THE LEYDIG CELLS OF THE TESTIS ORIGINATE FROM THE MICROVASCULAR PERICYTES

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ABSTRACT

*This review offers some clarifying thoughts about the nature and origin of the fetal and adult Leydig cells, supporting the conception that the pericytes (the periendothelial cells) and the smooth muscle cells of the microvasculature, that represent the main omnipresent adult stem cell population of the mammalian organism, are the Leydig cell ancestors. Our attention is specifically dedicated to the numerous contradictions as well as ambiguities concerning the hypotheses that the mesenchymal stromal cells (MSCs), the neural crest stem cells (NCSCs) and the peritubular myoid stem cells (PMCs) represent the stem ancestors of the Leydig cells. In effect, it becomes evident that the only pluripotent stem cell-like cells in the vertebrate body, including the testis, are the pericytes. The pericytes are derivate of the embryonal epiblast and retain its pluripotency within the microvascular niches where they are disseminated during the embryo- and fetogenesis and are stored as a resting adult stem cell population for tissue generation, maintenance, repair and regeneration. The pluripotency of the epiblast and the pericytes themselves are responsible and explain the neural features of the Leydig cells. Thus, both NCSCs and PMCs are not the ancestors of the pericytes, respectively of the Leydig cells. **Biomed Rev 2017; 28: 1-21.***

Keywords: Leydig cells, origin, microvascular pericytes, smooth muscle cells, Leydig stem cells

INTRODUCTION

During the last four decades a lot of new information about the testosterone producing Leydig cells of the mammalian testis has been published. It became evident that the Leydig cells are not pure endocrine cells because they express also characteristic marker substances of nerve and glial cells, which allowed their designation as neuroendocrine cells (1-9). This fact evoked again the question regarding the origin (the ancestors) of Leydig cells. It was unequivocally evidenced that Leydig cells originate from stem cells (10-13). Most authors represented the opinion that these cells are of mesodermal origin. Recently, it was revealed that Leydig stem cells are the pericytes and the smooth muscle cells of the testis microvasculature (14, 15). Beside the fact that these results were confirmed for nearly all organs of the body (14, 16, 17), until

now contradictory interpretations exist concerning these two important topics. Even in the newest very informative articles by the group of Ge (18-20) the hypothesis of peritubular myoid stem cell origin of Leydig cells was favored.

ORIGIN OF THE LEYDIG CELLS

The ancestors of the adult Leydig cells are the testicular microvascular pericytes and smooth muscle cells. The pericyte ancestors are produced by the pluripotent epiblast cells which originate from the blastocyst inner cell mass in the very early embryogenesis (21). The pericytes are disseminated throughout the whole organism with the developing blood vessels (22, 23) and become stored within microvascular niches (24) as a resting, reserve population for tissue generation, physiological tissue maintenance and repair as well as for regen-

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Figure 1. Depicts the very early stages of the zygote and the blastocyst differentiation. Note that the first cell populations derived from the epiblast are the primordial germ cells, the hematopoietic stem cells and the pericytes whereas the neural crest originates a bit later from the one of the primitive embryonic germ layers, the ectoderm (epiblast/ectoderm).

eration in the adult organism (5, 6, 25-30).

The epiblast is a derivative of the internal cell mass of the blastocyst. It arises during the postimplantation gastrulation in the very early embryogenesis (31-36). The epiblast produces firstly three cell phenotypes, namely, the pluripotent primordial germ cells (PGCs), the multipotent hematopoietic stem cells (HSCs) and the pluripotent pericytes (15, 37). Shortly after that, the epiblast generates the three primary germ layers: ectoderm, mesoderm and endoderm that are responsible for the building of all tissues and organs in a vertebrate organism (Fig. 1; 43). It is well known that the ectoderm is the ancestor of the neural crest (see below).

Until now, different ancestors of the Leydig cells were supposed, such as: mesodermal (mesenchymal-like) stem cells (4, 5, 9, 25, 44-52), peritubular myoid/fibroblast stem cells (19, 20, 53-60), peritubular or perivascular fibroblast-like stem cells (45, 50, 51, 53-55, 61, 62, 64-70), neural crest stem cells (48, 71-77), perivascular stem cells (63, 78, 79), endothelial cells (63, 80), bone marrow cells (81, 82), the pericytes and the smooth muscle cells of the microvasculature (14, 15). Also multiple origins are presumed (19, 20, 52,

58, 83). In this respect connective tissue cells that include lymphatic endothelial cells, perivascular fibroblast-like cells and pericytes were mentioned (54, 61, 84, 85)

