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Infrastructure of the telocytes from tumor stroma in the skin basal and squamous cell carcinomas

N. MIRANCEA[1)], ANA-MARIA MOROŞANU[1)], G.-V. MIRANCEA[2)], F. D. JURAVLE[2)], V. S. MĂNOIU[3)]

[1)]*Department of Plant and Animal Cytobiology, Institute of Biology Bucharest of Romanian Academy, Bucharest*
[2)]*"Carol Davila" University of Medicine and Pharmacy, Bucharest*
[3)]*National Institute of Research and Development for Biological Sciences, Bucharest*

Abstract

In this paper, we focus our interest on the ultrastructure of telocytes (TCs) present inside of tumor-stroma in basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). Tumor–stroma cooperation is necessary for tumor growth, invasive behavior and ectopic development of microtumors. There is a plethora of reports about the role of different stromal cell types in tumor evolution in the human body. In this line, almost nothing is known about the recently identified interstitial cell type called telocyte (TC). To our best knowledge, this is the first study to publish TCs in malignant tumors, namely BCC and SCC. Here, we described the infrastructural aspects of TCs as well as their relationships with other tumor stroma components. TC from the tumor stroma has cell body where the nucleus is located and exhibits two (rarely more) very long cell extensions of tens (over 60–100 μm) termed telopodes. A telopode appears as an alternation of very thin segments called podomers and dilated segments called podomes, which accommodate mitochondria, rough endoplasmic reticulum, cytoskeleton, caveolae, as well as coated vesicles. TCs establish homocellular junctions leading to a 3-D network inside of peritumoral stroma. TCs may play an important role in intercellular signaling *via* stromal synapses and shed microvesicle transfer. Comparative evaluation with normal dermal skin showed that telocytes from tumor stroma have a very restraint number of heterocellular junctions. The limitation of TCs heterocellular junctions suggests a possible involvement in induction of cell–cell communication alterations into the peritumoral stroma and, consequently, into the whole tumor mass.

Keywords: telocytes infrastructure, basal cell carcinoma, squamous cell carcinoma, homo- and heterocellular junctions, synapses.

Introduction

The large majority of human cancers have an epithelial origin. There are two main categories of skin cancer: (1) melanoma and (2) non-melanoma. Melanoma (also known as malignant melanoma) is one of the most dangerous cancer phenotype, but the frequency is less common than non-melanoma skin cancers. Non-melanoma skin cancers are mainly comprised of (1) basal cell carcinoma (BCC) and (2) squamous cell carcinoma (SCC). BCC is the most common and the least dangerous. After BCC, SCC is the second-most common cancer of the skin. Overexposure or chronic sun exposure is the strongest environmental risk factor for both BCC and SCC, but mention must be made that immunosuppressive applied to individuals as organ transplant recipients, also increases the incidence of SCC. HPV infection is often associated with non-melanoma cancer. Usually, SCC arises from mutated ectodermal or endodermal cells lining body cavities; consequently, SCC can be developed in the skin, lips, mouth, esophagus, prostate, penis, lung, vagina, cervix, etc. One considers that 90% of cases of head and neck cancer are due to SCC.

A tumor is a very complex ecosystem mostly represented by (1) neoplastic genetic altered cells and (2) tumor stroma represented by (a) different (other) cell types (fibroblasts, fibrocytes, mast cells, inflammatory cells, endothelial cells, naked or mielinated nerves, etc.) as well as (b) extracellular matrix (basal lamina, collagen and elastic fibers and soluble molecules). Autocrine and paracrine factors offer the necessary support for tumor growth and invasive behavior, a pre-requisite for ectopic location and growth of malignant cells by forming secondary tumors. Many reports documented about the tumor–stroma cooperation, including tumor angiogenesis [1–6]. Nowadays, is largely known that for a malignant tumor initiation, as well as for tumor growth and invasion, a permanent cross talk between malignant cells and peritumoral stroma is necessary.

Recently, a new interstitial (stromal) cell type called telocyte (TC) was identified and described in different normal tissue types [7–13]. A TC is defined as a special interstitial cell (pheno)type, having a relative small cell body with 1–5 (more frequently 2–3) specific long (several tens to hundreds of micrometers), cell extensions named telopodes. Telopodes have a moniliform aspect: dilated segments (podomes) alternate with thin segments (podomeres) less than 200 nm thickness. Usually, podomes accommodate mitochondria, endoplasmic reticulum and caveolae (Ca^{2+} uptake/release units). Telopodes may perform homo- and/or hetero-cellular junctions. Moreover, by their telopodes,

TCs release small microvesicles (mean diameter of 180 nm) as a single or a pool of shedding membrane vesicles; the delivered macromolecules from those microvesicles are considered to play a paracrine role by sending signals to neighboring cells and eventually modifying their transcriptional activity [7]. Sometimes, telopodes follow a convoluted aspect [7] as is also the case of such phenotype in stromal adipose tissue (personal ultrastructural unpublished data).

So far, the presence of telocytes as a special cell phenotype present into the tumor stroma and their involvement in skin carcinomas development was not reported. To our best knowledge, this is the first study to publish TCs in skin BCC and SCC. We will first deal with the general ultrastructural alterations at the tumor–stroma interface. Next, the focus will be turned to the ultrastructural features of TCs and their relationships status with different other stromal cell types (homo- and hetero-cellular junctions) as well as their presumptive effects in the context of tumor status.

Materials and Methods

In order to perform transmission electron microscopic (TEM) investigations, small cutaneous tissue fragments about 2–4 mm^3 resulted by surgery as curative option/therapy from patients suffering from BCC and SCC (surgeon got patients consent) were processed following the routine TEM protocol [14]. After pre-fixation in fresh ice-cold 4% glutaraldehyde in sodium cacodylate buffer, pH 7.4 for three hours at 4^0C, tissues were six times washed in 0.05 M sodium cacodylate buffer (pH 7.4) at 4^0C, post-fixed in 2% osmium tetroxide in 0.1 M sodium cacodylate at 4^0C for 2.5 hours, stained *en bloc* with 0.5% aqueous uranyl acetate overnight at 4^0C and washed with 0.05 M sodium cacodylate buffer. After dehydration in graded series of ethanol and infiltration with propylene oxide, specimens were embedded in Glycid ether (Epon 812-equivalent) and finally polymerized at 60^0C for 48 hours. Semithin sections were stained with 1% toluidine blue for light microscopy. Ultrathin sections (80–100 nm) were cut using a diamond knife and collected on 200 mesh grids, and double counterstained with uranyl acetate and subsequently lead citrate. The grids were examined by a Philips transmission electron microscope EM 208S and an electron microscope JEOL JEM-1400 operated at an acceleration voltage of 80 kV. In order to highlight the presence of TCs, several electron microscopic images were digitally colored.

Results

Infrastructural abnormalities of the dermal epidermal junctional zone (DEJZ) in BCC and SCC

Previously, we published our results concerning the ultrastructural aspects of skin tumors harvested from patients diagnosed with BCC or SCC [5, 6, 15]. Relevant infrastructural abnormalities were recorded at the DEJZ. Similar aspects were detectable in the present study (Figures 1–6).

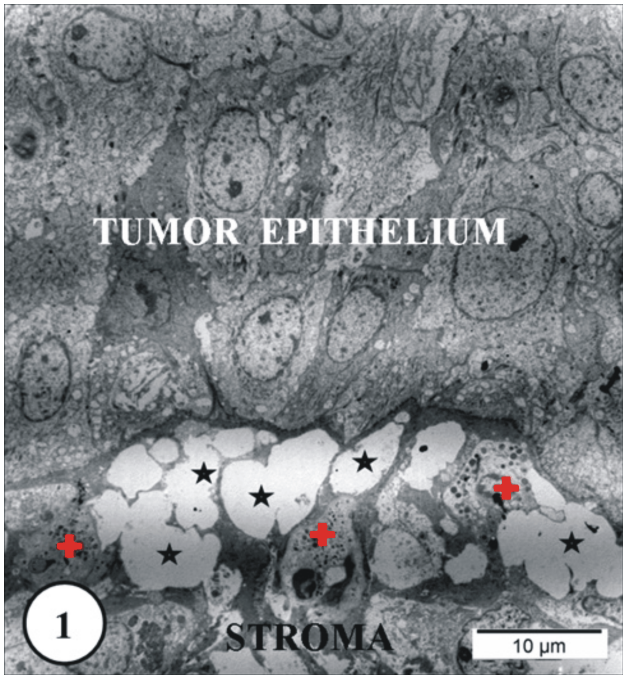

Figure 1 – ***Overview of a tumoral epithelial SCC and adjacent stroma shows tumor cells with large euchromatic and nucleolated nuclei. Infiltrated peritumoral dermal cells are mostly represented by inflammatory cells (red crosses), probably involved in large lysis areas formation (asterisks) below the tumor.***

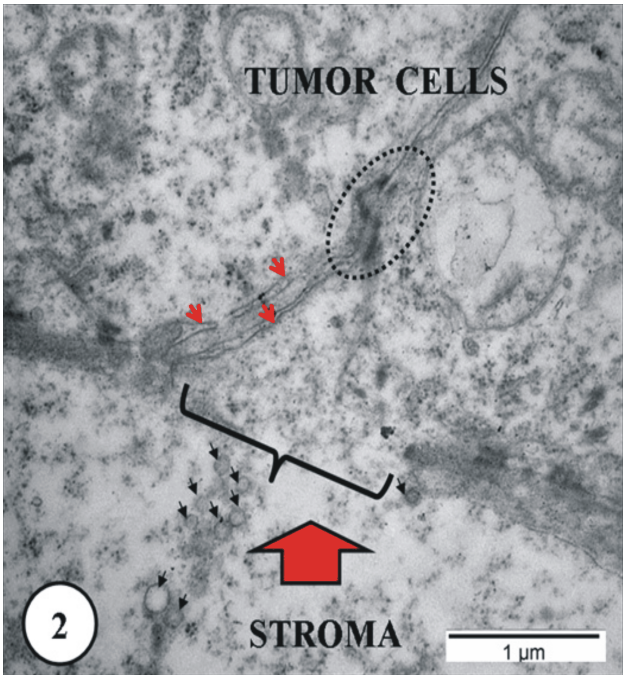

Figure 2 – ***Tumor–stroma interface (large red head arrow) showing a partial dissolution of plasma membrane (accolade) where basal lamina is destructured. At this level, shedding microvesicles are detectable (black small arrows). Duplication of plasma membrane (red arrows) suggests the attempt of tumor cell to repair the damaged basal profile facing peritumoral stroma. Desmosomes between tumor cells are missing except for the one with altered infrastructures encircled by the ellipsoidal area. (SCC).***

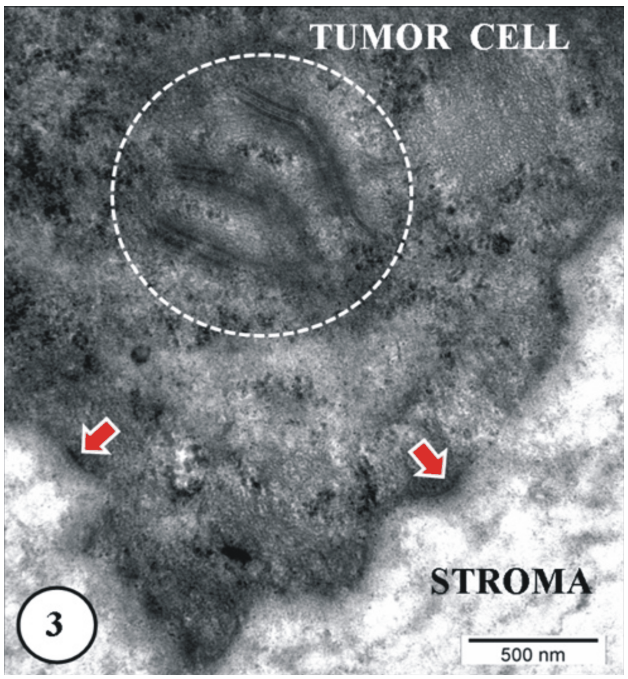

Figure 3 – *At the tumor cell-adjacent stroma level the basal lamina is almost missing but some densely electron microscopic deposits can be seen. Hemidesmosomes (arrows) are defective for the inner plaque, but internalized desmosomes (dotted encircled area) can be seen. (BCC).*

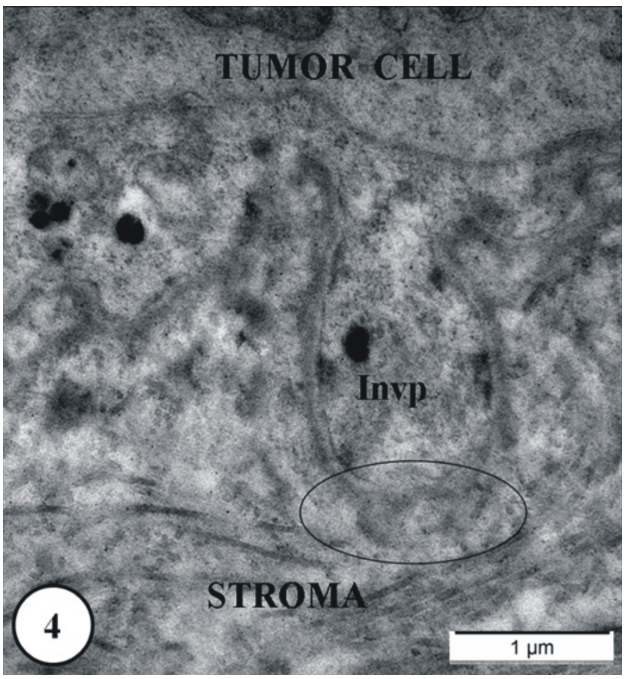

Figure 4 – *At the tumor–stroma interface, hemidesmosomes are almost missing. An invadopodia (Invp) rises from a tumor cell. At this level, plasma membrane as well as basal lamina exhibits a discontinuity (ellipsoidal area). (BCC).*

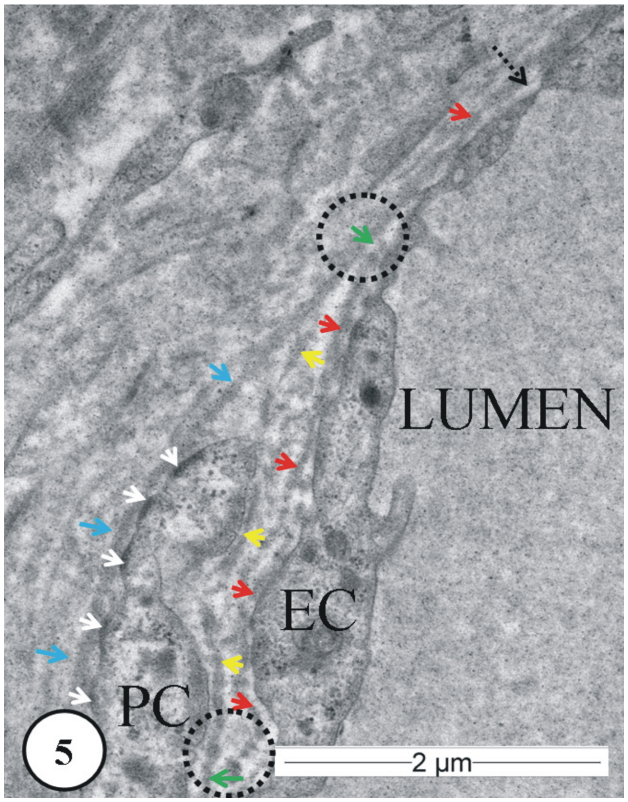

Figure 5 – *A sector of a blood microvessel exhibits a fenestration (dotted black arrow). The basement membranes around the endothelial cell (EC, red arrows) as well at the periphery of pericyte (PC, blue arrows) are continuous. An additional basement membrane is facing the pericyte on the small circumference (yellow arrows). Green arrows (dotted encircled areas) mark the place where periendothelial basal lamina fuses with pericytic basal lamina. Specific dense material attached to the inner face of pericytic plasma membrane is visible (white arrows). (BCC).*

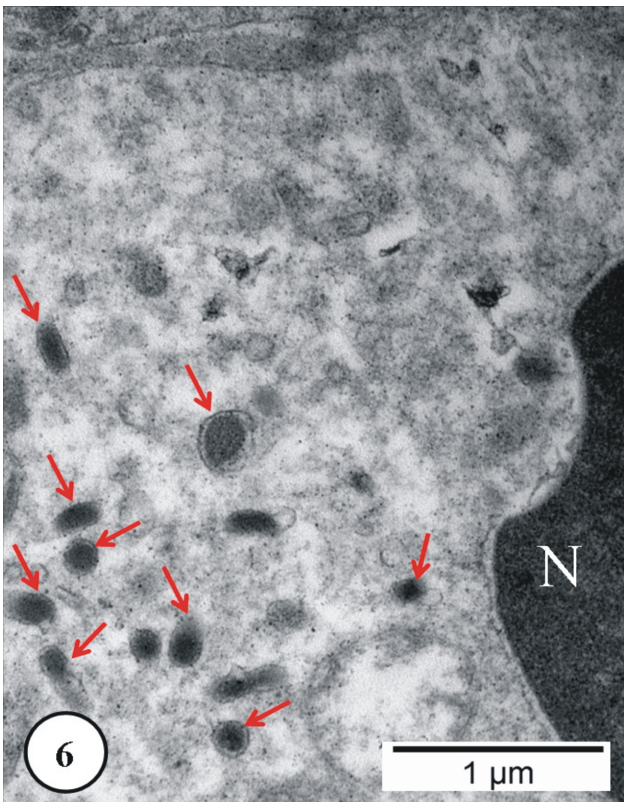

Figure 6 – *Small sector of an extravasated inflammatory cell is full of lysosomes (red arrows). (BCC).*

Massive infiltration of the tumor stroma with inflammatory cells delivered their lysosomal content, probably involved in the large lysis areas formation (by destroying the adjacent extracellular matrix) (Figure 1; see also Figure 6). Basal lamina and hemidesmosomal junctions as well as the associated cytoskeleton are severely altered (Figures 2–4). Tumor cells exhibit cell extensions termed invadopodia (Figure 4). Moreover, intercellular junctions are also affected: desmosomes are missing for long profile of the adjacent epithelial cells, being internalized (Figure 3). Dissolution of tumor cell plasma membrane can be detected (Figures 2 and 4). Interestingly, destructured plasma membrane contributed to shedding membrane vesicles formation (Figure 2). Abnormal fenestrated capillary inside of the peritumoral stroma are detected (Figure 5). Previously, we reported that in such kind of epidermal tumors, concomitantly with fenestrated capillary, some endothelial gaps may occur [16], which may facilitate the inflammatory cells extravasation beneath the epithelial tumor (in this study, Figures 1 and 6).

Telocyte – a cell phenotype identified as tumor stromal (interstitial) component

An interesting cell phenotype present in peritumoral stroma, so far not described in BCC or SCC, is represented by so-called TC. TCs are prevalently located inside of the (peri)-tumoral fibrotic stroma. Most telocytes described in this paper appears as bipolar cells having an ovoidal nucleus with predominant euchromatin; heterochromatin appears prevalently attached to the inner membrane of nuclear envelope (Figure 7).

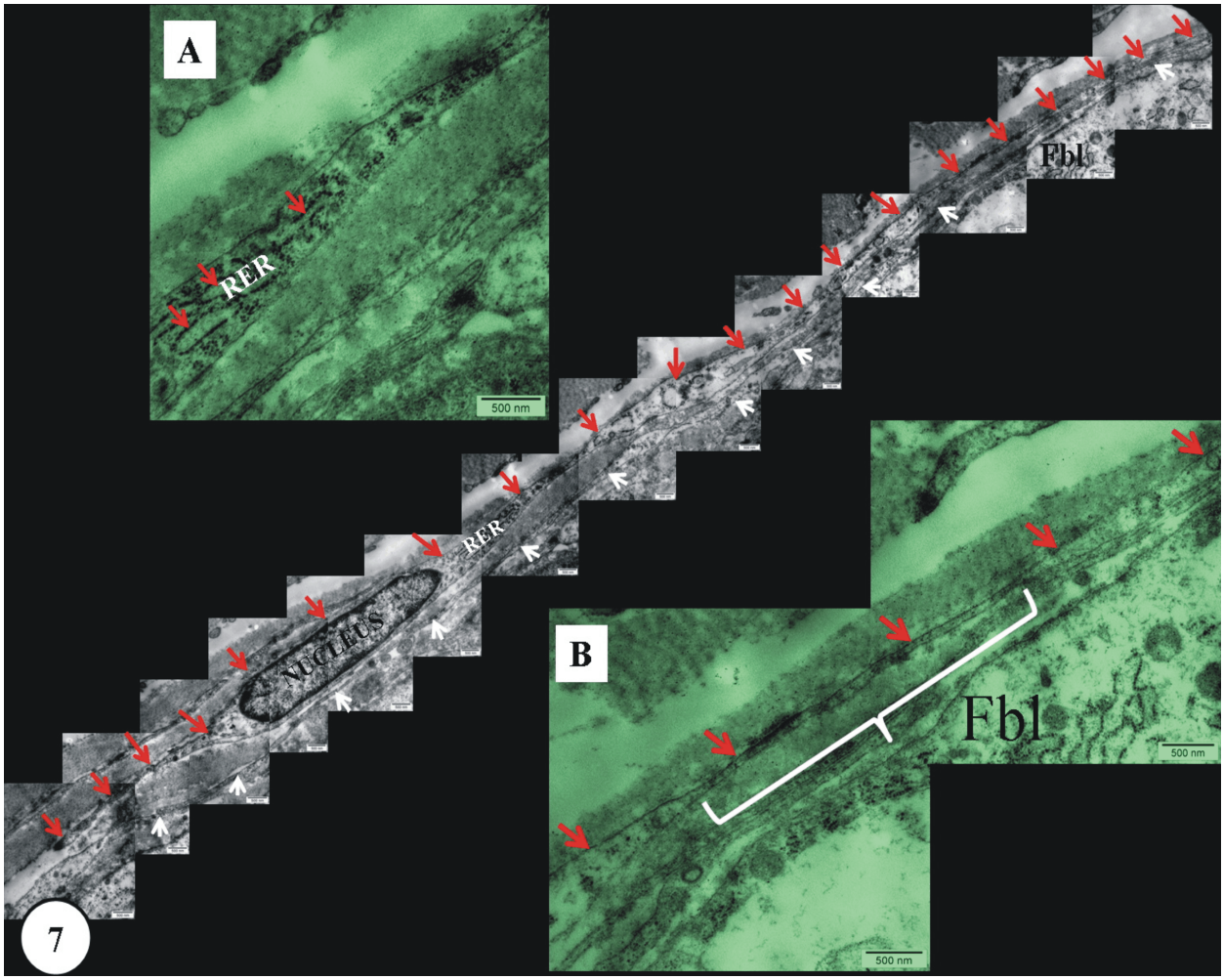

Figure 7 – ***A bipolar telocyte has an ovoidal nucleus with predominant euchromatin; heterochromatin appears prevalently attached to the inner membrane of nuclear envelope. Body cell together with the two cell extensions (telopodes) (red arrows) measured approximately 50 µm; one telopode is very long. An extremely long slender telopode (white head arrows) belonging to another telocyte whose cell body (including nucleus) is not visible, runs parallel with the nucleated telocyte. Rough endoplasmic reticulum (RER) is located in close vicinity of the nucleus (A). In (B), a detail for the long telopode (red arrows) shows the alternation of dilated segments (podomes) and thin segment (podomere) (accolade). Fbl = Fibroblast. (A) and (B) digitally colored in green. (BCC).***

Body cell together with the two cell extensions called telopodes has approximately 50 µm. Often, rough endoplasmic reticulum (RER) is located in close vicinity of the nucleus (Figure 7A). Similar to RER, Golgi apparatus was mainly located in the perinuclear region (not shown). Telopodes shows the alternation of dilated segments (podomes) and thin segments (podomeres) (Figure 7B). Usually, telocytes are embedded inside of collagenous fibrotic stroma. Sometimes, telocytes are not far from the front of growing tumor cells. Telopodial extensions of tumor telocyte run

parallel with tumor front of growth at only 1–3 μm distance (Figure 8A). The thickness of the quite long profile of the podomere is about 200 nm (Figure 8B). Hemidesmosomes are almost missing; when present, they are defective for the inner plaque and consecutively, their connection with intermediate filaments (keratin) is missing. Mention must be made that some hemidesmosomes look normally (Figure 8C).

