

Telocytes are the common cell of origin of both PEComas and GISTs: an evidence-supported hypothesis

Carmen Ardeleanu^a, Gianni Bussolati^{a, b, *}

^aVictor Babes Institute, Bucharest, Romania

^bDepartment of Biomedical Sciences and Human Oncology, University of Turin, Turin, Italy

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Abstract

We advance the hypothesis that the telocyte might be the cell of origin of both PEComas (perivascular epithelioid cell tumours) and GISTs (gastro-intestinal and extra-gastrointestinal stromal tumours). The hypothesis is supported by data from the literature reporting that both PEComas and GISTs, as well as telocytes, share the expression of several markers. These data were supplemented by original immunohistochemical tests on selected series. Specifically: (1) Melanoma markers (Melan A, MiTF) typical of PEComas are expressed by a substantial fraction of GISTs. A fraction of GISTs was also found positive for CD63, a tetraspanin protein originally described in melanomas and marking exosomes. (2) c-KIT (CD117), proper of the vast majority of GISTs, can be expressed by PEComas (as well as by telocytes). (3) Markers described in telocytes (CD34, S-100, smooth muscle actin and vascular endothelial growth factor) have been reported as positive in cases of PEComas and GISTs. Telocytes show distinctive ultrastructural features with thin, extended, telopodes and are likely involved in inter-cellular signalling *via* paracrine secretion as well as by shed vesicles and exosomes. These cells have been described in many locations (cavitary and non-cavitary organs) and might display potentialities of a wide spectrum of differentiation (and function). In conclusion we propose that telocytes could be the common cells of origin for both PEComas and GISTs.

Keywords: telocytes • immunohistochemistry • GIST • PEComa • c-kit • CD34 • VEGF

Introduction

The term 'telocytes' [1] has been selected to designate cells characterized by thin, extended, moniform projections best recognized in electron microscopy which have been described in the stroma of several organs as heart [2–5] and skeletal muscles [6], vessels [7, 8], placenta [9], duodenal lamina propria [10], pleura [11] and lungs [12], being mainly located in perivascular areas. Their light-microscopical detection is linked to the expression of several markers such as c-kit (CD117), CD34, smooth muscle actin (SMA), S-100 protein, vimentin and vascular endothelial growth factor (VEGF).

Although they seemed similar to interstitial cells of Cajal, they are not identical. However, they (or perhaps only a fraction) might be engaged in the process of stem cell proliferation, because the presence of exosomes suggests that they share features with

mesenchymal stem cells [13] and direct physical nanocontacts were reported [12].

No pathological lesions have so far been referred to neoplastic or reactive alterations of telocytes.

We advance the hypothesis that these cells might indeed share a prominent interest in pathology, and that they might represent the common cell of origin of a variety of stromal tumours and non-neoplastic conditions, whose cell of origin is presently unclear, comprehensive of tumours supposedly derived from perivascular epithelioid cells (s.c. PEComas) and of gastrointestinal and extra-gastrointestinal (GISTs, and E-GISTs) [14]. Our hypothesis surmises that a phenotypic spectrum (likely linked to different specific functions) is acquired by telocytes in different organs and locations, and suggests a common histogenesis of different pathological lesions. This hypothesis finds support in a series of serendipitous observations in the literature (see below) which otherwise would remain unexplained. In addition, we looked, in a limited number of selected cases, for additional experimental data supporting our hypothesis and thus we checked, in a series of lesions and foetal organs for the alternative expression of markers presently regarded as proper, if not specific, of either PEComas or GISTs.

*Correspondence to: Professor Gianni BUSSOLATI,
Victor Babes Institute, Bucharest,
Romania Spl. Independentei 99-101,
050096 Bucharest, Romania.
Tel.: 0040-213192732
Fax: 00390116635267
E-mail: gianni.bussolati@unito.it

Material and methods

Tissue specimens were routinely fixed in buffered 10% formalin and embedded in paraffin.

