Proliferative Activity of Adipocytes in Adipose Tissue Tumors

E. G. Kolokol'chikova, A. A. Pal'tsyn, A. I. Shchegolev, A. A. Adamyan, and N. V. Chervonskaya

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Electron-autoradiographic study of normal and tumor-transformed adipose tissue (common lipoma and destructive lipoma, *i.e.* infiltrating and degrading lipoma) showed the capacity of adipose tissue cells in lipomas, especially in destructive lipomas, to proliferation and differentiation. *In vivo* synthesis of DNA in mature adipocytes not observed previously is described. The role of microvascular wall cells as mesenchymal multipotent precursors in the formation of the adipose tissue is discussed. The involvement of the bone marrow mesenchymal stem cell in this process cannot be ruled out.

Key Words: *adipocyte formation; precursor cell; lipoma; autoradiography*

Biology of the white adipose tissue attracts special interest in recent years, because this tissue not only regulates the energy balance in the body, but also acts as a dynamic secretory endocrine organ with numerous functions [9,11]. Disorders in adipose tissue content (obesity or lipoatrophy) are closely associated with cardiovascular diseases, diabetes, and, presumably, with the formation of malignant tumors. Hence, the importance of investigating physiological and pathophysiological mechanisms underlying the formation of adipose tissues causes no doubts. The interest to adipose tissue increased during recent years due to the appearance of reports in which adipose tissue is regarded as an alternative (to the bone marrow) source of mesenchymal stem cells for cell replacement therapy [15].

Histogenesis of the adipose tissue is little studied. Many regularities of physiological processes manifest most brightly under conditions of various pathologies; there are great potentialities for investigation of adipose tissue pathology (specifically, tumors) under conditions of surgical clinic; therefore, the aim of our study was evaluation of the morphology and function of adipose cells in normal and tumor tissue.

A. V. Vishnevskii Institute of Surgery, Russian Academy of Medical Sciences, Moscow

MATERIALS AND METHODS

Adipose tissue biopsy specimens and operation material (tumors) from 10 patients (4 men and 6 women aged 40-64 years), including repeated biopsy specimens (2 and 4 times) from 2 patients, were studied by electron microscopy and autoradiography. Tissue specimens were incubated in medium 199 containing ^{3}H-uridine in a dose of 100 μCi/ml (specific activity 26.0 Ci/mmol) or ^{3}H-thymidine in a dose of 20 μCi/ml (specific activity 21.6 Ci/mmol) at 37-38°C for 1.5 h. After incubation and washing from free precursor the material was fixed in 2.5% glutaraldehyde and 1% osmium tetroxide and embedded in epon-araldite. The sections and autoradiographs were prepared as described previously [2] and examined under Leitz light microscope and Phillips CM 10 electron microscope.

RESULTS

Histological analysis of biopsy specimens diagnosed lipoma in 14 cases. By the differences in macroscopic structure, the tumors were tentatively divided into common and intramuscular lipomas (infiltrating, degrading, and destroying the adjacent tissues, *i.e.* destructive lipomas).

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The bulk of normal adipose tissue and tissue of all lipomas consisted of large mature adipocytes, larger than any other cell in the body. Their cytoplasm was filled with fat of homogeneous structure; a small nucleus was shifted to the plasma membrane (ring-like cells). Adipocytes in lipomas were of different size, particularly in destructive lipomas. Destruction of adipocytes of different degree was often observed in these latter tumors.

Changes in the content of adipose tissue in the body can be due to enlargement of adipocytes (hypertrophy) and to increase in their number (hyperplasia) [10]. Adipocyte is a highly specialized cell, which reached the final stage of differentiation, and therefore normally it is not dividing and does not synthesize DNA. The number of adipocytes increases at the expense of proliferation and differentiation of adipocyte precursors (preadipocytes).

Morphologically differentiation of a preadipocyte into adipocyte consists in fusion of numerous lipid droplets into one lipid vacuole, *i.e.* transformation of a multilocular cell into monolocular or unilocular cell. Molecular studies showed that acquisition of the adipocyte phenotype is associated with expression of more than 2000 genes [9].

