Commentary

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Immortalization of Human Adipose-Derived Stem Cells as Useful Tool to Produce Cell-Secreted Paracrine Factors for Future Applications in Cell Therapy and Regenerative Medicine

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Multipotent mesenchymal stem cells (MSCs) were isolated for the first time by Friedstein from adult bone marrow [1]. During the following years, MSCs were identified and isolated from other tissues such as trabecular bone [2], skeletal muscle [3], skin [4] and umbilical cord [5]. Since from their discovery, MSCs became an attractive field of research for Cell-based Therapies (CT) and Regenerative Medicine (RM). Indeed, it has been widely recognized that MSCs exert immunomodulatory, pro-angiogenic, anti-apoptotic and pro-survival activities and have the ability to regenerate damaged tissues. It is now ascertained that the regenerative properties of MSCs are related to their capability to secrete cytokines and growth factors (paracrine mechanism), rather than to their differentiation potential.

Human Adipose-derived Stem Cells (hASCs) are MSCs isolated from adipose tissue. Compared to other MSCs, hASCs have the great advantage to be obtained from human fat specimens with relative feasibility, greater stem cell yields than from other stem cell reservoirs and, most important, minimal invasive procedures.

Several studies have demonstrated that hASCs secrete multifarious cytokines and growth factors. Vascular endothelial Growth factor (VEGF), Hepatocyte Growth factor (HGF), transforming growth factor-beta (TGF-β), Stromal Cell derived Factor-1 α (SDF-1α), and Interleukins 6 and 8 (IL-6,-8) are the most prominent molecules [6-9], however such proteins represent only a minimal portion of the whole hASCs secretome.

Taken together, all the above mentioned points render these cells a very interesting tool for CT and RM [10,11].

To date, hASCs and stromal vascular fractions (SVFs) have been or are currently under investigation in several clinical trials to ascertain their suitability in CT and RM [10]. Over 40 clinical trials using adipose-derived cells conducted in 15 countries have been registered with the NIH, the majority of which are Phase I or Phase I/II safety studies [12]. These studies make use of freshly isolated or cultured and expanded cells; moreover, their administration is by an autologous or allogeneic manner. Thus, some considerations about cell yields, characterization, manipulation and time required to expand cells and patient safety should be done. In fact, in clinical trials involving freshly isolated hASCs defining a precise cell number to be administered and characterizing them (i.e. phenotype, caryotipe and DNA analysis for heterologous applications, etc.) is almost difficult. On the other hand, in clinical trials involving hASCs culture and expansion, the time required to obtain cells could represent an important obstacle in some pathologies where it is important to urgently intervene. Nevertheless, items such as risks associated to technical operations and hypothetical biological aberrations (e.g. DNA mutations) should be evaluated.

Another drawback related to *in vitro* hASCs expansion could be the difficulty to obtain elevated amounts of cultured cells for repeated administrations. In fact the phenomenon termed "replicative senescence", which naturally occurs in cells plated for long time, leads to an irreversible growth arrest and subsequent impossibility to proliferate [13]. Different stimuli are able to induce a senescent state [14]; at some extent, this process depends on the "molecular clock" theory, which considers progressive shortening of chromosomes ends (telomere ends) as sign for cells to cease divide and proliferate with consequent arrest in G1/S phase of the cell cycle [15-17]. From a different point of view, this phenomenon guarantees undesirable cell-cycle control escape, which could promote tumorigenesis [18,19].

By considering all the above reported aspects, direct cell

administration could negatively affect CT and RM, at least in part.

A potential innovative hypothesis to fully exploit stem cell paracrine properties in CT and RM could be conditioned media administration. There are several pre-clinical studies showing the effectiveness of secretome treatment. For example, some findings have elucidated a possible role of cell-conditioned media with regenerative activities in models of wound-healing [20-22], chronic kidney disease [23], stroke [24] and lung injury [25]. More in general, soluble factors and microvesicles could represent better therapeutic strategies for regenerative medicine [26]. Cell-free media carrying a therapeutic potential comparable to stem cell transplantation could offer an obvious advantage for a clinical translation in the future. Indeed, conditioned media (containing secreted factors) can be manipulated and managed easier than cells in terms of manufacture, freeze-drying, packaging and delivery. More importantly, avoidance of direct cell administration largely limits tumorigenicity and rejection problems [27].

Interestingly, at the moment two clinical trials are currently evaluating hASC secretome therapeutic effectiveness on hair follicle regeneration and wound healing [28,29].

Under this pioneering perspective, the only limitation might be the senescence occurring during hASCs expansion to obtain elevated cell number and, subsequently, conditioned media.

A possible alternative to circumvent cell expansion limitations imposed by replicative senescence is cell immortalization. This strategy requires abrogation of p53 and pRB-mediated terminal proliferation and/or activation of a telomere maintenance mechanism [30]. Several methods have been developed for immortalizing cells *in vitro* [31]. Among these, introduction of viral genes [32], such as SV40 [33] or HPV E6/E7 genes [34] and hTERT gene [35,36] have been widely used.

Based on the above premises, we recently proposed the "hybridoma-like" method as an alternative strategy to unify hASCs paracrine properties, cell immortalization methods and conditioned media administration. For this reason we aimed to immortalize human hASCs, compare and characterize them with non -immortalized cells by phenotypic and functional *in vitro* evaluations [37]. Moreover, by administering serum-free conditioned media *in vivo* to assess angiogenic and regenerative potential of immortalized hASCs secretomes, we observed that the media alone were highly effective to induce a response [unpublished data].

A previous study demonstrated that MYC-mediated hESC-MSC immortalization was effective in producing an infinite supply of cells to produce exosomes as either therapeutic agents or delivery vehicles [38]. At least in part, these findings confirm that cell immortalization might be the right strategy to obtain large amounts of secreted factors and microvesicles. In addition, the relatively recent generation of safer lentiviral particles for transducing cells has been described [39,40]. Thus, employment of these lentiviruses to immortalize hASCs could further contribute to render the "hybridoma-like" method more affordable and not dangerous.

In conclusion, this "hybridoma-like" model could represent a new strategy to produce large amounts of soluble factors and to bypass limits imposed by senescence occurrence (process scale-up), and a proper manner to maximize hASCs therapeutic efficacy avoiding direct administration of stem cells and potential adverse effects. In fact, production of large quantities of conditioned media could allow a deeper characterization of secreted factors, a more accurate quality control and, possibly, an allogeneic administration. Although further studies will be needed to better understand this model and its potentialities, nevertheless it may represent a useful tool to produce cell-secreted paracrine factors for future applications in CT and RM.

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