Thus the problem of the origin of the Leydig cells remains still controversial. There are two main reasons for this fact. In the most studies the fast transdifferentiation (14, 15, 86) of the stem cell progeny (the transit amplifying cells) into immature Leydig cells was not really documented. This caused a serious difficulty in the establishment and understanding of the Leydig cell genetic lineage. Further, a subpopulation of human perivascular cells that express both pericytes and mesenchymal stem cell (MSC) markers *in situ* was identified (22). If isolated in culture this population expand and show clonally multipotent features, providing evidence that MSCs found throughout fetal and adult tissues are members of the pericyte family of cells. Thus blood vessel walls harbor a reserve of stem/progenitor cells that may be integral to the origin of the elusive MSCs and other related adult stem cells (78, 87). Taking into account the spatially and temporally association of the microvasculature and the adult (Leydig) stem cells and that the cardiovascular system

Figure 2. The diagram shows the relationship between the testicular blood vessels and the seminiferous tubule. IV – intertubular vessels; PTV- peritubular vessels. The green oval blood vessel components illustrate the pericytes. Note the close relationship between the blood vessels/pericytes and the tubule wall and that parts of the tubule surface are free of blood vessels.

becomes the first functioning system in the embryo (88-90), it is surprising that in the most studies no comparison between the course of the testicular vasculature (91-94) and the localization of the stem Leydig cells was done. This close relationship between Leydig cells and blood vessels is obligatory taking into consideration that the Leydig cells are neuroendocrine cells (95). Even the important close spatial, functional and organizational relationship between the testis vasculature and the seminiferous tubules from early embryogenesis to adult was not really respected (60, 96, 97) (Fig. 2). The ignorance of this fact led to the impression for the peritubular location of the stem Leydig cells (see below). However, numerous authors draw attention that Leydig cells are located in the vicinity or in close contact with testicular blood vessels (87, 98-100) but this fact was not interpreted satisfactory and in details.

ORIGIN OF THE PERICYTES

The pericytes are a brilliant achievement of the biological nature aiming to supply every organ with an omnipresent population of pluripotent adult stem cells. The pericytes originate very early in the embryogenesis from the epiblast (primitive ectoderm). The pericytes are pluripotent stem cells (such as

the epiblast; 23). They possess ectodermal, endodermal and mesodermal lineage progeny. The adult stem/progenitor pericytes (the epiblast representatives) of the adult vertebrate organism are located in authentic microvascular niches as reserve (resting, silencing) adult stem cell population for tissue/organ generation, maintenance, repair and regeneration.

For a long time the origin of the pericytes was presented as multiple (15, 101). Meanwhile a lot of information accumulate that the pericytes originate from the pluripotent epiblast and represent pluripotent stem cells (22, 23, 41, 42). They retain the possibility to produce cells characteristic for all three embryonal germ-layers: ectoderm, mesoderm and endoderm. This is also the reason why the progeny of the pericytes is so meaningful and variable and why these cells in different stages of differentiation and variable environments may have mesenchymal, neural and epithelial characteristics. As Dore-Duffy (25) emphasized, once isolated, pericytes rapidly differentiate along multiple lineages depending on the regulatory signals present in the microenvironment. It is this pluripotentiality and the ability to migrate as well as the lack of pericyte specific markers that has led to the enormous confusion about this cell type. This also provides serious difficulties for the scientist to follow a logical lineage differentiation or pathway of the pluripotent pericytes. Moreover, in cell cultures these cells may behave different as *in vivo* which was also evidenced for the mesenchymal stem cells and the mesodermal progenitor cells (17, 102-104). In this respect Zwaka and Thomson (21) pointed out that it is remarkable that permanent pluripotent embryonic stem cell lines can be derived from preimplantation embryos at all, because, *in vivo*, pluripotent cells of the early mammalian embryo proliferate only briefly before becoming cells with a more restricted development potential. Moreover, the authors (25) underline that the embryo-derived stem cells are in some sense tissue culture artifacts (105-107) that do not exist in this form and functions in the living organisms. As discussed below, the representatives of the embryonal epiblast become disseminated through the whole organism with the differentiated blood vessels and remain in specialized niches of the vascular wall as reserve adult stem cell population named pericytes (108). Additionally, evidence accumulate that the pericytes are the adult stem cells in the vertebrate organisms (22, 26, 27). They are the ancestors of the mesenchymal stem cells (MSCs) (22, 28, 100, 109, 147).

