the newly formed vessels.

CD117/c-kit is a transmembrane receptor tyrosine protein kinase, which is expressed by several cell types, such as interstitial Cajal cells (ICC)s or Cajal-like cells (ICLCs), telocytes, mast cells, melanocytes, progenitor cells, neurons, and glial cells [43-49]. CD117/c-kit regulates survival, proliferation and cell differentiation [50, 51]. In our study, the retinal Müller cells were continuously or discontinuously labeled by CD117/c-kit antibodies. Only the inner segments of the Müller cells were CD117/c-kit positive in the retinal sectors unrelated to the tumor. In this regard, the expression of CD117/c-kit seems not reliable as nestin to indicate situses of retinal maintenance. CD117/c-kit- and nestin-positive labeling of Müller cells could be viewed as a reactive gliosis of injury, as was previously suggested, but only for nestin [34, 52, 53].

The tip cells extend filopodia of variable length, the long ones appearing moniliform in transmission electron
microscopy [3]. Numerous filopodia emerging from a
tip cell are highly suggestive for an active migratory
capacity of this high-specialized endothelial cell; these
filopodia guide the tip cell migration, and lead the extent
and direction of the new vessel, under the control of
vascular endothelial growth factor A (VEGF-A) gradients
[7, 54, 55]. The ETCs have a minimal or inexistent
capacity of proliferation, but are able to recruit non-
vascular cells [7]. During pathological angiogenesis, the
ETCs and the number of filopodia increase [7]. Anti-
angiogenic therapy is a promising option for cancer and
metastasis management. The evaluation of the angio-
genesis may be a predictor of the melanoma behavior
and could be used to evaluate the cancer prognostic.

Conclusions

While nestin could be a reliable marker for retinal
damage, the CD117/c-kit phenotype of retinal Müller cells
needs further investigations. As target to control the cancer
invasion and metastasis, angiogenesis may provide new
clinical treatment strategy for choroidal melanoma.

Figure 1 – Nestin immune labeling of the choroidal melanoma (M) and adjacent retina (R) (A). The Müller cells in
the retina adjacent to the melanoma (R) are positively labeled by nestin antibodies (A and B). However, Müller cells in
the retina (R') opposite to the melanoma are nestin-negative (C). Nestin-positive endothelia are identified in the retina
adjacent to the melanoma (B), within the melanoma (D), but not in eye structures unrelated to the melanoma, such as
the opposite retina (R' – C) or sclera (S – E, the arrowhead indicates the episclera). Non-pigmentary melanoma cells
are also nestin-positive (F). Tumoral sprouting angiogenesis is proven by tip cells' filopodia (D, inset, arrows).
Figure 2 – CD34 antibodies label (A) endothelial cells (arrowheads) within the melanoma and the adjacent retina (R). Endothelial tip cells intrinsic to the melanoma, but not retinal, project filopodia (B, arrows).

Figure 3 – Beneath the posterior pigment epithelium (arrowhead) endothelial tip cells project into the iridial stroma moniliform filopodia (arrows).

Figure 4 – (A) CD117/c-kit antibodies labeling of the retina (R) adjacent to the melanoma (M) discontinuously labeled the retinal Müller cells; the outer plexiform (arrow) and the inner plexiform (arrowhead) layers being poorly labeled. (B) Towards the periphery of the tumor the retina (R) the Müller cells have a continuous immune positive phenotype (the arrowhead indicates the outer granular layer and the arrow indicates the inner plexiform layer). (C) In the retina, which was not directly related to the melanoma (R’), the CD117/c-kit antibodies partly labeled the Müller cells within the inner retina (arrow), but not in the outer retina (arrowhead).
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References


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