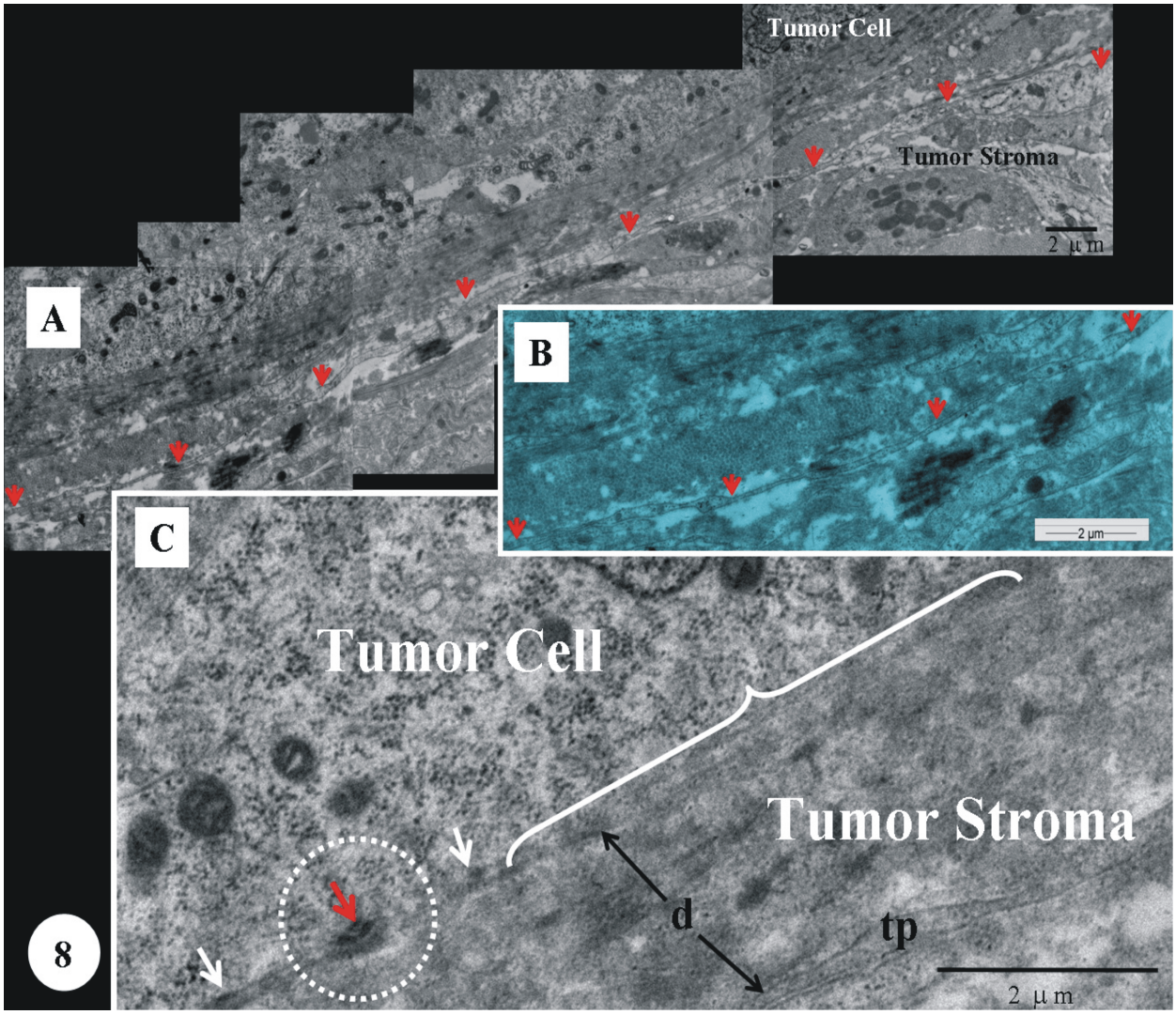

Figure 8 – ***Inside of peritumoral stroma, a long profile of a telopode (red arrows) embedded in collagen stroma is seen (A). In (B), a higher magnification allows easy to see the very thin telopode. In (C), the basal profile of a tumor cell shows that hemidesmosomes are almost missing (white accolade); when present, they are defective for the inner plaque (white arrows). Here, only one hemidesmosome looks normally (red arrow in dotted encircled area); d marks the distance (cca. 2 μm) which separates the telopode (tp) of the telocyte from the tumor cell. (B) is digitally colored in blue. (BCC).***

Interestingly, few telocytes established stromal synapses by the end of their telopodes. Figure 9 depicted two-dimensional (2-D) sequenced concatenation of 18 serial electron micrographs showing telopodes of three telocytes connected by homocellular junctions (stromal synapses) (Figure 9, A and C; for details, see also Figures 13, 13A as well as Figure 15, including inset). The nuclear body of all three telocytes is not visible at this level of incidence sectioning angle. The longest telopode have approximately 55 μm. Some telocytes (TC2 in Figures 9 and 9C, detailed in Figure 15) may have concomitantly two kinds of junctions: homocellular junction (between two telocytes, TC1 and TC2) and a heterocellular junction, *i.e.* TC2 with a naked nerve edge. Usually, telocytic podome accommodates mitochondria and cytoskeleton represented by microfibrils type intermediate filaments (Figures 10 and 11). Inside of podomes, clathrin coated vesicles and caveolae (Ca^{2+} uptake/release units) are also detectable (Figure 12). Sometimes, the podomeres are extremely thin (cca. 20 nm thickness) as can be seen in Figure 13 and 13B. At the level of each homocellular junctions (somatic synaptic zone), both telopodes exhibit electron dense microscopic material attached to the inner face of the plasma membranes (Figures 13A and 15, including inset). Not all the homocellular junctions between two adjacent telocytes exhibit such kind of electrondense material, but a recombination of plasma membrane can be detected. Moreover, at this level coated caveolae and shedding microvesicles (cca. 75–100 nm diameter) in different stages of formation and deliverance into extracellular matrix can be seen (Figure 14). The absence of TCs basal lamina in peritumoral stroma is

remarkable. Very seldom, only small profiles/patches of basement membrane associated to one side of telocyte from the tumor stroma can be detected (Figure 14). Sometimes, plasma membranes of the two connected telocytes (a homocellular junction) become fused, as is shown in Figure 14 where telocyte TC1 and telocyte TC2 performed plasma membranes recombination. A telocyte may develop concomitantly homo- and heterocellular junctions. In Figure 15, telocyte 2 (TC2) have a homotypic cell junction as direct somatic junction with another telocyte (TC1) and a heterocellular synaptic junction with a naked nerve edge. Inside of the nerve edge, polymorphic presinaptic vesicles are visible. A relative long profile of a synaptic cleft as a narrow gap of approximately 30–50 nm delimited by the presynaptic axolemma and plasma-membrane of telocyte 2 (TC2) in postsynaptic cell position is visible. A heterocellular connection of a telopode with a nerve edge is detectable (detail in Figure 15, inset). A dense intracellular attachment plaque connects the telopode of the telocyte 2 (TC2) with small area of nerve edge. The largest contact of the TC2 with nerve edge is performed by a long tight contact between plasma membranes of the telopode and the naked nerve edge. Our comparative evaluation concerning the heterocellular junctions, usually telocytes may realize with other cell type in normal dermis, underlines that tumor stroma telocytes express a limited number of such connections. In this context, we observed that in both BCC and SCC, TCs become very seldom in close vicinity with endothelial cells or pericytes (not shown). Heterocellular junctions of telocytes with mast cells are almost missing in case of BCC while, in SCC such kind of junctions are extremely encountered. When this is happened, the direct contact between telopode and microvillous extension of mast cell is very narrow, suggesting plasma membranes recombination (Figure 16).