It is assumed that the main route of adipocyte formation is transformation of fibroblast-like cells into preadipocytes [9]. Accumulation of fat in fibroblasts can be often seen not only in adipose tissue (Fig. 1, *a*). The fibroblast can further transform into a fibroblast-like cell (Fig. 1, *b*); the cell then acquires a round spherical shape. It retains the features of a fibroblast (well-developed granular cytoplasmic reticulum at the periphery), but the number of lipid vacuoles in it increases. We suppose that RNA synthesis in cell nucleus can provide protein production essential for differentiation of a fibroblast-like cell into preadipocyte. This latter one should receive appropriate mitogenic or adipogenic signals and, depending on the predominance of this or that type of signals, it will divide or differentiate into a mature adipocyte [9]. The microenvironment largely determines the fate of the cell: the cell-cell and cell-matrix interactions. The hormones and growth factors positively or negatively influencing adipocyte differentiation were determined [8].

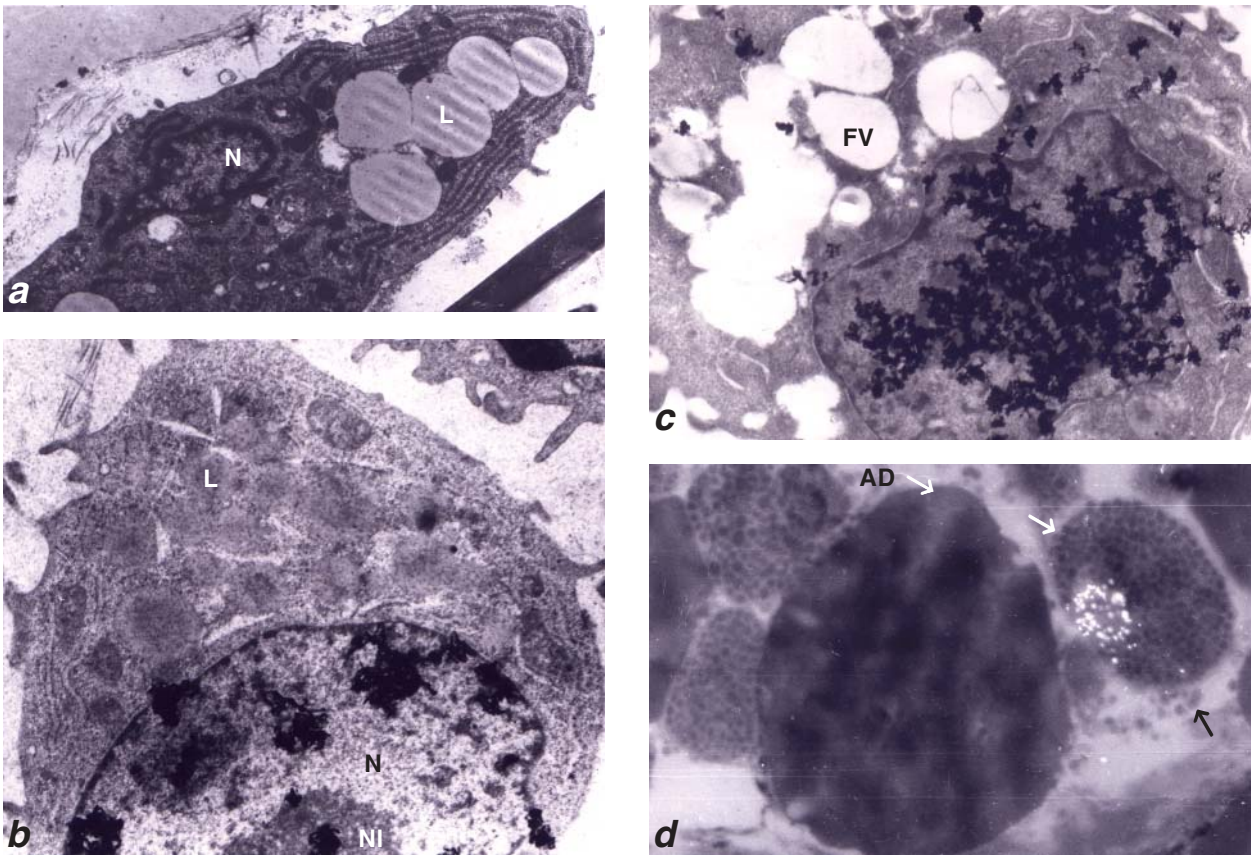

Fig. 1. Preadipocyte differentiation and proliferation in lipomas. *a*) fibroblast with lipid incorporations in the cytoplasm, ×7800; *b*) fibroblast-like cell with active synthesis of RNA in the nucleus (^{3}H-uridine incorporation: black silver grains), ×10,400; *c*) intense production of RNA in preadipocyte nucleus, ×16,000; *d*) DNA synthesis (^{3}H-thymidine incorporation: white silver grains; photograph in reflected light) in preadipocyte nucleus (arrows). Semithin section. Toluidine blue staining, ×1000. L: lipid incorporations; N: nucleus; Nl: nucleolus; FV: fatty vacuoles; AD: adipocyte.

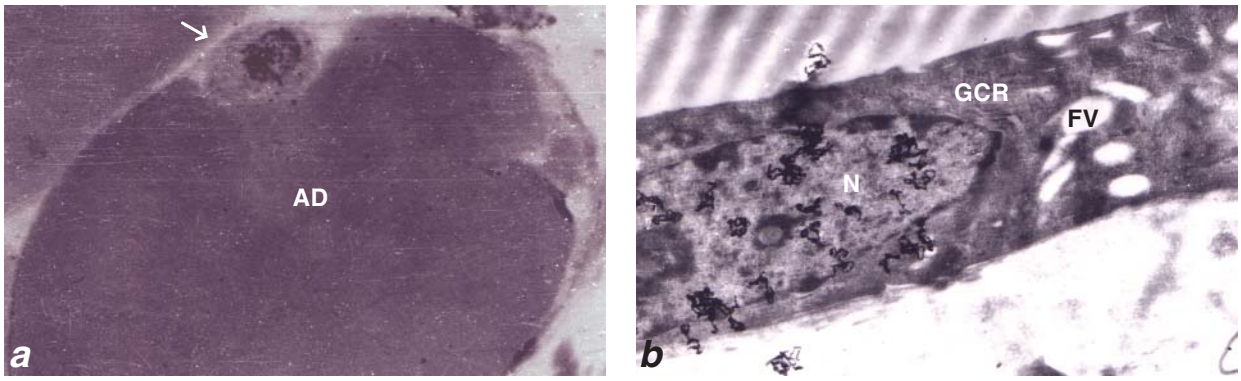

