The role of adipocyte G0/G1 Switch Gene 2 (G0S2) in the crosstalk between cancer cells and adipocytes

JULIE CRÉMER, LAURA BROHÉE , ALAIN COLIGE AND CHRISTOPHE DEROANNE

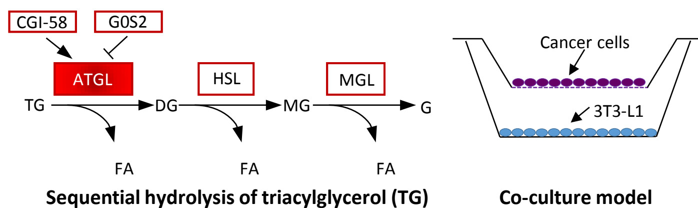
Laboratory of Connective Tissues Biology, GIGA-Cancer, University of Liège, Belgium

Introduction

In solid tumors, cancer cells are intermingled with a microenvironment consisting in stromal cells, immune cells and a large assortment of extracellular matrix proteins. Among the stromal cells present in the tumor microenvironment, adipocytes were reported to up-regulate cancer cells migration and invasion by providing fatty acids and cytokines to tumor cells. On the other hand, tumor cells were reported to alter adipocyte phenotype notably by increasing lipolysis. The molecular mechanisms triggered in adipocytes are however still poorly characterized. In this study, we aimed at identifying key adipocyte genes involved in the crosstalk between tumor cells and adipocytes.

Results

We evaluated the consequences on differentiated 3T3-L1 pre-adipocytes of the co-culture with prostate cancer cells, in particular by determining the expression of lipin1 and of 3 genes regulating the first and rate-limiting step of triacylglycerol hydrolysis: the adipose triglyceride lipase (ATGL), its coactivator CGI-58 and its inhibitor G0S2 which locks the system and prevents triacylglyceride hydrolysis (Yang et al., *Cell Metab* **11**, 194-205).



Sequential hydrolysis of triacylglycerol (TG)

Co-culture model

(1) Co-culture of 3T3-L1 with prostate cancer cells (PC3) induced lipolysis (Fig 1). In parallel, a strong decrease of the expression of G0S2 was noticed in 3T3-L1 upon co-culture with PC3 while the expression of the other genes was not, or only barely, affected. This strong regulation of G0S2 in 3T3-L1 was also observed upon co-culture with murine breast cancer cells E0771. At the end of these experiments, we also noticed an acidification of the culture medium, which prompted us to evaluate the effect of acidosis on gene expression in 3T3-L1.

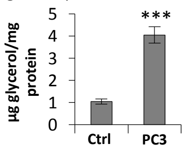


*Fig 1: Free glycerol produced by 3T3-L1A after 48h in presence or not of prostatic tumor cells was measured with the « Free Glycerol Kit » from Sigma. *** p<0,001.*

Gene	Fold increase
Lipin1	0,90
ATGL	1,22
G0S2	0,33
CGI-58	0,90

Table 1: Lipin1, ATGL, G0S2 and CGI-58 expression in 3T3-L1A cultured for 48h in presence or not of prostatic tumor cells was measured by RT-qPCR. Results are expressed as the expression level in 3T3-L1 pre-adipocytes co-cultured in presence of prostatic tumor cells relative to control.

(2) G0S2 expression at both mRNA (Table 2) and protein levels (see Fig.2) was repressed upon acidification to pH value commonly observed in the tumor microenvironment. This regulation of G0S2 was associated with an enhanced lipolysis (Fig.3).

Gene	pH 7,4	pH 7,0	pH 6,7	pH 6,5
Lipin1	1,00	0,87	0,53	0,30
ATGL	1,00	1,05	1,22	1,72
G0S2	1,00	0,88	0,34	0,06
CGI-58	1,00	0,82	0,69	0,52

Table 2: Lipin1, ATGL, G0S2 and CGI-58 expression in 3T3-L1A cultured for 24h at the indicated pH. Results are expressed as fold increase relative to the pH 7,4 condition taken as 1.

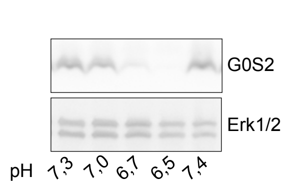


Fig 2: Western blot analyses with specific anti-G0S2 antibodies of total lysates of 3T3-L1A cultured for 24h at the indicated pH. Total ERK1/2 was used as loading control.