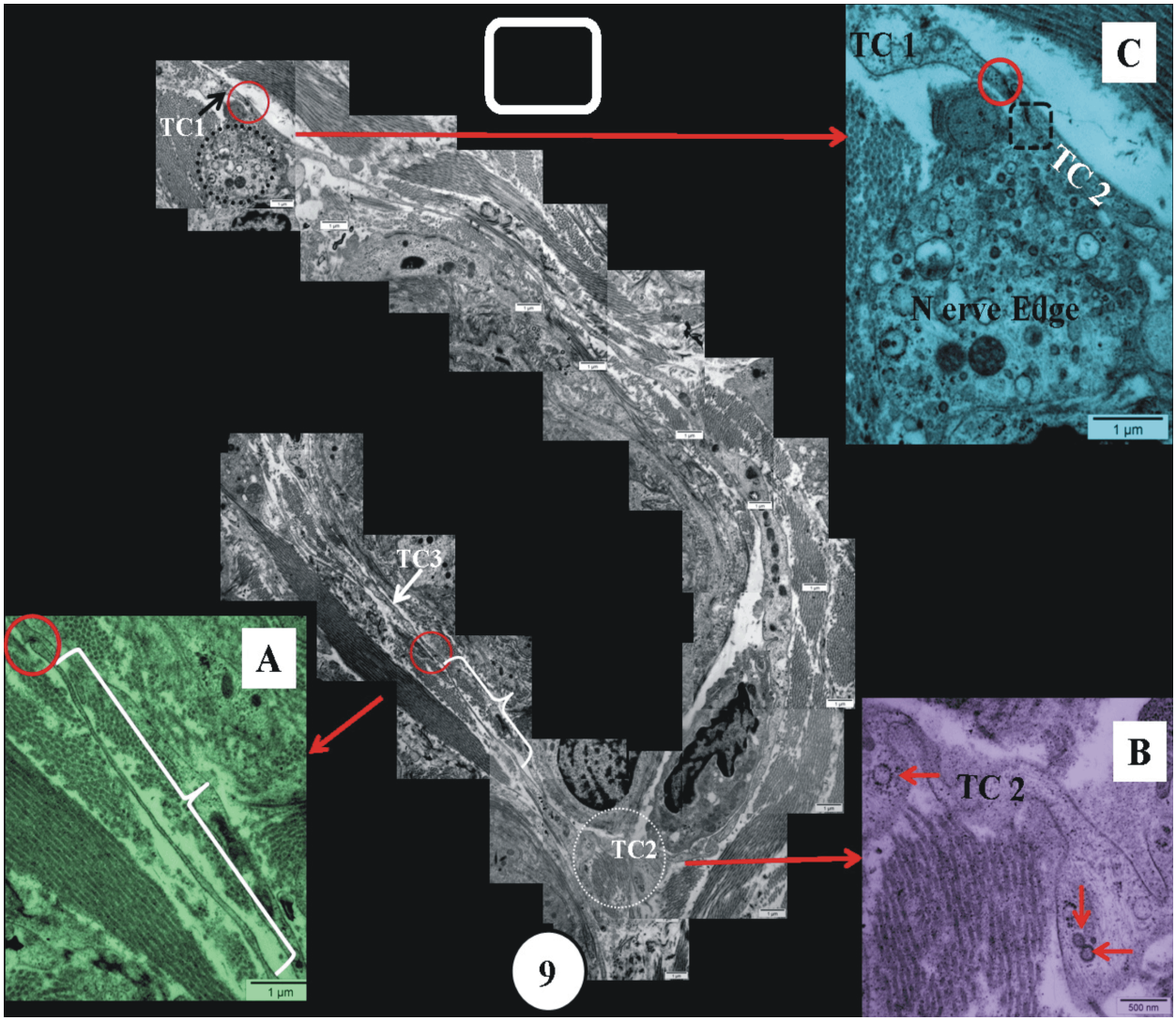

Figure 9 – ***Two-dimensional (2-D) sequenced concatenation of 18 serial electron micrographs. Inside of the peritumoral dermal tissue from BCC of the skin, three telocytes are connected each other by homocellular junctions/stromal synapses (red circles areas). The nuclear body of telocytes is not visible. The longest telopode measured approx. 55 µm. In (A), detail for a thin telopode (accolade) of the telocyte TC2 connected by a somatic synapse (encircled area) with telocyte TC3 (digitally colored in green and much detailed in Figure 13). In (B), detail for the white dotted encircled area in Figure 9, showing clathrin coated vesicles (red arrows) (digitally colored in pink and much detailed in Figure 12). In (C), telocytes TC2 makes synapses with TC1 and a naked nerve edge (digitally colored in blue and much detailed in Figure 15). (BCC).***

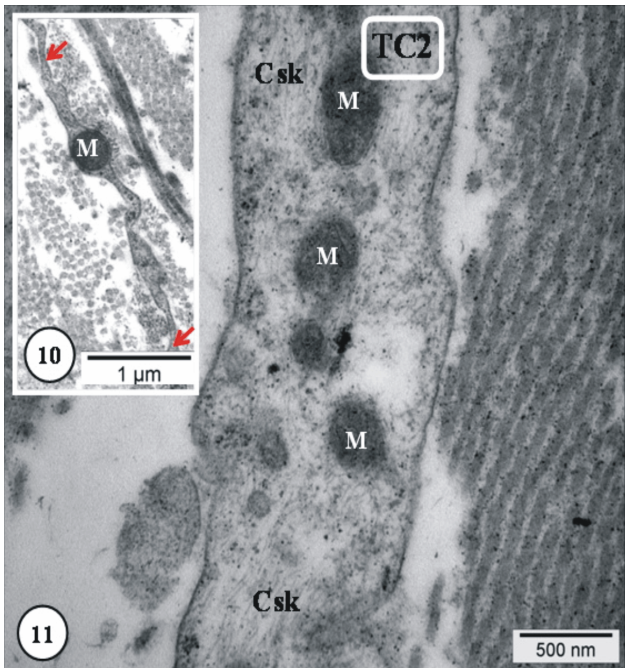

Figure 10 – *A mitochondria (M) is visible inside a telocytic podome between two podomeres (red arrows). (BCC).*

Figure 11 – *Inside of a podome, few mitochondria (M) and a rich microfibrillar cytoskeleton (Csk) are housed. TC2 = Telocyte. (BCC).*

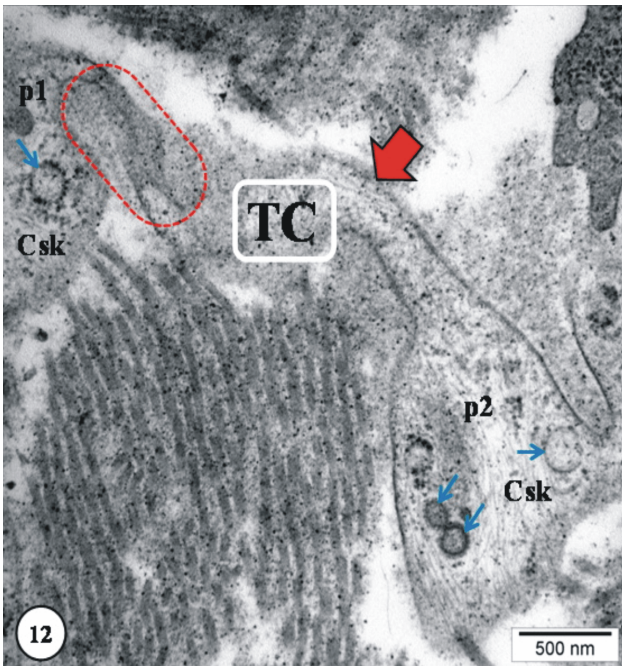

Figure 12 – *Two podomes (p1, p2) are connected by a podomere (large head arrow) of the telocyte (TC). Inside of the podomes of telocyte TC, cytoskeleton (Csk) and clathrin coated vesicles (blue arrows) are detectable. Dotted ellipsoidal delimited area indicates a region of the podomere sectioned tangentially. (BCC).*

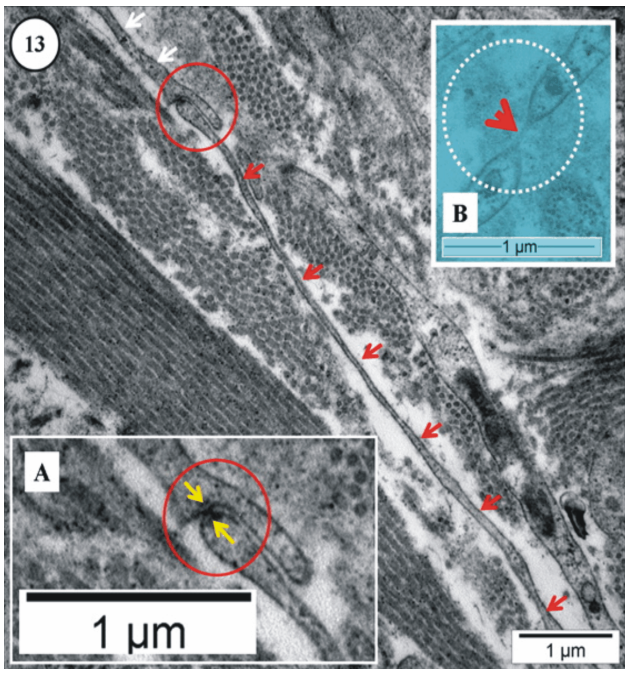

Figure 13 – *Two telopodes belonging to telocyte 2 (red arrows) and telocyte 3 (white arrows) (overview in Figure 9) establish a homocellular connection (encircled area) detailed in inset (A): to some extent, at the synaptic zone, both telopodes exhibit electron dense microscopic material (yellow arrows). Red head arrow in (B) marks an extremely thin (cca. 20 nm thickness) segment of a telopode (digitally colored in blue). (BCC).*