Fig. 2. Proliferative activity of mature adipocytes in fatty tumors. *a*) DNA synthesis (^{3}H-thymidine incorporation: black silver grains; photograph in transmitted light) in the nucleus inside a detached fragment (arrow) of huge cytoplasm of the adipocyte (AD) in destructive lipoma. Semithin section. Toluidine blue staining, ×1000; *b*) DNA synthesis (black silver grains) in adipocyte nucleus (N) surrounded by a fragment of its cytoplasm with a characteristic feature of a fibroblast-like cells: well-developed granular cytoplasmic reticulum (GCR), ×10,000.

Presumably, preadipocytes actively differentiate into mature adipose cells in lipomas. Examined lipomas (but not all lipomas) often contained preadipocytes. Preadipocytes in lipomas were characterized by pronounced polymorphism, mainly by the cell size and nucleus shape and size. Intense incorporation of labeled uridine in their nuclei (RNA synthesis) presumably reflects normal process of preadipocyte differentiation into adipocyte (Fig. 1, *c*).

Proliferative activity of preadipocytes in some lipomas was expressed primarily by incorporation of labeled thymidine (DNA synthesis; Fig. 1, *d*). Moreover, we observed hyperplastic processes in preadipocytes: increase of their size in comparison with, *e.g.*, analogous cells in a wound and appearance of bi- and multinuclear cells.

Division (or at least DNA synthesis) was detected in mature adipocytes in destructive lipomas, though these cells normally and even in many fatty tumors never divide and never synthesize DNA. Their division is difficult to imagine morphologically, considering adipocyte structure, on the one hand, and dynamics of mitotic process, on the other. DNA synthesis in mature adipocytes looked as follows. A small (in comparison with the huge size of the entire mature adipose cell) fragment of the cytoplasm surrounding the nucleus was separated and DNA was synthesized in this fragment (Fig. 2, *a*). We can say that this fragment of cytoplasm with the nucleus underwent dedifferentiation and was transformed into a preadipocyte synthesizing DNA.