PERIENDOTHELIAL *VERSUS* PERIVASCULAR LOCATION OF THE STEM CELLS

A real problem in the publications concerning not only the Leydig cells is the absence of the exact location of the presumed stem cells. It is not equal whether a cell has a periendothelial (embedded within the basement membrane of arterioles, capillaries and post-capillary venules and in contact with the endothelial cells) or perivascular position in the adventitia of the vessel wall (outside the basal lamina and being not in contact with the endothelial cells). In the first case the stem cell is situated in an authentic vascular niche (15, 24, 110-112), whereas, the adventitia contains daughter cells of the vascular stem cells (the pericytes as ancestors) that proliferate, migrate, transdifferentiate and further, in the perivascular space ("stem cell niche of the vascular adventitia"; 78) and the distant interstitium, differentiate towards transit amplifying (51), immature and mature cells. This means that the pericytes are true pluripotent stem cells, whereas their progeny (transit amplifying cells) termed in the adventitia "mesenchymal stem cells" (MSCs or adventitial progenitor cells – APCs; 113, 114) are multipotent. Thus, in the vascular wall pericytes and endothelial cells coexist with MSCs both of which are interpreted by (115) as possibly progenies of adipose tissue-derived stem cells (ADSC) or more precisely as vascular stem cells (VSC) that are multipotent progenitor cells with reduced potency (116, 117). The capability of the transit amplifying cells, respectively the mesenchymal stromal cells to migrate away from the vascular adventitia (and/or vascular *tunica adiposa*) explain the existence of extravascular, in addition to the perivascular positioned MSCs (114).

PERICYTES OR MESENCHYMAL STEM CELLS (OR ACTUALLY, MULTIPOTENT MESENCHYMAL STROMAL CELLS)?

A main problem is also that numerous authors do not make difference between pericytes and multipotent or mesenchymal stromal cells (MSCs) (30, 118-120). As mentioned, the pericytes have a periendothelial position, whereas the MSCs are situated perivascularly (218). Numerous studies postulate that pericytes and mesenchymal stromal cells (MSCs; until now dubbed mesenchymal stem cells) are the same cells or that all mesenchymal stem cells are pericytes (22, 24, 26, 27, 52). This view is partially correct but there is now strong evidence that the pericytes are the ancestors of mesenchymal stromal cells (22, 24, 27, 48, 121-124), because especially the earlier MSCs - descendants share close similarities with their pericyte ancestors. Pericytes are highly undifferentiated

pluripotent cells (125) whereas the MSCs as more differentiated are multipotent. Furthermore a lot of authors interpret the existence or the absence of one or two marker substances as enough to define a different lineage of cells, which are in fact pericyte daughter cells in various stages of differentiation. There are results showing that the MSCs (APCs: adventitial progenitor cells) in cell culture conditions possess the capability to de-differentiate toward pericytes: "centripetal" relationship (17, 19, 126). The reason for such events is the great plasticity of the stem cells appearing especially during experimental or pathological conditions.

ARE THE PERICYTES STEM CELLS, PROGENITOR CELLS OR BOTH?

The answer of this question provides some difficulties for the scientists to define the character of the ancestor cells, although in the literature these terms are clearly defined. Concerning the Leydig cells it is necessary to define their stem cells and their progenitors. As our studies demonstrate, the stem cells of the testicular Leydig cells are pericytes and smooth muscle cells of the microvasculature. From the view point that the pericytes are epiblast derivatives that at the beginning differentiate to pericyte, the pericytes are progenitor cells. Taking into account that pericytes are the representatives of the epiblast situated in the vascular niches, the conclusion is that they are adult stem cells with pluripotent qualities that are able to differentiate toward all tissues of the vertebrate body. Thus the pericytes are both stem and progenitor cells.

There is strong evidence that the pericyte progeny change the biochemical composition during its commitment to immature and further differentiation to adult, mature Leydig cells. The leading event is the process of fast transdifferentiation (127) in which the early progeny of the pericytes become transformed to transit amplifying cells, immature and mature Leydig cells that lose their similarities with the stem cell ancestors. This process affects also the potency of the stem/progenitor cells.

It seems very likely that one of the earliest changes of the pericyte progeny in the testis is the expression of the PDGFR- α , followed by the expression of other factors such as Coup – TFII, CD51, CD90, 3 β -HSD and additional enzymes involved in the process of steroidogenesis. These cells are correctly qualified as progenitor Leydig cells (19, 20) because these are migrating transit amplifying cells in process of maturation. However, in numerous publications they are qualified as stem cells (58). It is interesting to note that Kil-

coyne et al (58) established that the Coup-TFII cells express also androgen receptors. This result provides additional evidence that the cells under study are differentiating progenitor cells and not stem cells.

THE PERICYTES BEHAVE AS STEM CELLS. THEY ARE RESTING IN AUTHENTIC MICROVASCULAR NICHES AND IF ACTIVATED UNDERGO ALL CHANGES CHARACTERISTIC FOR TYPICAL STEM CELLS

The pericytes behave principally as stem cells because, located in the vascular niches of various organs (25, 29, 95, 125, 132-136), after activation, they self-renew and give rise to a large number of different cell types, including the Leydig cells. Thus, pericytes of the microvasculature, generally acting as stem/progenitor cells in the body, represent precursor cells in individual organs (nervous system, muscle, skeleton, skin, liver, testis), where they generate population of restricted progenitor, intermediate and differentiated adult organ-specific cells (137-140).