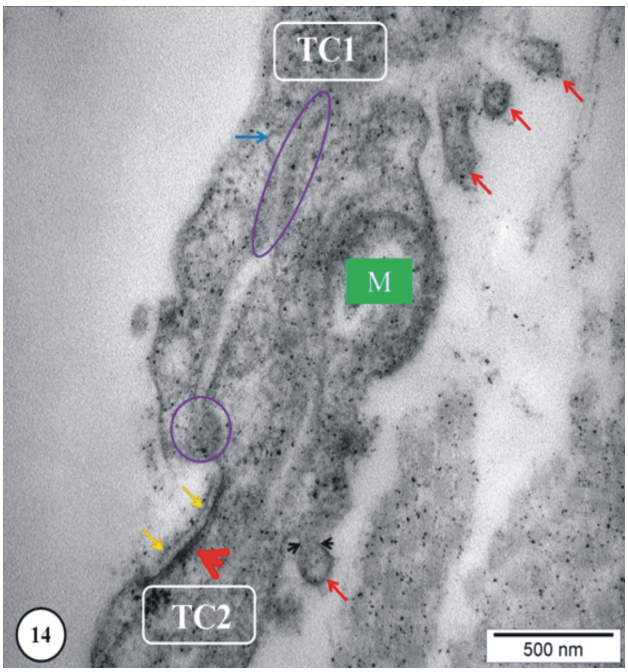

Figure 14 – *Two telocytes (TC1 and TC2) realized homocellular junctions (ellipsoidal and encircled areas). Red arrows indicate large (cca. 125–150 nm diameter) shedding vesicles originated from the telocyte 1 (TC1) and telocyte 2 (TC2); one of them is in way to be detached (black head arrows) from the telocyte 2 (TC2). Blue arrow indicates a coated caveolae while yellow arrows indicate a small profile of basement membrane along the telocyte 2 (TC2) faced by an electron dense material (red head arrow) attached to the inner surface of plasma membrane of TC2. Encircled area indicates the point where plasma membranes of connected telocytes become fused. M = Mitochondria. (BCC).*

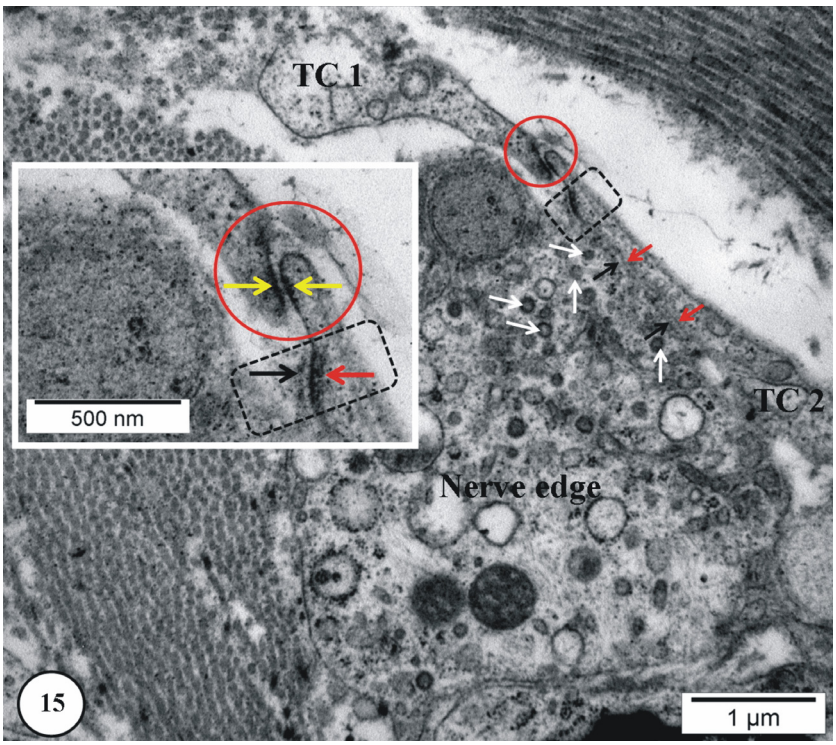

Figure 15 – *Two telocytes (TC1 and TC2) are connected by homocellular junction (encircled area). Heterocellular junctions (dotted square), including a synaptic junctions between the telocyte TC2 and a nerve edge is visible. Inside of the nerve edge, polymorphic presinaptic vesicles (white arrows) are visible. A relative long profile of synaptic cleft as a narrow gap of cca. 30–50 nm delimited by the presynaptic axolemma (black arrows) and plasma membrane of telocyte 2 (red arrows) in postsynaptic cell position is visible. In inset: electron dense plaques (yellow arrows) are visible attached to the inner face of plasma membrane at the homocellular junctions. Red arrow indicates an electron dense plaque belonging to the telocyte while black arrow indicates a slightly dense plaque attached to the plasma membrane of nerve edge. (BCC).*

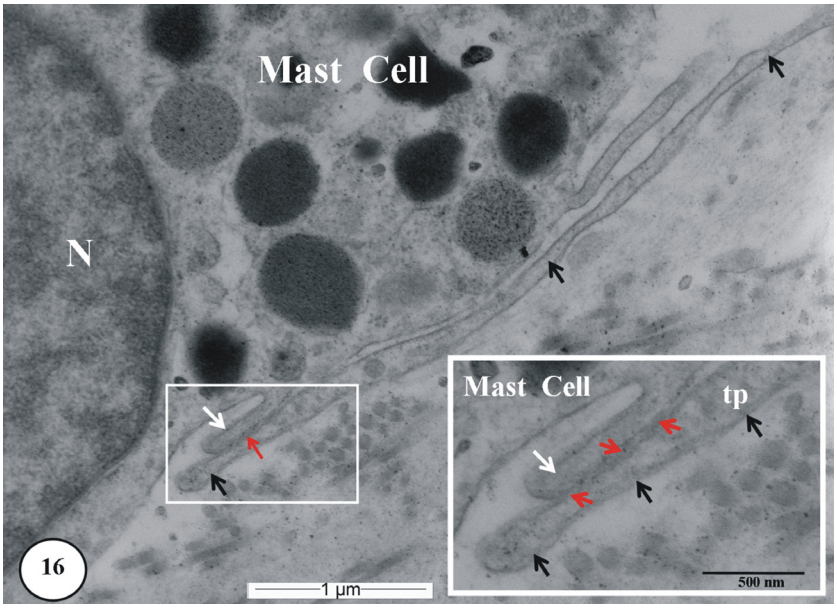

Figure 16 – *A heterocellular junction (red arrow) between a telopode (black arrows) belonging to a telocyte whose cell body is not visible here and a microvillous cell extension of a mast cell (white arrow), detailed in inset. Inset: red arrows indicate the very narrow direct contact between telopode (tp) and mast cell. N = Nucleus. (BCC).*

Discussion

Ultrastructural abnormalities at the tumor–stroma interface

Almost all human cancers (cca. 80%) derive from the epithelial tissues. A neoplastic epithelial tissue is represented by (1) tumor cells and (2) tumor stroma. It is well established the major role of stromal tissue in supporting the tumorigenic process of the epithelial cells in which these are involved. Previously, we published our results concerning the ultrastructure of tumors surgically excised in some cases of patients diagnosed with BCC or SCC, especially the infrastructural changes and some associate molecular alterations at the tumor–stroma interface [5, 6, 15]. Such kind of alterations of extracellular matrix (including basal lamina lysis), as well as of the epithelial tissue *per se*, recorded as focally dissolution of tumor cell plasma membrane, internalization of desmosomes, invadopodia formation, also briefly presented in this paper depicted in Figures 1–4, lead to the loss of cell polarization and consequently increase cell mobility, abnormal behavior of the tumor cell affronted to the peritumoral stroma, by facilitating the spreading and release of cancer cells to generate metastases. One considers that at the base of an invadopodia developed by tumor cells there is a matrix-degrading activity [17]. Extravasated inflammatory cells facilitated by abnormal fenestrated blood capillary inside of peritumoral stroma (Figures 5 and 6) make them an active support for tumor growth.