We collected from the archives of our institutions: five cases of pulmonary lymphangiomyomatosis, five cases of angiomyolipomas of the kidney, 25 cases of GISTs and thoracic block (comprehensive of heart and lungs) from three cases of 17-week-old fetuses (from medically oriented abortions).

Four-micron-thick paraffin sections were stained using the FLEX-EnVision visualization system by Dako (Glostrup, Denmark) following the procedure recommended by the producer. The following reagents were used as primary antibodies:

HMB45 (clone HMB45, 1:50; Dako), Melan A (clone A 103, 1:50; Dako), microphthalmia transcription factor (Mitf) (clone 34CA5, 1:20; Novocastra, Newcastle-upon-Tyne, UK), CD63 (clone NK1/C3, 1:100; Novocastra), CD117 (polyclonal, 1:400; Dako), S-100 protein (polyclonal, 1:400; Dako),

smooth muscle actin (SMA, clone 1 A4, 1:50; Dako), CD34 (clone Qbend, 1:50; Dako).

The immunohistochemical reaction was developed using DAB as chromogen and nuclei were counterstained in Haemalum.

Results

We first checked in GISTs for the presence of melanoma markers, which are regarded as diagnostic of PEComas. In a preliminary study on 10 cases of GIST, none was found positive for HMB45. We then tested additional melanoma markers on a selected tumour series (Table 1) and 58.33% were found positive for Mitf (14 out of 24 cases of GISTs of the spindle, epithelioid or mixed type) and 68.18% were positive for CD63 (15 out of 22 tested

Table 1 Cases of GISTs tested for immunohistochemical markers

Nr crt	Nr case	Histological type	MITF	CD63	CD117	CD34	Others
1	122476/12524	Spindle cell	+	++	+++	+	DOG1+, Tau+
2	125572/170691	Spindle cell	++	+++	+++	–	DOG1+Nest+D2-40+
3	122620/158407	Spindle cell	++	+++	+	+++	Nest+, Tau+Act-S-100-
4	172861/19229	Spindle cell	+	+	nd	nd	DOG1+, Act+, S-100+, PDGFRA–
5	121339/9042	Epithelioid	++	–	++	+	
6	125039/97356	Spindle cell	+	+++	++	++	Act+, S-100+, Tau+
7	120007/7556	Spindle cell	–	+	–	++	DOG1+, Nest+, Act+, S-100+, Tau+
8	140581/194914	Spindle cell	–	nd	++	++	Nest+, Act+, S-100+
9	119213/736	Epithelioid	–	nd	+++	+++	Nest–, Act–, Tau+, S-100-
10	159971/141910	Spindle cell	–	–	+++	+++	Nest+++D2-40 +/-, Cav + PDGFRA +/- Act–, S-100–
11	176172/83466	Spindle cell	++	–	–	+++	S-100–, Act+
12	171246/169333	Spindle cell	–	++	+++	+++	DOG1+, Nest+, S-100+, Act+, D2-40–, PDGFRA–
13	162922/147189	Spindle cell	–	+++	+++	nd	
14	172056/172088	Mixed	–	–	+++	+++	PDGFRA+, Nest+, S-100+, Act–, D2-40–
15	172238/148390	Mixed	++	+++	+++	+++	Nest+, PDGFRA+, D2-40+
16	180373/83467	Spindle cell	+	+	+++	+++	Act+, S-100-
17	167839/67394	Epithelioid	–	+	–	++	Act+, S-100+
18	167842/148389	Mixed	+	+	–	+	Act+, S-100+
19	172240/159475	Epithelioid	–	–	+++	–	Nest+, PDGFRA+, D2-40–
20	148649/176069	Spindle cell	+	nd	+++	+++	Nest+, Act+, S-100+, Tau+
21	172242/172909	Spindle cell	+	–	+++	+++	DOG1+, Nest–, D2-40-, PDGFRA–
22	148504/113074	Mixed	+	++	++	nd	DOG1+, Nest+, D2-40–
23	121339/9042	Epithelioid	+	–	+	++	Nest+, Tau+
24	149944/5032	Epithelioid	–	++	+++	–	DOG1+, Nest+, Act+, D2-40–
25	152303/37203	Spindle cell	nd	+++	+++	–	DOG1+, Nest+, Act+, S-100+, PKC?+, HMB45–