After activation the pericytes self-renew, proliferate, migrate, disrupt the vessel basal membrane, leave the stem cell niche and as transit amplifying daughter cells enter the perivascular space (14, 33) and transdifferentiate to provide committed young (immature) Leydig cells. From the moment in which a daughter cell of the divided pericytes enter the perivascular space, it becomes transit amplifying cell (51), that still possess some pericyte markers, but under the influence of the chemical composition and factors of the perivascular space, rapidly prolongs to differentiate, changes its gene activity and becomes a different phenotype that may do not have any similarity with its ancestor. So there is the possibility that the early changes from the resting pericyte state starts still in the microvascular niche after the activation and proliferation of the stem cell, depending on its dislocation within the niche at the beginning of its migration, as described for the skin bulge stem cells by (111). Some of the activated pericytes become microvascular smooth muscle cells (myofibroblasts), thus pericytes and smooth muscle cells share a common lineage and the unified term pericytes or mural cells is used more often (29, 30, 126). Now, it becomes evident that the pericytes are the periendothelial adult stem cells (e.g. the Leydig stem cells) whereas they progeny, the transit amplifying cells represent the perivascular progenitor cells (e.g. the Leydig cell progenitors). To avoid some confusing interpretations Lin and Lue (141) suggest the term vascular stem cells (VSCs) as a more accurate term for mes-

enchymal stem cells (MSCs). This term includes both the periendothelial pericytes and the adult perivascular/adventitial stem/progenitor cells (APCs). This is important because the most experimentally isolated and cultured mesenchymal stem cells consist of a mixture of both: pericytes and adventitial stem/progenitor cells.

“Multipotent stem cells”, partially cells of the vascular endothelium, were firstly mentioned by Friedenstein *et al* (142, 143). According to (17) because of their high heterogeneity the MSCs have been re-named “multipotent mesenchymal stromal cells” modifying the term “stem” into “stromal” in order to maintain the same acronym and avoid possible over-interpretation of their limited pluripotent potential (121, 144, 145). Recently (17) defined a new perivascular cell population the “multipotent stromal cells” (MSCs). Before that, these authors described the “mesodermal progenitor cells” (MPCs) as a subpopulation of the MSCs (103, 104). Petrini *et al* (103) describe these cells as peculiar large cells, which show embryonic markers, high aldehyde dehydrogenase activity (ALDH) activity, and the potential to differentiate into several mesodermal lineages including mesenchymal and endothelial cells and identify them as “mesodermal progenitor cells” (MPCs). The MPCs do not show features of pericytes and adventitial progenitors. However, that's not the case in the testis, because the pericytes express both Nestin and CD31/PECAM and exactly as the described MPCs lose these markers with their further differentiation and are involved in angiogenesis. Moreover, there is evidence that MPCs act as pericytes within experimental tumors (146, 147).

In addition, only cultured pericyte progenitors and not MSCs in culture are highly immature cells with increased plasticity that yielded neuronal differentiation potential (116, 148). Furthermore, only pericytes, but not MSCs, have the capability to migrate through a mimetic basement membrane and to interact with endothelial cells for stabilization of the tubular vascular structures (33, 149). However, (13) and (149) emphasized, that a controversy remains, as the MSCs don't express several molecular markers specific to the pericytes such as PDGFR- β (platelet-derived growth factor receptor- β), desmin, NG2 (chondroitin sulfate proteoglycan 4), and α -SMA (α -smooth muscle actin) which suggests the existence of cell functions specific only to pericytes or to MSCs, despite the shared markers between these cell types. In this respect, it must be noted, that the MSC are transit amplifying cells which are in process of differentiation leading to changes in the marker expression characteristic for the

pericytes. This shows that the similarity between the ancestor cells and the daughter cells disappear with the progression of their maturity. However, for the pericytes and the Leydig cells there is one exception because the Leydig cells retain the neural markers also in their mature state (14, 15). In addition, the MSCs, as progeny of the pericytes, are multipotent and not pluripotent like their ancestors. In contrast to pericytes, there is no clear evidence for transdifferentiation capacity of MSCs (121).