The capacity of adult adipose cells to proliferation was shown in studies carried out by Japanese scientists, who also used labeled thymidine [13]. *In vitro* cultured cells at first dedifferentiated into fibroblast-like cells, proliferated, and then again redifferentiated into adipose cells. Active synthesis of DNA was observed in adipocyte nucleus (Fig. 2, *b*). However, the nucleus was surrounded with the cytoplasm, with characteristic features of a fibroblast-like cell (well-developed granular cytoplasmic reticulum), *i.e.* in this case it was also dedifferentiation of a fragment of adipocyte cytoplasm, but even deeper than in the described case with destructive lipoma.

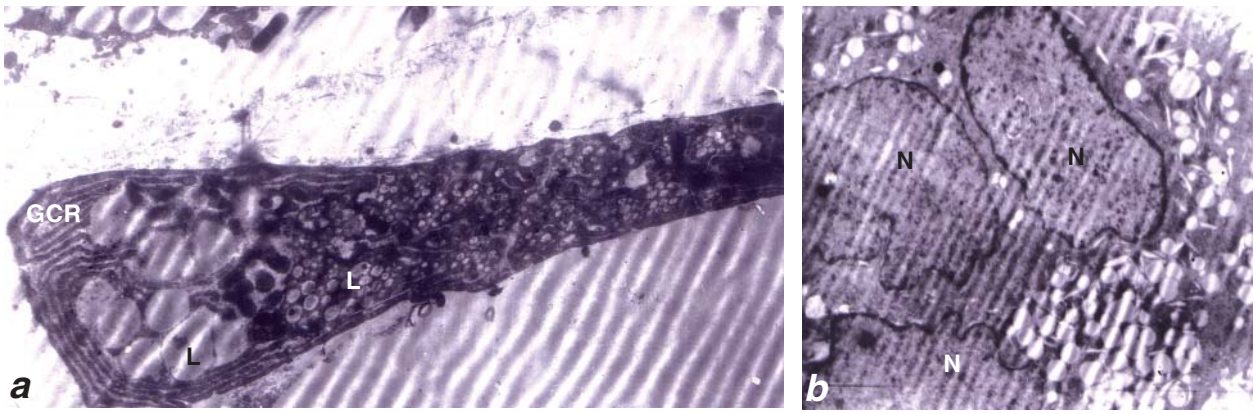

Fig. 3. Morphology of cells in destructive lipomas. *a*) fibroblast-like preadipocyte with well-developed GCR and numerous lipid incorporations (L), ×6000; *b*) three-nuclear preadipocyte, ×11 600.

Fibroblasts in the examined lipomas were characterized by pronounced polymorphism, demonstrating a variety of cellular and nuclear shapes. The cells often retained the characteristic feature of the fibroblast (well-developed granular cytoplasmic reticulum) and at the same time had numerous lipid incorporations (Fig. 3, *a*). The cell (Fig. 3, *a*) can be called a fibroblast-like preadipocyte, demonstrating a histogenetic relationship between fibroblast and adipocyte. These fibroblasts were found in the giant lipoma of the suprapubic area and in the derma of one female patient; preadipocytes remarkable for extreme polymorphism and multiple nuclei were found in the subcutaneous fat of this patient (Fig. 3, *b*). Hence, in this patient active adipogenesis was detected not only in the lipoma, but also in the connective tissue of the skin.

Lipomatosis was diagnosed in two cases. The tissue of these tumors contained many capillaries and, in contrast to other lipomas, their role in adipose tissue formation was clearer.

The stromal-vascular (angiogenic) origination of adipocytes can now be considered proven. *In vitro* studies showed proliferation and differentiation of stromal vascular cells derived from human adipose tissue into mature adipocytes under the effects of many factors [9]. In addition, differentiation of these stromal vascular cells into chondrogenic, osteogenic, and myogenic cells in the presence of line-specific inductors was attained [9,15].

