Adipose tissue in breast cancer: not an idle bystander but an active participant in breast cancer progression

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Introduction

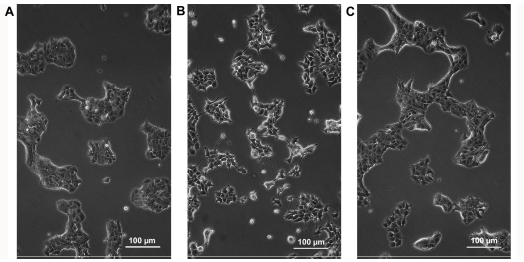
Since a few years it has come to our understanding that adipose tissue is a dynamic organ that secretes a plethora of molecules called adipokines. In contrast with many other cancer types, breast cancer is unique for its direct interaction between breast cancer cells and adipocytes. Moreover, obesity is a known negative prognostic marker for postmenopausal women. A direct influence of adipokines on breast cancer cells is strongly suspected and is of great interest for scientific experimentation.

Materials & Methods

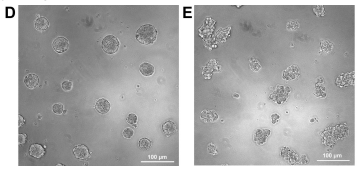
Pieces of adipose tissue were collected from patients undergoing a mastectomy for breast cancer according to the recommendations of the local Ethics Committee. After removal of macroscopic blood vessels and connective tissue, the pieces of adipose tissue were incubated in culture medium. The medium was removed after 24h and checked for its quality by determining the concentration of total proteins, leptin, adiponectin, TNFalpha and triglycerides. This conditioned medium of adipose tissue (CM AT) was used for in vitro experimentation with MCF-7 breast cancer cells. We evaluated the influence of CM AT on the morphology, aggregation, proliferation and invasion of MCF-7 cells.

Results

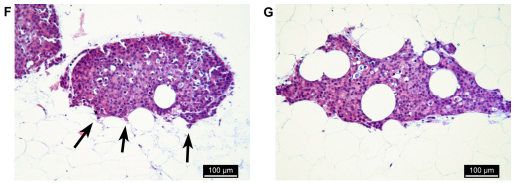
1. Influence of CM AT on morphology and aggregation of MCF-7 cells



When MCF-7 cells are grown in a culture flask, they tend to form smoothly edged compact islands (A). CM AT stimulates cell scattering, a process characterized by cell spreading and loss of cell-cell contacts (B). A rescue experiment in which CM AT was removed showed a reorganization of the smoothly edged island formation (C).

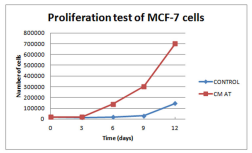


In the slow aggregation assay, MCF-7 cells form compact aggregates under control conditions (D). Aggregate compaction is lost when the cells are treated with CM AT (E).



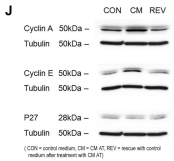
H&E stained sections of MCF-7 spheroids placed adjacent to (F) or inside (G) adipose tissue. Single MCF-7 cells tend to migrate into the adipose tissue (arrows) (F), while the MCF-7 spheroid placed inside adipose tissue shows a massive reorganization into an irregularly shaped mass (G).

2. Influence of CM AT on proliferation of MCF-7 cells



I

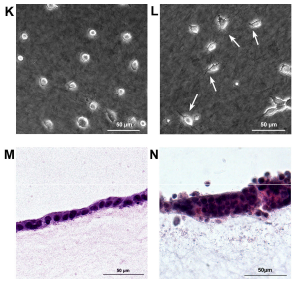
	Control	CM AT
G0/G1	42,2%	50,8%
G2/M	2,5%	5,0%



MCF-7 cells treated with CM AT have a higher rate of proliferation than MCF-7 cells in control medium (H). This was confirmed by cell cycle analysis which revealed a doubling of cells in G2/M phase (I), and by Western blot which showed an upregulation of cyclin A and cyclin E, both positive regulators of the cell cycle (J).

3. Influence of CM AT on invasion of MCF-7 cells

A 24h collagen type I invasion assay revealed invasive characteristics of MCF-7 cells treated with CM AT (formation of polarized cells and extensions, indicated by arrows) (L) while MCF-7 cells in control conditions are round and non-invasive (K).



In contrast, a 14 day transwell invasion test showed no invasion of MCF-7 cells in the collagen gel in the CM AT condition (N). However, the growth pattern was clearly disorganized when compared to the control situation (M).

Conclusion

We demonstrated that adipose tissue secreted factors are able to stimulate the proliferation of MCF-7 breast cancer cells and change their morphology into a pro-migratory phenotype. Unraveling the mechanism behind these observations may provide vital information regarding the link between obesity and poor prognosis in postmenopausal breast cancer.