However, recently published results do not agree with the notion that the pericytes are stem cells (119, cf. 120). These authors (119) admit that their conclusion concerns only the organs under their investigation (brain, heart, tibialis anterior skeletal muscle, and multiple adipose depots) and make sure that in other organs this may be not the case. In this respect, we evidenced that the ancestors of the Leydig cells are the microvascular pericytes (14). This was confirmed in experiments with ectopically autografted adult mouse testes by (9). Also for other organs it was shown that the pericytes play the role of adult stem cells. As Cano *et al* (120) underline, the main problem in the study of (119) is the unsatisfactory distinction of the pericytes and the mesenchymal stem cells. In this respect our results and the results of other authors (22, 26, 149, 150) show that the pericytes and the mesenchymal stem cells possess very high similarity in expressing stem and progenitor cell marker molecules. Detailed interpretation allows the conclusion that the pericytes are the ancestors of the mesenchymal stem cells (paper under preparation). The MSCs are a product of the proliferating and migrating pericytes. Under the influence of local factors the early transit amplifying cells undergo a fast transdifferentiation and convert towards multipotent, premature (intermediate, young) cells which gradually lose any morphological, biochemical and functional relationship (similarity) with their ancestors (219-221) and differentiate further as typical for the organ mature cells (14, 15, 151).

POTENCY OF THE STEM/PROGENITOR CELLS

The pericyte progenitors together with the primitive (primordial) germ cells and the hematopoietic stem cells are the earliest lineage descendants of the epiblast (41, 42).

The true adult stem cells are pluripotent. Beside the primordial germ stem cells, the only effective pluripotent adult stem cell phenotype in the differentiating and adult organism is the pericyte (41).

A lot of authors do not describe precisely the pluripotent

Figure 3. Bromodeoxyuridine (BrdU) in rat pericyte nuclei 2 days after EDS injection. The capillary is designated by the red asterisk and the arrow. Note the close relationship between the vessel/pericytes and the tubules (T) walls. X 550

or multipotent qualities of the members of a cell lineage as well as the restriction of the potency of a perivascular daughter progenitor cell during its further differentiation. It have to be noted that the pericytes behave as pluripotent stem cells (25, 35, 125), whereas the MSCs are multipotent and with their further differentiation they lower the potency becoming oligopotent and unipotent (22, 27). Thus MSCs are the progeny (the daughter cells) of the activated, proliferated and migrating pericytes. Consequently, pericytes represent in fact the true adult stem cells of the vertebrate organism. Only the pericytes lie within authentic vascular niches and are able to disrupt the basal lamina and migrate toward the interstitium of an organ (33, 149, 152-154). In accordance with this explanation, MSCs represent the transit amplifying cells of the transdifferentiated pericytes, which as progenitors differentiate further to premature and mature somatic cells with a changed phenotype. Altogether, MSCs are pericyte-derived multipotent progenitor cells.

THE HISTOLOGY (MICROSCOPIC SHAPE) OF THE ADULT STROMAL STEM CELLS

Numerous authors use the histological shape of a cell as criterion for the determination of its phenotype or cell lineage. Accordingly, the Leydig stem cells have been described as interstitial or peritubular elongated fibroblast-like or small spindle-shaped cells with mesodermal origin (55, 63, 64, 66, 155, 156). It is actually like that the Leydig cell progenitors are spindle-shaped. However, these scientists have ignored the fact that the activated pericytes are also spindle-shaped,

Figure 4. Nestin immunoreactivity in activated pericytes 14 days after EDS application. Note the spindle shaped form of the pericytes on the capillary wall (thick arrows). The migrating pericytes form aggregates in the vicinity of the vessels. Some pericytes have still contact with the vessel wall (thin arrow). X 950

regardless of that they are derivatives of the pluripotent epiblast cells (Fig. 4, 5). Moreover, the space orientation of the stem, progenitor and Leydig cells depends to a great extent from the orientation (intertubular or peritubular) of the testicular blood vessels. In this respect (97) and (60) showed that the testis vasculature (especially the endothelial cells) is responsible during embryogenesis to organize the position of the seminiferous tubules with which they are in very close contact. Our study on the postnatal development of Leydig cells in the rat testis has shown that even on the 60th postnatal day the differentiating Leydig cells are spindle-shaped and keep the contact with the blood vessels *via* their long processes that also cover the surface of the microvasculature (14, 15). Probably this relation is retained during the whole life (Fig.5). As seen in the Figure, in cross or bevel sections the Leydig cell bodies appear round or oval.