–: negative; +: sparse positivity; ++: zonal positivity; +++: diffuse positivity; nd: not done

Fig. 1. Cases of gastro-intestinal stromal tumours (GISTs) stained with anti-microphthalmia transcription factor (Mitf) (A) and with anti-CD63 (B). Numerous tumour cells are positive (staining for Mitf located in the nucleus). Nuclei counterstained with Haemalum (200×).

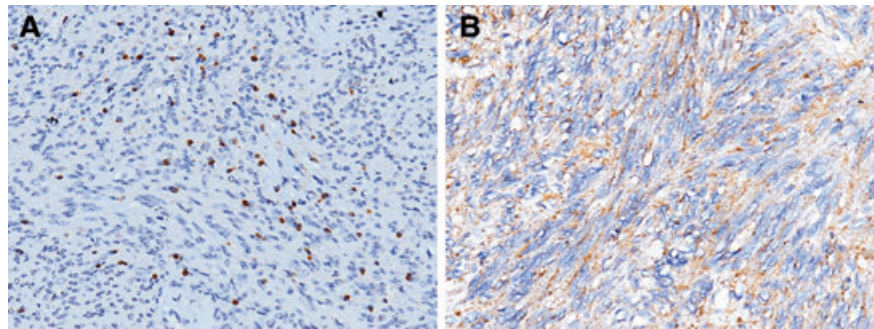
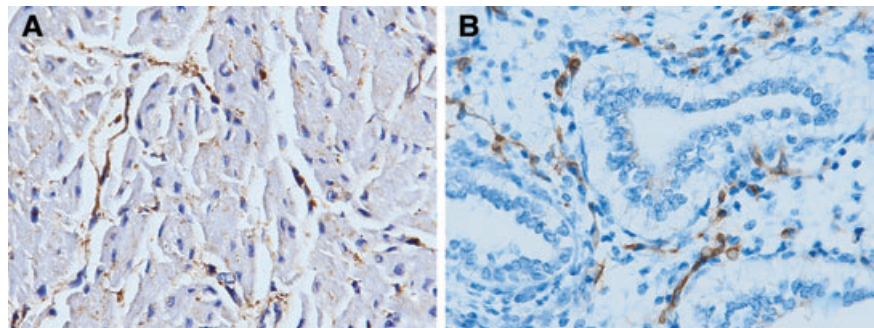


Fig. 2. In the foetal heart, elongated cells sharing the structure and location of telocytes are marked by CD63 staining (A). In the foetal lung, numerous irregularly arranged spindle-shaped cells, with long and thin projections are detected in the interstitium by CD-117 staining (B). Nuclei counterstained with Haemalum (400×).



cases). The staining was either limited to a few dispersed cells or extended to the vast majority of tumour cells (Fig. 1A and B). The kidney PEComas tested for CD63 were also positive, similar to other melanocytic markers.

Then we evaluated CD117 expression in PEComa-related lesions. Cytoplasmic projections or sparse spindle cells, morphologically reminding of telocytes, were CD117-positive in focal intra-alveolar areas typical of lymphangioliomyomatosis of the lung.

In the foetal heart we detected by CD63 staining some elongated telocyte-like cells in perivascular areas (Fig. 2B), presence of Mitf-positive elongated cells in the atrial wall and a very limited number of cells, mainly located in sub-pericardial areas, were found to be CD117-positive. On the contrary, in areas of foetal lung, no CD63-positive cells were observed, while numerous haphazardly arranged spindle-shaped cells, with long and thin projections, were detected in the interstitium by CD117 immunostaining (Fig. 2B).