Pronounced proliferation (mitoses, binuclear cells, incorporation of labeled thymidine, *i.e.* DNA synthesis) was observed in endotheliocytes, pericytes, and cells located near the vessels (Fig. 4, *a*). These were fibroblasts or undifferentiated cells. Mitoses were also seen in undifferentiated cells. Presumably, these proliferating cells provided adipose tissue growth. This

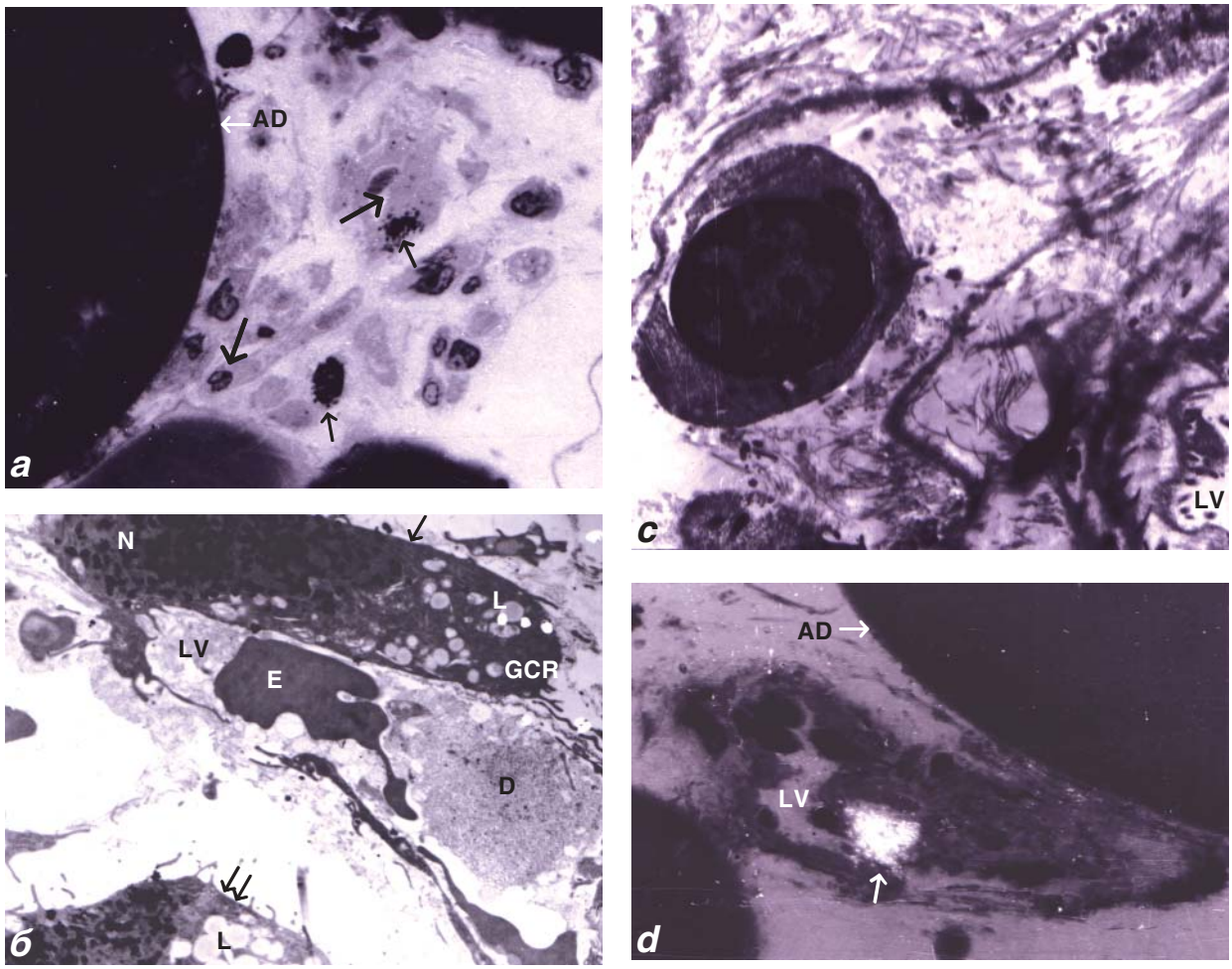

Fig. 4. Differentiation and proliferation of capillary cells in adipose tissue tumors. *a*) DNA synthesis (black silver grains) in vascular endotheliocyte and the cell situated near capillary wall (short arrows). Vascular lumen: long arrows. Semithin section. Toluidine blue staining, ×1000; *b*) intense synthesis of RNA (black silver grains) in the nucleus of a fibroblast-like cells (arrow) in the wall of a destroyed vessel at the site of endotheliocyte and in the preadipocyte nucleus (double arrow), ×8000; blood cell in vascular wall, ×6000; *d*) DNA synthesis (white silver grains) in the cell (arrow) situated in vascular lumen. Semithin section. Toluidine blue staining, ×1000. E: erythrocyte; D: detritus; LV: vascular lumen: AD: adipocyte.

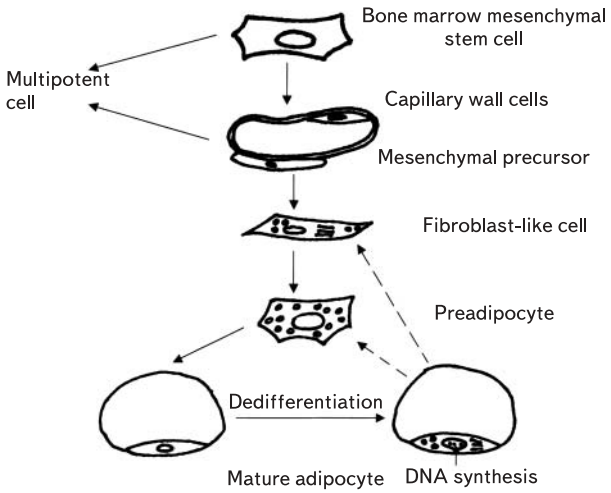

Fig. 5. Stages of adipocyte differentiation in adipose tissue tumors (scheme).

was confirmed by not only active proliferation, but also differentiation of capillary wall cells. The presence of a fibroblast-like cell with lipid incorporations in the cytoplasm and intense RNA synthesis in the nucleus in the wall of destroyed vessels indicated active differentiation of this cell into preadipocyte (Fig. 4, *b*). Preadipocyte near the same vessel, also producing RNA (*i.e.* differentiating) confirms our hypothesis.