It is interesting to note that the peritubular localization of new, from the vessel wall arising, Leydig progenitor cells, after ethane dimethanesulfonate (EDS) destruction of the original Leydig cell population (Fig. 6), depends on the distribution and orientation of the peritubular vessels that supply two neighboring tubules (Fig. 3). In this respect it is very probable that the Leydig progenitor cells become in their further differentiation influences from regulatory signals of the tubular structures (Sertoli cells, germ cells, and myofibroblasts of the lamina propria) (Fig. 3-7) and of the microenvironment that determine their migration, proliferation and differentiation (19, 20, 25). The close contact between the peritubular capillaries and the seminiferous tubules make it indeed difficult to

determine whether cells of *lamina propria* or cells of vascular wall are stem cells. However, our results show unequivocally that the stem cells are pericytes and smooth muscle cells of the microvasculature and that the cells surrounding the seminiferous tubules are their progeny in form of early migrating Leydig cell progenitors (partially transit amplifying cells; 14, 15) expressing steroidogenic factors. At one established period of the testis development the peritubular immature Leydig cells reduce in number, become atrophic or destroyed. In some publications this phenomenon is explained with back

Figure 5. Photo montage of Rat testis interstitium. Cytochrome P450 cholesterol side chain cleavage enzyme (Cyp450scc) immunoreactivity on day 60 of postnatal development. Numerous positive young (immature) Leydig cells possess spindle-shaped form (large arrows). Their outgrowths show contacts with the vessel walls or cover the walls over long distances. The bodies of cross-sectioned Leydig cells in contact with the vessel (asterisk) are round or oval (small arrows). T – tubules. X 380

migration of these cells toward and accumulation within the interstitial space of the testis. It seems more likely that at this time of the fetal development fewer hormones are needed for the composition, maintenance and functioning of the seminiferous tubules which leads to the dedifferentiation, atrophy and disappearance of the unnecessary cells. As consequence the interstitial perivascular cells increase in number and become enlarged. They remain in contact with the blood vessel wall (Fig.5) and supply hormones for the male forming organism.

THE PERICYTES AND THE LEYDIG CELLS ARE NOT PROGENY OF THE NEURAL CREST

To explain some events with the pericytes and keeping in mind that the Leydig cells possess neural properties, we used information concerning the situation in the central nervous system. As mentioned above there is a great similarity between the biology of the developing and adult Leydig cells and the progenitors /immature/mature nerve and glial cells. Also in the nervous system until recently the brain vasculature/pericytes were neglected. However, the first evidence was provided by Palmer *et al* (95) which found that adult neurogenesis occurs within an angiogenic niche (222). Later it becomes evident that in the telencephalon a very well developed subventricular vascular plexus exists (157, 158) and the theory on the neurovascular unit was broadly accepted (159, 160, 161). According to (162) pericytes and astrocytes play important role for the maintenance of the cerebral microvasculature. In this respect, it seems very probable that the brain microvascular pericytes possess the capability to transdifferentiate into radial astroglia (163-166). Thus the conclusion can be made that the pericytes of the subventricular plexus are the neural stem cells (174) which generate the B progenitors (GFAP+ astrocytes; radial glia), that build the C (transit amplifying) cells and the A (neuroblast) cells which are surrounded by an glial tunnel that lead them towards the olfactory bulb (167-169). This hypothesis is also supported by the observation that Nestin and NG2-positive cells are precursors of neurons, oligodendrocytes and astrocytes in the gray matter (170, 171). Similar conditions were reported for the subgranular zone of the hippocampal dentate gyrus (164, 172, 216; Fig. 9).

As evidenced both the pericyte progenitors and the neural crest stem cells (NCSCs) represent very early phenotypes in the embryogenesis which show remarkable similarities in their expression activities (21, 23, 42). This was the reason for the presumption that both cell types originate from the same

earlier ancestor. The similarity between the NCSCs and the pericytes is an unequivocal fact (1, 10, 173, 175-178) what allowed to determine the Leydig cells as neuroendocrine cells and lead to the presumption that the pericytes, resp. the Leydig cells are of neural crest origin (14, 15, 52). However, we were convinced by new results (see below) that the reason for this similarity is the close embryologic timing in arising of these cell types (Fig. 1).

Consequently, the neural crest cells arise at the border of the neural plate and the epidermis at the time of neural tube closure (179, 180). As new data show, in the embryonic ectoderm of the neural fold two different cell populations coexist: authentic neural crest cells and mesectodermal stem cells (181-183). The neural crest cells, derived from the epiblast/ectoderm possess some restricted multipotent characteristics and represent a migratory cell population that generates diverse cell types during vertebrate development (184-186). In comparison, the pericytes originate something earlier direct from the epiblast whereas the neural crest stem cells arise a bit later from the primary germ layer, the ectoderm, which is also a derivate of the epiblast (epiblast/ectoderm; Fig. 1). In this respect (187) reveal that the epiblast cells possess different transcriptomes in comparison with the epiblast/ectoderm. Thus NCSCs arise indirectly from the epiblast and as recent results show they are not pluripotent, but multipotent and are able to produce different cell types with neural qualities, but not mesenchymal cells (4, 83, 183, 223, 224).