Discussion

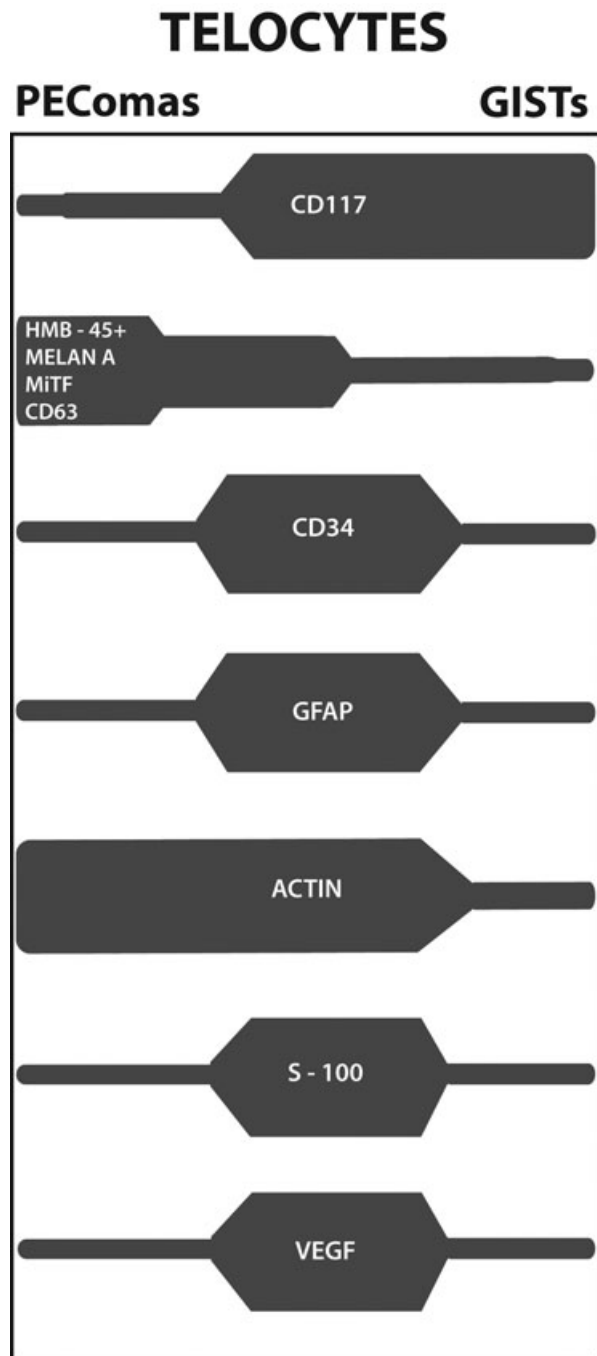
PEComas is a general term indicating a family of tumours including renal and hepatic angiomyolipomas, clear cell sugar tumours of the lung and clear cell myxoid melanocytic tumours, as well as pulmonary lymphangioliomyomatosis [15]. These lesions are sup-

posedly originating from perivascular epithelioid cells, but their normal histological counterpart is presently unknown [15, 16]. The common and unifying feature of PEComas is the remarkable (and so far unexplained) modulation of immunohistochemical markers, with co-expression of SMA and myosin and melanocytic markers (HMB 45, Melan A). The histotype of the tumours varies, from spindle to epithelioid. Focal and occasionally remarkable expression of S-100 protein, SMA, desmin, CD117 (c-KIT) and CD 34 has been noted in these tumours [16, 17]. PEComas have a widespread distribution, with reports of cases occurring in sites such as gastro-intestinal tract, soft tissues and female genital organs, but also in the heart [18], laryngopharynx [19] and pancreas [17].