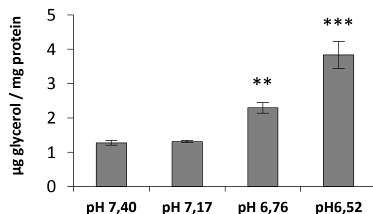


*Fig 3: Free glycerol produced by 3T3-L1A after 24h of culture at the indicated pH. ** p<0,01; *** p<0,001.*

(3) The inhibition of G0S2 expression can be reversed by PPARγ activators. Chromatin immunoprecipitation experiments (ChIP) suggest that CEBP/α is involved in G0S2 regulation.

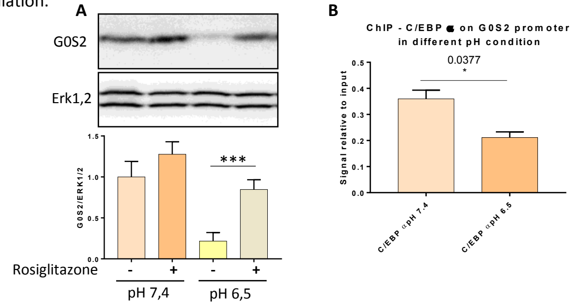


*Fig 4: **(A)** Western blot analyses of total lysates of 3T3-L1 cultured at the indicated pH with (+) or without (-) 2µM Rosiglitazone with specific anti-G0S2 antibodies. Total ERK1/2 was used as loading control. **(B)** Chromatin ImmunoPrecipitation analyses of lysates of 3T3-L1 cultured at the indicated pH. *p<0,05; ***p<0,001.*

(4) To further analyze the role of G0S2 in acidosis-induced lipolysis, we generated 3T3-L1 overexpressing G0S2 in a doxycycline-dependent way. The re-expression of G0S2 barely affected basal lipolysis but strongly reduced acidosis–induced lipolysis (Fig.5) and was sufficient to decrease the pro-migratory effect exerted by adipocyte on cancer cells (Fig.6).

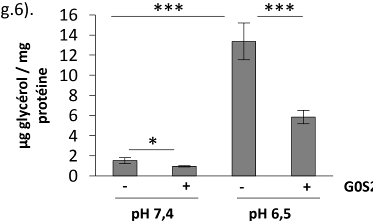


*Fig 5: Free glycerol produced by 3T3-L1mG0S2 cultured for 24h at the indicated pH with (+) or without (-) G0S2 overexpression. *p<0,05; ***p<0,001.*

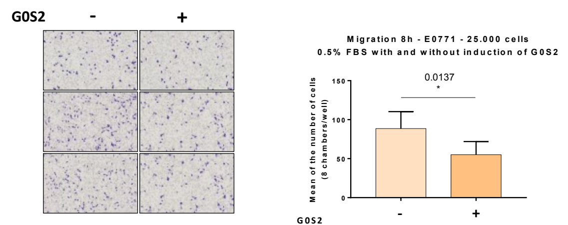


*Fig 6: Breast cancer cells (E0771) were seeded on transwell and co-cultured during 8h with 3T3-L1 overexpressing (+) or not (-) G0S2. **(A)** representative fields illustrating E0771 cells migrating through the transwell. **(B)** number of cells counted in 8 fields per conditions (n=6), *p<0,05.*

(5) The re-expression of G0S2 in adipocytes antagonizes the pro-survival effect exerted by adipocytes on cancer cells treated with the chemotherapeutic agent doxorubicin (Fig.7)

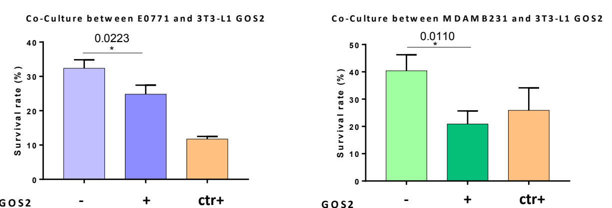


*Fig 7: Breast cancer cells (E0771 or MDA-MB231) were treated with doxorubicine and cocultured with 3T3-L1 overexpressing (+) or not (-) G0S2. Breast cancer cells treated with doxorubicine and cultured alone (ctr+) were included in each experiment to visualize the protective effect exerted by adipocytes. *p<0,05.*

Conclusions

Our results support a key role for adipocyte G0S2 in the crosstalk between adipocytes and cancer cells. Its regulation is necessary and sufficient for adipocytes to modulate the phenotype of tumor cells and to enhance their resistance to chemotherapeutic agents. Analyses of the effects, on tumor growth and metastasis, of a specific overexpression of G0S2 in adipocytes are ongoing.

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