There is also evidence that the neural crest cells cannot produce pericytes (9, 83, 102, 182, 183, 188). Therefore the only way that remains is that the pericytes are the progeny of the epiblast whereas the neural crest is the daughter structure of the a bit later arising epiblast/ectoderm (42). Thus, the epiblast arises a little earlier from the ICM (189) than the epiblast-derived germ line, the ectoderm, which is the ancestor of the neural crest (190). The mesenchymal-like cells are product of a sharp from the neural crest stem cells bordered cell group in the neural fold (non-neural epithelial domain: (191). These cells possess the ability to perform an epithelial-to-mesenchymal transition and were termed metablast (9, 181-183, 191, 192) or ectomesenchyme (mesectoderm). As Weston *et al* (182) show, within the neural fold, a sharp boundary exists between E-cadherin-expressing non-neural epithelium and the neural epithelium of the dorsal ridge. In addition, they found that cells co-expressing E-cadherin and PDGFR α are present in the non-neural epithelium of the neural folds. These observations raise the possibility that at least some PDGFR α

ectomesenchyme originates from the lateral non-neural domain of the neural fold epithelium. This seems to be consistent with previous reports (193, 194) that mesenchymal cells emerge precociously from an epithelial neural fold domain resembling the primitive streak in the early embryonic epiblast. The authors underline their hypothesis that neural crest and ectomesenchyme are developmentally distinct progenitor populations and that at least some ectomesenchyme is metablast-derived rather than neural crest-derived tissue. It was also found that the pericytes and the endothelial cells are not neural crest derivatives (102). We presume that the described metablast cells are of epiblast origin and resemble pericyte precursors.

Of note, the neural crest cells arise and migrate after the origin of the developing vasculature and use the blood vessels and the associated extracellular matrix as leading structures (195). Thus, the pericytes are disseminated before the migration of the neural crest stem cells and are not produced by them. With other words the pericytes, resp. the Leydig cells are not neural crest derivatives.

THE PERICYTES AND THE PERITUBULAR MYOID CELLS

There is strong evidence that during normal development and after EDS treatment, new Leydig cells can be seen in peritubular position as sheaths surrounding the seminiferous tubules (15; Fig. 6, 7). A number of authors presume that these Leydig cells arise from the peritubular myoid cells (196, 197). However, some of the authors designate these cells as Leydig cell progenitors (PDGF-A positive) that is more accurate (59). Studies concerning the testis development describe that the peritubular myoid cells are interstitial stromal cells that are attracted by the Sertoli cells, both building the basal membrane that stabilize the differentiating seminiferous tubules. We presume that the peritubular myoid cells are also pericyte progeny (198, 199). As mentioned the activated pericytes build myofibroblasts under different physiological and pathological circumstances (200-202). After the seminiferous tubules become created, developing Leydig cells in form of transit amplifying cells become attracted, to build an additional fetal type Leydig cell sheath around the tubules which

Figure 6, 7. Immunoreactivity for CytP450scc in young rat Leydig cells on day 21 and 30 after EDS. Note the massive peritubular accumulation (Fig. 6) and the lowering the number of the positive peritubular cells on day 30 (Fig. 7). X 450

provide the tubules and the peritubular myoid cells with hormones necessary for the final differentiation of the tubular structures. The close relationship between the blood vessels and the seminiferous tubules provide the best conditions for exchange and influence of substances. Thus, the migrating pericyte progeny move along the peritubular vessels and remain in contact with the tubular surface after being attracted and linked by the Sertoli cells and the peritubular myofibroblasts. In this respect, the Sertoli cells, the peritubular myoid cells and the Leydig progenitor cells exchange signals that regulate these events. The most important among them are PDGF-proteins and their receptors, the androgen receptors and some other factors. For example, it was reported that in the rat fetus PDGF-A expression corresponds with the rapid proliferation (59, 203 in fetal and adult human testis) and the testosterone expression of the Leydig cells. PDGFs were demonstrated in rat Sertoli cells which chemoattract PDGFR-expressing peritubular myoid cells (PMCs) in close proximity of the seminiferous tubules (204, 205). Mariani *et al* (206) found that PDGF-B/PDGFR- β interactions may be involved in the proliferation and migration of peritubular myoid cell (PMC) precursors from the intertubular space to the peritubulum. Ergün *et al* (87, 99) provide evidence that endothelin (ET) produced in Leydig and Sertoli cells can act in a paracrine manner *via* ET-B receptors in the human testicular mi-

Figure 8. Fragment of a seminiferous tubule of a rat testis on day 30 after EDS treatment. The arrow points to a young Leydig cell accumulation showing ubiquitin immunoreactivity. The cells surround a small peritubular capillary (arrow). X 500