Peritumoral stroma – tumor–stroma interaction – plays a major role in tumor cell behavior. That is the reason why many investigators focused their interest on different cell types and soluble factors as components of tumor stroma involved in tumorigenesis [2, 4, 6, 16, 18, 19].

In this context, a putative important player belonging to stromal (interstitial) cell component, as telocyte is, should be investigated.

Telocyte – a new cell phenotype identified as stromal (interstitial) component

Popescu's group [20] in Bucharest (Romania) have been described a new type of interstitial (stromal) cell termed interstitial Cajal-like cells (ICLCs) because of their morphologic aspect, at first glance, similar to canonical (gastrointestinal) interstitial cells of Cajal (ICCs). Ultrastructurally, the ICCs are heterogeneous in appearance: their phenotype range from cells closely resembling smooth muscle cells [21, 22] to those similar to fibroblasts/fibrocytes. Nonetheless, ICCs exhibit some general traits (cytological characteristics): inside of the nucleus euchromatin is prevalent, rough endoplasmic reticulum and Golgi apparatus are mainly located perinuclear, a lot of mitochondria are located throughout the cytoplasm, and cytoskeleton is represented by intermediate filaments, mostly vimentin and some microtubules [23]. Moreover, along the plasma membrane, caveolae can be identified and, outside the cell membrane run some short profiles of basal lamina. ICCs are derived from non-neuronal cells and have mesenchymal origin [7, 23]. To some extent, TCs seem to share some ultrastructural features of ICCs. This was the reason why, formerly TCs were considered to be interstitial Cajal-like cells (ICLCs). Recent, ultrastructural studies proven that TCs represent a special class of interstitial (stromal) cells with a distinct phenotype. Indeed, as many laboratories discovered that ICLCs are present in different tissues, it becomes clear that formerly described interstitial cells as ICLCs can not be assimilated to the canonical ICCs or to any other resident and non-resident interstitial (stromal) cells (fibroblasts, fibroblast-like cells, myofibroblasts, macrophages, etc.). Step by step, becomes clear that TCs represent a distinct population of interstitial (stromal) cells. Popescu LM and Faussone-Pellegrini MS [24] coined the terms TC for the ICLCs and telopodes for their extremely long but slender prolongations. So far, formerly ICLCs, renamed TCs were described in a wide variety of vertebrate (including humans) cavitary and non-cavitary organs: mammary glands [20, 25], heart [8, 12, 26, 27], lungs [28], pleura [29], skin [10, 30], trachea [11], skeletal muscle [31], duodenum [13], pancreas [32], myometrium [33], vasculature [8], etc.

Morphologic aspect of the TCs

Morphologically, the TCs are identified by a small cell body where the nucleus is located and one to maximum five very long (tens of μm) thin cell prolongations (less than 200 nm in diameter) called telopodes – slender, occasionally convoluted. The most specific ultrastructural features of TCs are: (1) each telopode comprises thin fibrillar-like segments termed podomeres in alternation with dilated, cistern-like regions/segments termed podomes, which accommodate mitochondria, (rough)endoplasmic reticulum profiles and caveolae. The alternation podomere–podome gives telopodes a moniliform aspect [7, 34]. Cytoskeleton is represented by intermediate filaments, mostly vimentin [35, 36] and scarce microtubules. TC have a mesenchymal origin [7, 34]. Essentially, all the above-mentioned ultrastructural characteristics are identifiable to the TCs we detected inside of tumor-stroma in BCC and SCC (Figures 7–14). Moreover, TCs are c-kit/CD117 and CD34-positive [12, 28, 30, 35–38]. Mention must be made that canonical ICCs exhibit also a positive immune reactivity to anti-c-Kit antibody [23]. Unfortunately, so far there is no report about a reliable immunolabeling specific to TCs, so that, the ultrastructural cell phenotype identification remains the only possibility to discriminate TCs from other stromal cell mimicking the telocytic phenotype. Care should be taken to discriminate between TCs and other interstitial (stromal) cells capable to form thin cell extensions. It is worthy just an example: TCs may not be assimilated with endoneurial dendritic cells of peripheral nerves in the skin, which also exhibit few long cell extensions, to some extent, mimicking telopodes and having somatic synapses with other endoneurial dendrocytes as well as with perineurial epithelial cells and Schwann cells (unpublished personal ultrastructural observations). Nonetheless, now there is a body of evidence that TC is a special interstitial (stromal) cell phenotype described in different tissue types. The present study confirms that TCs are clearly interstitial cellular component of tumor stroma in both BCC and SCC.

Intercellular communications of telocytes inside of tumor stroma in BCC and SCC (a comparative evaluation with normal human skin telocytes)

In a recently published study, we reported about the morphological aspects of dermal TCs, their homo- and hetero-cellular junctions infrastructure in the normal human skin [10]. The question we address here is: there are any particular infrastructural aspects associated to TCs in BCC and SCC comparing with TCs from normal human cutaneous tissue? Present study evaluates differences between normal skin and tumor skin (BCC and SCC). We describe the telocytes ultrastructure and their relationships within the peritumoral stroma in BCC and SCC of the human skin.

The most interesting phenomenon we underline about the telocytes' behavior is related to their ability to perform intercellular communications. Our electron microscopic study of TCs from tumor stroma revealed homo- and heterotypic cell junctions. Indeed, like in normal stroma tissues, TCs in tumor-stroma of BCC and SCC are able to realize (1) direct cell–cell communications/junctions, either (a) homocellular junctions (somatic/stromal synapses) or (b) heterocellular junctions (somatic/stromal synapses) as well as (2) indirect cell–cell communications by shedding microvesicles.

Cell–cell and cell–extracellular matrix play a key role in cell growth, tissue morphogenesis, maintaining cell polarization, as well as in tissue physiopathology, including renewal and repair [39–42]. There is a plethora of data, which documented that in case of normal or tumor epithelia, adjacent stromal tissue (by its cellular and extracellular components) exerts direct and indirect influences on epithelia behavior and destiny. After clearly identification of TCs as interstitial (stromal) dermal components, raised the question: what is the role of TCs? In this respect, there are some preliminary data, but TCs' functional role remains largely unknown.

As a general rule, mention must be made that telocytes are strategically located in between blood vessels (microvasculature, including capillaries), nerve endings, and other specific resident or non-resident cell populations of a given healthy tissue/organ [7–10]. One considers that a TC interacts with neighboring interstitial cells (adjacent TCs or other interstitial cells), either directly by cell–cell contact, creating 3-D network, or indirectly, by shedding microvesicles involved in cell signaling. There is a body of evidence that, irrespective of tissue type TCs belong to, these establish homo- or heterotypically cell junctions [7, 9–11, 30, 34].

Tumor–stroma telocytes perform end-to-end connections (homocellular junctions)

Here we document the existence of both modalities of direct or indirect cell–cell communications inside of tumor-stroma. Connected with each other *via* homocellular junctions, TCs form an interstitial 3-D network (Figures 9 and 11–15). Intercellular space, which separates two apposed telocytes involved in a somatic homocellular junction, is within macromolecular interaction range (cca. 10–30 nm). So far, there is no report about the nature of molecules involved in homocellular communication.

Detailed ultrastructural aspects of somatic synapses realized by end-to-end TCs apposition showed that a dense electron microscopic material is located attached to the inner face of plasma membranes (inner plaques) of both TCs (Figure 15). There is no report about the molecular composition of such kind of inner plaques.

Fusion of plasma membrane telocyte at the homocellular junction

Another interesting aspect so far we did not encountered with TCs in normal tissues [9–11] but reported by Gherghiceanu M and Popescu LM in the healthy human heart [8] is the fusion of plasma membrane TCs at the cell-cell junction (a heterocellular junction between TC and an adjacent cardiomyocyte). In the present study, encircled area from Figure 14 indicates the point where plasma membranes of the two connected TCs (a homocellular junction) become fused, TC1 and TC2 performing plasma membranes recombination. Membrane fusion is a physiologic process often encountered to normal cells (endogenous cytomembrane biogenesis, intracellular traffic, cell secretion, synaptic transmission, syncytia formation, etc.). Previously, we also reported about high fragility of tumor cell plasma membrane leading to recombination membranes between malignant cells and adjacent stromal (interstitial) cells [6]. Could be the above-mentioned observation of TCs plasma membranes recombination related to the fact that often plasma membranes of tumor cells exhibit such kind of behavior, performed, either between two adjacent malignant cells or between a malignant cell and an adjacent stromal cell from the peritumoral stroma [6]? In the case of malignant cells, this aspect can be associated with the fact that the plasma membranes of tumor cells are more fragile and prone to perform cell–cell fusion [6, 15, 43].