Hence, capillaries in tumor tissue were centers of proliferation, the processes of formation and differentiation of new cell elements were observed around them. Cells of hematogenic origin were also seen near the vessel (Fig. 4, *c*). The study of cell morphology suggests that this is a bone marrow mesenchymal progenitor or precursor cell capable of differentiating into adipose cell.

It is known that bone marrow stroma contains, apart from hemopoietic stem cells, multipotent mesenchymal stem cells capable of differentiating into different types of connective tissue [5,12]. The number of these cells is presumably not high (1/100,000-1/1 000,000), but they can circulate in the peripheral blood and, if necessary, replenish the pool of tissue mesenchymal stem cells in their "tissue niche". The data on vascular wall capillaries suggest that the capillary wall serves as this "tissue niche" for adipose tissue mesenchymal precursors.

A cell actively producing DNA (cell capable of proliferation) is situated in the vascular lumen. Presumably, it is a progenitor mesenchymal cell capable of incorporating into the capillary wall (Fig. 4, *d*). Published data indicate that bone marrow cells can replenish various tissues, including the vascular endothelium [7,14].

We summed up these data on the origin and differentiation of adipose tissue cells and tumors (Fig. 5).

Hence, our findings make us change the idea of adipose tissue in general as inert mass. The diagnosis of lipoma unites tumors of different types. Proliferation and differentiation capacity of adipose tissue cell most clearly manifests in lipomas, particularly in destructive ones. Blastomatous changes in these latter tumors can lead to heretofore not observed *in vivo* production of DNA in mature adipocytes. Capillaries are the centers of proliferation and differentiation. "Resident" multipotent cells are situated in capillary walls. The pool of multipotent mesenchymal cells of capillary wall can be replenished by undifferentiated mesenchymal (stem) cells from the bone marrow.

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