Figure 9. Nestin immunoreactivity in the subgranular zone of the mouse hippocampal dentate gyrus. The positive stem cells show great similarity with the radial glia of the cerebral cortex. These cells have a close contact with the microvasculature of this zone. X 300

crovasculature and the peritubular myofibroblast. In addition, (98) found in *lamina propria* of human testis intramural vessels which are partly fenestrated and possess pericytes. This seems to be partially true for the rat testis in which after EDS treatment accumulation of peritubular regenerating Leydig cells around capillaries closely associated with tubules were observed (Fig. 8; 14, 15). Earlier study provide evidence that the testicular pericytes are PDGFR- α and PDGFR- β positive and that at 1 week of postnatal development spindle-shaped cells in the testicular interstitium, positive for PDGFR- α , differentiate to adult Leydig cells which become immunoreactive for all PDGFs and their receptors (197).

Concerning the androgen receptors (AR) it was shown that testicular AR mRNA exists in Leydig cells, pericytes, peritubular myoid cells, and Sertoli cells (207). The Leydig cells are maximally sensitive to androgen during puberty, which is consistent with the hypothesis that androgens facilitate their differentiation. As mentioned, Kilcoyne *et al* (58) found that the interstitial Leydig cell progenitors express androgen receptors (44). It was also established that the androgen action *via* testicular peritubular myoid cells (PMC) is essential for normal testis function, spermatogenesis, and fertility in males (208). Moreover, the androgen action *via* smooth muscle cells of testicular arterioles is important for Leydig cell differentiation, function, vasomotion and fluid dynamics (209, 210). In this respect, (211) presume that tes-

ticular blood vessels could be a target-organ for androgens and may mediate some of the effects of androgens in the testicular microcirculation. It was also found that the suppression of AR elevates the EGFR (epidermal growth factor receptor) and enhances the self-renewal of the MSCs (212). Peritubular myoid cells contain ARs and are involved in retinol processing, which is important for the maintenance of the spermatogenesis (196). There is also no convincing proof that the PMCs are the Leydig stem cells.

In conclusion, the peritubular myofibroblasts may be also derived by the pericyte transit amplifying cells. In combination with the Sertoli cells the PMC become peritubular cells that are important for the differentiation of the seminiferous tubules and the steroidogenesis of the newly arising Leydig cells as well as for the fetal microcirculation. Important significance for these processes seems to have PDGFs and their receptors, also ARs and COUP-TFII and its receptor H3K27me3 (56, 58). The PTM-AR signaling is important for normal development, ultrastructure, and function of the adult Leydig cells (44, 205, 208, 209, 214; 215 - in human adrenals).

CONCLUDING REMARKS

Until now four main sources of the testicular Leydig cells were presumed, namely the mesenchymal stromal cells, the peritubular myofibroblast cells, the neural crest stem cells, and the pericytes. New results provide evidence that the neu-

ral crest stem cells and the peritubular myofibroblast are not able to produce Leydig cells. As the most realistic source remain the pericytes and the smooth muscle cells of the testis microvasculature. Thus, the pericytes are the only effective adult pluripotent epiblast derivative that produces the neuroendocrine Leydig cells of the testis. The pluripotent epiblast cells migrate with the very early developing vasculature and become disseminated and stored throughout the whole organism as pericytes within authentic vascular niches and remain as a pluripotent resting (reserve) adult stem cell population for tissue generation, maintenance, repair or regeneration of different morphologically and functionally cell phenotypes, depending on the existing environmental factors. The neural features of the Leydig cells are result of their origin from the pluripotent epiblast derivatives and not from the neural crest. The similarity between the neural crest stem cells and the Leydig cells is based on the close timing of their origin in the early embryogenesis.

The Leydig cells are so far not neural crest and peritubular myoid cells progeny. The peritubular myofibroblasts, together with the Sertoli cells, contribute for the seminiferous tubules differentiation and the steroidogenic differentiation and function of the Leydig cell progenitors. Finally, the pericytes are the ancestors of the migrating perivascular and mesenchymal stromal cells (until now termed mesenchymal stem cells). Some cells of these populations may possibly de-differentiate “centripetal” to pericytes only under special (experimental or pathological) conditions. Actually, the mesenchymal stromal cells are transit amplifying cells of the activated pericytes that further differentiate toward cell types characteristic for the tissue or organ in which they are situated.

The exact knowledge of the nature and functions of the stem cells and their progeny is of great importance of the understanding of the genesis of a normal organism and the mechanisms of development of a pathological conditions, and raises new ideas in the field of practical medicine.

CONFLICT OF INTEREST STATEMENT

The author certifies that he has no affiliations with or involvement in any organization with any financial interest in the subject matter discussed in this review article.

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