Telocytes perform heterocellular junctions

Due to their heterocellular junctions (heterocellular connections), TCs are considered to be involved in intercellular signaling. TCs cooperate in a promiscuous manner with different interstitial cell types. Indeed, by their telopodes, TCs frequently establish stromal synapses with terminal naked peripheral nerves (nerve edges), mast cells, fibroblasts, Schwann cells, adipocytes [10, 44]. Occasionally, telopodes are closely associated with stromal dermal acellular (anhist) components as elastic and collagen fibers [10]. In their recently published paper, Gherghiceanu M and Popescu LM [8] emphasized the pivotal role of TCs cardiac network as integrator of the overall information from the vascular system (both endothelial cells and pericytes), nervous system (Schwann cells), immune system (macrophages, mast cells), interstitial milieu (fibroblasts, extracellular matrix) and working cardiomyocytes in the healthy human heart.

Like in normal skin [30], an important heterocellular junction which TCs performed inside of tumor stroma from skin cancer is represented by TC-naked nerve edge synapse, as is illustrated by Figure 15.

Comparing with normal human dermal tissue [11], we observed that inside of the tumor dermal stroma from patients suffering of BCC and SCC homocellular junctions are still well represented, but the heterocellular contacts of TCs with other tumor stromal cells are less numerous. In this context, mention must be made that TCs are detectable far from the tumor blood microvessels, almost missing TCs-pericyte or endothelial cells junctions. In normal dermis, mast cells are relatively abundant [11, 45]. Recently published papers, [8, 10, 11, 20] reported that TCs form structural tandem with mast cells. In a previous study ([11] and personal unpublished data), we observed that in the normal human dermis, quite frequently TCs are closely associated with mast cells. In the present study, we underline that in EBC, TCs-mast cells direct contacts are absent while, in SCC these are very infrequently. Figure 16 depicted such kind of TC-mast cell junction in SCC. Mast cells are considered constitutive tissue residents. Because of their high amounts of inflammatory mediators deposited inside of cytoplasmic granules, mast cells are very important players in the pathophysiology [46]. In this context, raises the question: can be the paucity of heterocellular junctions of telocytes–mast cells in tumor stroma of EBC and SCC we observed (meaning an abnormal control of mast cell granule secretion/deliverance) involved in the overexpression of inflammatory mediators, as they usually are overexpressed in many tumor stroma? Different from the normal skin, we observed that in both BCC and SCC the TCs-endothelial cells or pericytes contacts are missing inside of peritumoral stroma. Occasionally, TCs can be detected in close vicinity of blood vessels, but not in direct contact (not shown). To some extent, we may only speculate, that paucity of the heterocellular junctions of TCs can be assimilated to the loss of functions at the tissue homeostasis (here, at the tumor-stroma) level. The limitation of TCs heterocellular junctions suggests a possible involvement in induction of cell–cell communication alterations into the peritumoral stroma and, consequently into the whole tumor mass. A limitation of cell–cell cooperation is a general hallmark of tumor cell phenotype, a prerequisite for invasive behavior. To date, the role of TCs in pathology remains largely

unknown. Zheng Y *et al.* [37] and Zheng Y *et al.* [35] reported that TCs may have potential roles in pathogenesis of pulmonary diseases.

It is well documented that inside of different tissue types, stem cells behavior is under the control of their microenvironment (different interstitial cell types and extracellular matrix) known as niches [30, 47–51]. Clearly, in normal tissues TCs were detected in close vicinity with putative stem cells or progenitors from various types of organs, like heart [34], lungs [28], human skin [30]. TCs are considered a special cell phenotype members of the stem cell niches/putative stem cell niches with a presumptive role of "nurse" (supportive) cells for other adjacent mesenchymal and epithelial putative stem cells [7]. Recently, we identified TCs or stromal telocyte-like cells inside of the stroma located between mature adipocytes from dysfunctional visceral adipose tissue related to obesity [44]. It seems that during obesity, human body needs new adipocytes for storage of excessive lipids. We have been supposed that TCs present in the adipose tissue stroma, the place where the cell reserve for new adipocytes formation is located, could be involved in supporting adipogenesis, may be, rather as "nurse" (supportive) cells for preadipocytes.

On the other hand, Ceafalan L *et al.* [30] consider that TCs in normal human skin may play a role in skin regeneration. Moreover, Manole CG *et al.* [52] showed that TCs may be involved in neo-angiogenesis after experimental acute myocardial infarction.

Concerning the composition of cancer stem cell niches little is known. It is worthy to mention that cancer stem cells in brain tumors, similar to normal neural stem cells, are mostly located in regions that are rich in micro-vasculature called "vascular niches", so that endothelial cells secrete factors that control cancer stem cells destiny [53]. Moreover, initiation and, especially ability of many malignant tumors to maintain their growth is attributed to a small population of cells called cancer stem cells. Like in normal healthy tissues, in different malignancies, stem cells have a so-called "cancer stem cell niche" [53]. In our present study, we did not identify TCs in direct contact with tumor cells or putative cancer stem cells. In the present investigated cases, TCs appear located at different distances from the malignant cells of the tumor (either BCC or SCC origin). In Figure 8 (A and C), the telopodial extension of a tumor-stroma TC runs at not far from 2–3 μm distance.

Indirect cell–cell communication mediated by special shedding microvesicles

Inside of podomes, clathrin coated vesicles and caveolae (Ca^{2+} uptake/release units) are also detectable (Figure 12). This is a sign that, like in normal tissues, TCs from the tumor stroma are active cells by trafficking membranes and calcium.

Recently published papers [8, 10, 28] showed that by their telopodes, TCs release small microvesicles (mean diameter of 180 nm) as a single or a pool of shedding membrane vesicles. The delivered micro- and macro-molecules from microvesicles are considered to play a paracrine role in long distance signaling by sending signals to neighboring cells [7, 8, 33]. Shedding membrane microvesicles are involved in some physiopathological processes by surface–membrane traffic and the horizontal transfer of proteins and RNAs among adjacent or neighboring cells – paracrine effects in order to perform rapid phenotype adjustments required by a variety of conditions [54]. Concerning indirect cell–cell communication mediated by special microvesicles delivered by TCs into peritumoral stroma, we indicate Figure 14 (a case of BCC) showing large (cca. 125–150 nm diameter) shedding micro-vesicles originated from the telocyte 1 and telocyte 2 connected each other by a somatic synapse. Is hard to evaluate the real significance of the shedding micro-vesicles by telocyte we observed both in BCC and SCC. A similar modality to disseminate signaling molecules by shedding microvesicles to neighboring cells (paracrine cell signaling) was described as a tumor cell behavior [6]. In agreement with [54], we consider that shedding plasma membrane vesicles by tumor cells facilitate motility of cancer cells, a prerequisite for tumor progression and metastasizing by secondary tumor formation.

As a very general conclusion, we appreciate that by their homo- and heterocellular connections, TCs make a 3-D network in the tumor stroma, which may be involved in long-distance intercellular signaling coordination. The possible functional role of the TCs as tumor-stroma cells remains largely unknown, being a matter of debate and, further investigations are required.

Conclusions

Like in normal human skin, TCs are detectable as distinct interstitial cells phenotype present inside of both BCC and SCC tumor stroma. TCs establish homocellular junctions by specialized somatic synapses leading to a 3-D network inside of peritumoral stroma. TCs play an important role in intercellular signaling *via* stromal synapses and shed microvesicle transfer. Comparing with TCs from the normal dermal skin, TCs from tumor stroma have a very restraint number of heterocellular junctions. The limitation of TCs heterocellular junctions suggests a possible involvement in induction of cell–cell communications alterations into the peritumoral stroma and, consequently into the whole tumor mass.

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Corresponding author
Nicolae Mirancea, PhD, Senior Scientist Grade I, Head of Department of Plant and Animal Cytobiology, Institute of Biology Bucharest of Romanian Academy, 296 Independenţei Avenue, P.O. Box 56–53, 060031 Bucharest, Romania; Phone +4021–221 92 02, Fax +4021–221 90 71, e-mail: nick_mirancea@yahoo.com

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