LETTER TO THE EDITOR

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Prolonged *in vitro* expansion alter the biological and morphological properties of adipose stem cells

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Dear Editor,

We have read with great interest the review article by Şovrea *et al.*, about human adipose stem cells (ASCs) with emphasis for stem cell-based therapies [1]. Authors provided many important information concerning ASCs biological properties, procurement and isolation techniques, differentiation capacity and their potential for clinical applications. This article has a high educational value for healthcare professionals working not only in the field of regenerative medicine and tissue engineering, but also for wide spectrum of the clinicians and scientists, as we believe that the constant promotion of this issue can facilitate the transfer of advanced medical approaches to clinical practice [2]. Nevertheless, we want to contribute information on the influence of *in vitro* expansion, which is necessary to provide sufficient number of ASCs on selected characteristics of these cells. Many works suggest that long-term cultivation may significantly alter morphology and biological characteristics of cells and thus may present a significant biological risk [3–5].

The results from our previous studies also support these statements. As present on Figure 1, the most noticeable changes were in morphology. The first alterations were recorded after the 7th passage. At this time, the cells became much larger, and we observed an increase in the number of significantly wider cytoplasmic projections. Other morphological changes were recorded as the number of passages increased. In higher passages (20–30), ASCs demonstrated irregular morphology as the cytoplasm became more granular with debris formation increasing within the culture medium. Transmission electron microscopy (TEM) showed accumulation of numerous electron-dense bodies and lamellar structures (Figure 2) within cytoplasm during later passages (25–30). The analysis of cell cycle and the expression of cell cycle and apoptosis regulators also revealed a significant influence of prolonged cultivation on the distribution of cell cycle phases as well as on expression of cyclin-dependent kinase 1 (CDK1) and B-cell lymphoma-2 (Bcl-2). In later passages, proliferation decreased while the expression of tumor protein 53 (TP53) reached high values that probably indicated the accumulation of deoxyribonucleic acid (DNA) damage and the initiation of apoptosis. This was also underlined by the fact that the majority of ASCs were arrested in G0/G1 phase (Figure 3). On the contrary, telomerase activity was not detectable even in the later passages and did not record any alternation in function of apoptosis regulators.

In summary, we would like to underline significance of ASCs as a promising tool of modern regenerative medicine. However, there are still several obstacles concerning mainly their biological safety that have to be resolved prior to ASCs translation into the clinical practice.

Conflict of interests

The authors report no conflict of interests.

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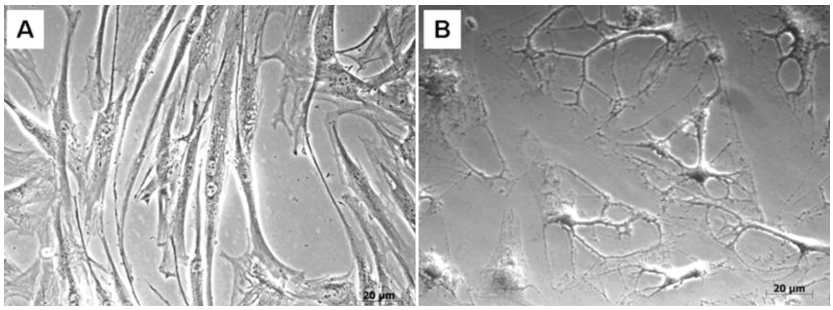

Figure 1 – ***Representative micrograph of human adipose stem cells grown* in vivo *after the 7th passage (A) and during the last 10 passages (P20–P30) (B). Scale bar: 20 µm.***

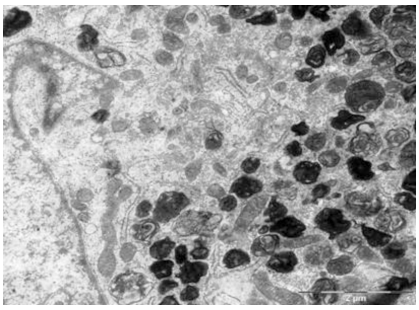

Figure 2 – ***Representative electro-micrograph of human adipose stem cells taken for processing during the last five passages (P25–P30). Scale bar: 2 µm.***

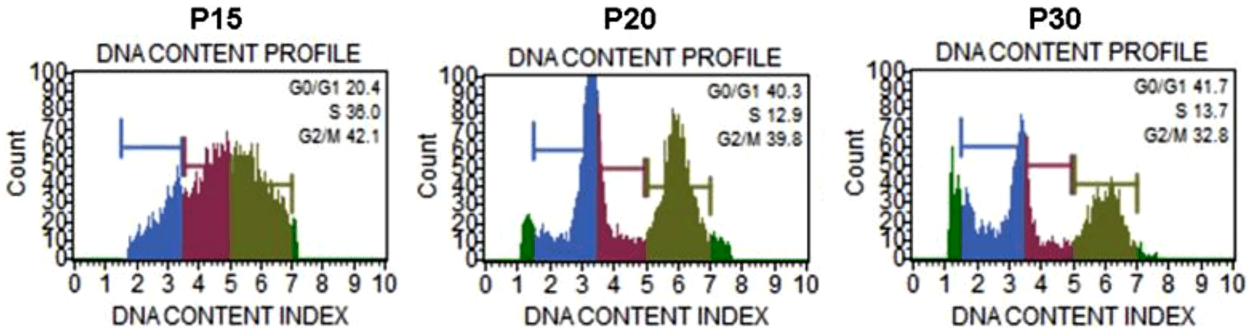

Figure 3 – ***The distribution of cell cycle phases G0, G1, S, G2 and M during prolonged* in vitro *expansion of human adipose stem cells as analyzed using Muse™ cell cycle reagent and detected by a Muse™ cell cycle analyzer. DNA: Deoxyribonucleic acid; P: Passages (15, 20 and 30, respectively).***

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