Occasional and so far unexplained observations, in both PEComas and GISTs, have been reported in the literature:

- (1) Expression of melanoma antigens, particularly of Melan A, has been noted in a substantial fraction of epithelioid GISTs [20]. We have here confirmed and further expanded these findings. In agreement with data of the literature [20] we did not detect positivity for HMB45, but a considerable number of GISTs were found positive with Mitf and especially with CD63, a marker characteristic, though not specific, of melanomas [21]. The significance of these findings, as far as type and clinical evolution of melanoma-marker positive GISTs, is presently unknown and warrants experimental study.
- (2) CD117 (c-KIT) expression is proper of the vast majority (over 95%) of GISTs, but it can occasionally be detected in

Table 2 Schematic representation of the expression in PEComas and GISTs of immunohistochemical markers proper of Telocytes



PEComas [16, 17, 22]. In the present work we observed CD117-positive cytoplasmic projections or sparse spindle cells, morphologically reminiscent of telocytes, in focal areas of lymphangioliomyomatosis of the lung.

- (3) SMA is expressed in GISTs [23], as well as in the vast majority of PEComas [16] (telocytes have positive expression of α SMA) [9].
- (4) S-100 expression has been reported in both PEComas [18] and spindle cell GISTs [20,24] (telocytes are also positive for S-100) [5].
- (5) PEComas as well as GISTs are occasionally CD34-positive [17, 23] (telocytes have positive expression for CD34) [5, 9, 25, 26].
- (6) VEGF is expressed in GISTs, in angiomyolipomas and in lymphangioliomyomatosis [27, 28] (telocytes have positive expression for VEGF [6, 9, 29, 30].

All these data, which would remain unexplained, are fitting with the hypothesis that the common cell of origin of both PEComas and GISTs has to be referred to telocytes, a cell type with ubiquitous distribution and distinctive structural features, likely involved in inter-cellular signalling *via* paracrine secretion. Telocytes would then give rise, in several organs, to a spectrum of lesions variably expressing at least some of the cells' differentiation markers. A schematic representation of this concept is presented (Table 2).

The present hypothesis suggests a common cell of origin of PEComas and GISTs, two tumour types with apparently unrelated pathogenesis, because the former are associated to genetic alterations of tuberous sclerosis complex (TSC), an autosomal dominant genetic disease due to losses of TSC1 (9q34) or TSC2 (16p13.3) genes which seem to have a role in the regulation of the Rheb/mTOR/p70S6K pathway, while the vast majority of the latter are due to mutations of KIT and PDGFRA genes. However, evidence that these tumour types share common metabolic pathways derives from evidence that they both are sensitive to agents targeting the mTOR pathway [31, 32].

Our hypothesis carries as well a prospect on the definition and significance of telocytes, which should then be regarded as an archetypal entity with potentialities switching from a perivascular stromal cell to a differentiated cell type with contractile and signalling properties. In the heart interstitium, a site where ultrastructural investigations detected a vast number of telocytes [25, 33, 34], we observed MITF, CD117 and/or CD63-positive cells. The morphology of these cells closely matches that of telocytes already described in this location. No data are presently available on telocyte-related pathological lesions of the heart, but the present hypothesis might imply a reconsideration of the histogenesis of cardiac stromal tumours whose nature and cell of origin are presently questioned, such as cardiac myxomas [35, 36]. The significance of the expression in GISTs and PEComas of CD63, a tetraspanin protein known to be typical of exosomes [37], remains to be established. It remains to be defined if the micro-vesicles filling up the thin, extended, cytoplasmic projections characterizing telocytes are indeed CD63-positive. Our finding of a remarkable number of CD117-positive stromal cells, morphologically fitting as telocytes, in the interstitium of foetal lung is equally awaiting further investigations. So far, telocytes have been described in the sub-pleural areas of the lung [7], and recently in lung [12].

In conclusion, a series of data from the literature as well as our focused immuno-histochemical investigations concurrently fit

with the hypothesis that a phenotypic spectrum (likely linked to different specific functions) can be acquired by telocytes in different organs and locations, and that these cells might constitute the cell of origin of pathological lesions occurring in different organs and specifically designated as PEComas and GISTs.

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