Non-adherent mesenchymal progenitors are present in the stromal vascular fraction of freshly isolated human adipose tissue and are able to self-renew in suspension when cultured on their niche

Mehrkens, A; Di Maggio N; Gueven S; Scherberich A; Heberer, M; Banfi, A; Martin, I;
Basel University Hospital, Basel, Switzerland
Senior author imartin@uhsbs.ch

ABSTRACT INTRODUCTION:

Recently, the presence of multipotent mesenchymal cells in the non-adherent fraction of human bone marrow (BM) cultures (1) has been reported and it has been shown that a population of non-adherent mesenchymal progenitors can be cultured in suspension preserving an uncommitted phenotype (2). Although mesenchymal progenitors cells were originally described in the bone marrow (3), they have also been found in the stromal vascular fraction (SVF) of adipose tissue and more recently in a variety of other tissues, such as placenta, synovium and dental pulp (4). This led to the current hypothesis that multipotent mesenchymal progenitors are present in all tissues of the body, possibly in a perivascular position, representing a common reservoir of regenerative cells. Our group could show that non-adherent progenitors of BM stroma (BM-NAMP) represent the most primitive compartment of MSC, capable of self-renewing without loss of proliferation and differentiation capacity when cultured with the initially adhering BM fraction (5). Based on these results, we sought to determine whether the NAMP class of progenitors was specific to BM stroma or could represent a common feature of other mesenchymal compartments, like for example the SVF of adipose tissue (AT-NAMP).

METHODS:

Subcutaneous adipose tissue was obtained from 12 healthy donors (21-69 years old) during routine liposuction, after informed consent from the patient and following protocol approval by the local ethical committee. To determine colony forming efficiency (CFE) and for serial replating experiments, nucleated cells of freshly isolated human adipose tissue were plated at clonal density (9 cells/cm²) and cultured in alpha-MEM with 10% serum alone or with additional FGF-2 (5 ng/ml). After 2 weeks, colonies were fixed in 3.7% formalin, stained with Crystal Violet and counted to assess colony forming efficiency (CFE).

The nucleated isolated cells were analyzed by cytometry for markers specific for mesenchymal, endothelial and hematopoietic lineages and the correlation coefficient of the frequency of positive cells with the CFE of the first replated AT-NAMP was calculated. Data are presented as mean ± standard deviation. The significance of differences was evaluated using analysis of variance (ANOVA) followed by the Bonferroni test for multiple comparisons. P < 0.05 was considered statistically significant.

RESULTS:

Nucleated cells were plated on cell culture dishes (Plate0) and after 3 days the non-adherent fraction was resuspended in fresh medium and replated in a new dish (Plate1). After 14 days the dishes were stained and Plate1 contained a number of colonies equal to 21.3±7.5% (n=8) of the colonies present in Plate 0. The replating of the non-adherent cells in the same dish (Plate0), instead, did not significantly increase the number of colonies (Plate0=12.6±3.3% vs. Plate0*=11.5±2.8%, n=4, p=m.s.), indicating that these CFU-f were stably non-adherent and not simply displaying delayed adherence. To investigate whether AT-NAMP could regenerate themselves as non-adherent progenitors, serial replating experiments were performed. The cells were replated (Plate0) and after 3 days the non-adherent fraction was collected and replated in a new dish (Plate1). At day 7, the non-adherent fraction was removed from Plate1 and replated in a fresh dish (Plate2) and so on until Plate4 after 14 days. The number of colonies steadily decreased as compared to the initial CFU-f (Plate0=100%, Plate1=16.0±9.0%, Plate2=8.4±9.7%, Plate3=1.6±2.9%, Plate4=0.1±0.3%, n=11)-Fig 1. The size of the colonies, instead, increased from Plate0 to Plate1 (4.3±0.5mm and 6.1±0.7 mm respectively). In the following two replating steps (Plate2 and Plate3), there was no increase in colony size (5.9±1.4mm and 5.9±0.8mm). However, the few colonies present in Plate4, displayed an increased diameter size (9.5±0.7mm). To determine whether the AT-NAMP could instead proliferate if cultured with the initially adhering SVF, nucleated cells were plated at clonal density in presence of FGF-2 and the non-adherent fraction was resuspended in fresh medium and replated into Plate0 at each medium change. After 7 or 14 days, the non-adherent fraction was plated into a new dish and the number of colonies and their diameter was assessed after 14 days. When kept in the original dish for 7 (Plate2*) or 14 days (Plate4*), AT-NAMP generated a higher number of colonies as compared to when they underwent a serial replating for the same time (Plate2=12.8±13.2%, Plate2*=56.3±46.5%; Plate4=0.1±0.3%, Plate4*=12.9±66.0%, n=4, p=0.01)-Fig 2a. The colony diameter assessment showed that, maintaining AT-NAMP in contact with the initially adherent SVF, it was possible to preserve their proliferation capacity. In fact, there was no significant decrease in the size of the colonies, despite AT-NAMP underwent proliferation in suspension (Plate0=4.3±0.8mm, Plate2*=5.4±1.1mm, Plate4*=6.1±1.8 mm, n=4, p=m.s.)-Fig 2b. A direct analysis of AT-NAMP phenotype is- so far- not possible due to their exceedingly low frequency in the primary isolated population. AT-NAMP frequency did, instead, significantly correlate with the initial colony forming efficiency (R²= 0.705, p=0.046)-Fig 3a, but not with the diameter of the initially adherent colonies (R²= 0.009, p=0.816)-Fig 3b.

DISCUSSION:

The results show that a population of non-adherent mesenchymal progenitors is also present in human adipose tissue SVF cultures. Similarly to BM-NAMP, AT-NAMP did not simply display delayed adherence, but were stably non-adherent. However, AT-NAMP were not able to maintain them-selves as non-adherent progenitors upon serial replating. When kept in contact with the initially forming niche, instead, AT-NAMP could expand in suspension while preserving their proliferation capacity, suggesting they were able to undergo self-renewing divisions in these conditions, and, therefore, a niche function for the initially adhering SVF. AT-NAMP frequency was only positively related to the initial CFE, indicating that they represent a fixed fraction of the initially adhering CFU-f. In conclusion, the presence of NAMP appears to be a common property of the mesenchymal progenitor compartment of different tissues and not specific to BM stroma alone.

REFERENCES: