Colorectal Cancer Induces Metabolic Reprogramming in Adipose Derived Mesenchymal Stem Cells by Impairing Mitochondrial Function

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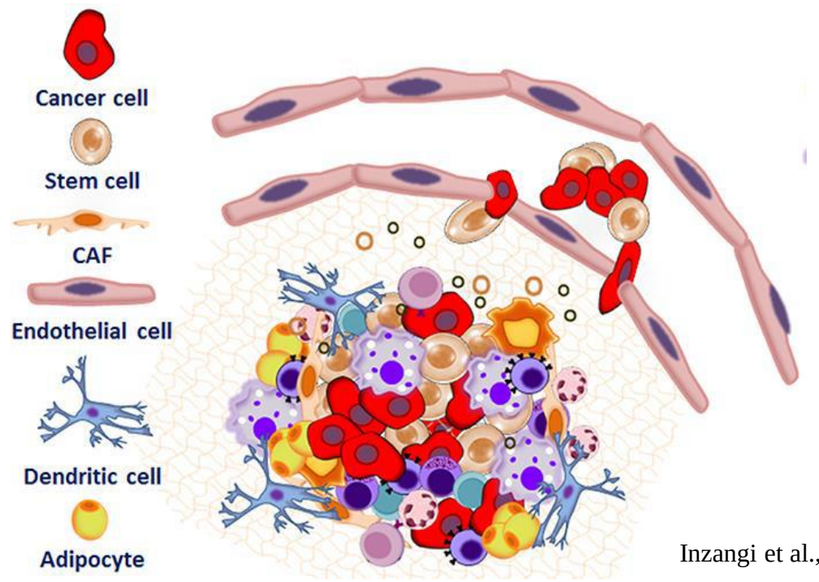
BACKGROUND

Obesity represents a major risk factor for many pathologies including colorectal cancer (CRC). White adipose tissue (WAT) is mainly involved in the development of these diseases, and adipocytes and MSCs represent important components in tumor microenvironment (TME). Evidence showed that MSCs can differentiate in both cancer associated adipocytes (CAA) and cancer associated fibroblast (CAF), sustaining tumor progression. Also, cancer can induce tumor-like metabolic reprogramming in MSCs. Conversely, studies report an anti-tumorigenic effect of MSCs.



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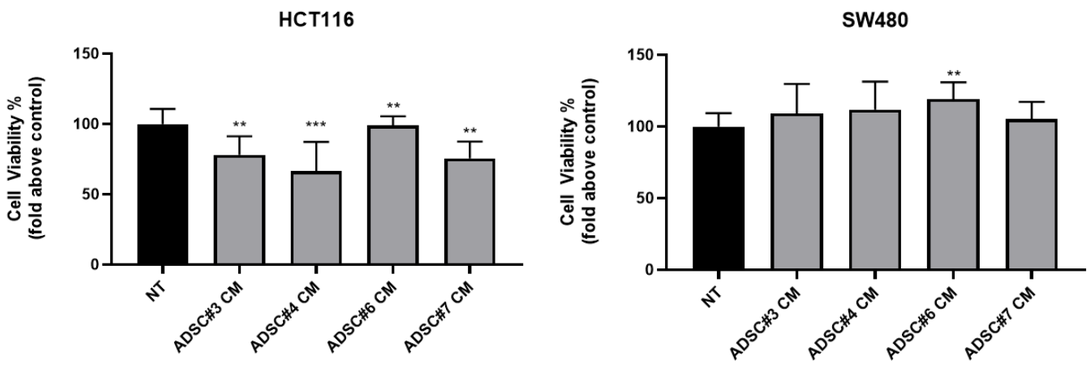
AIM of the STUDY

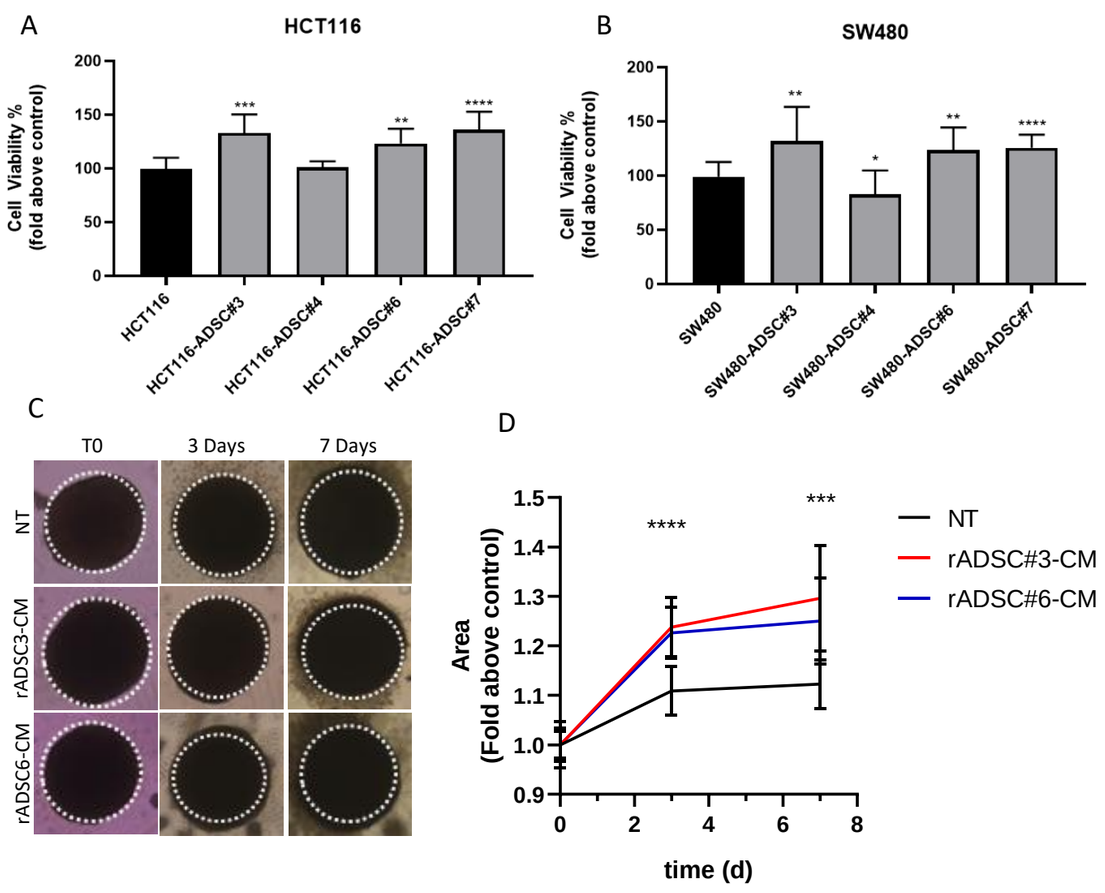
To investigate the interplay between colorectal cancer (CRC) cells and AT-derived MSCs (ADSCs) to clarify the molecular mechanism behind metabolic reprogramming of ADSCs.

ADSCs conditioned medium treatment reduces CRC cell viability



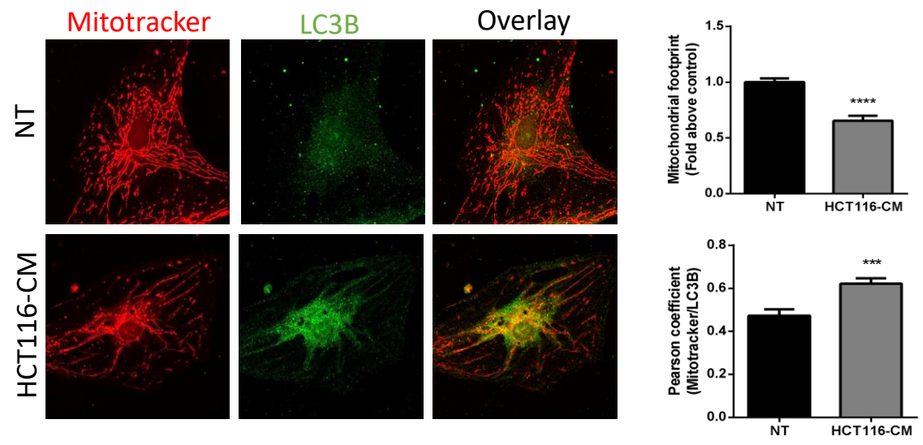
CRC cell line HCT116 and SW480 was treated for 72h with conditioned medium of four adipose derived mesenchymal stem cell lines (ADSC#3 , ADSC#4, ADSC#6, ADSC#7) Viabilities were assessed by CellTox green. **, Student's T-test $p < 0.01$; ***, Student's T-test $p < 0.0001$.

Reprogrammed ADSCs sustain CRC growth



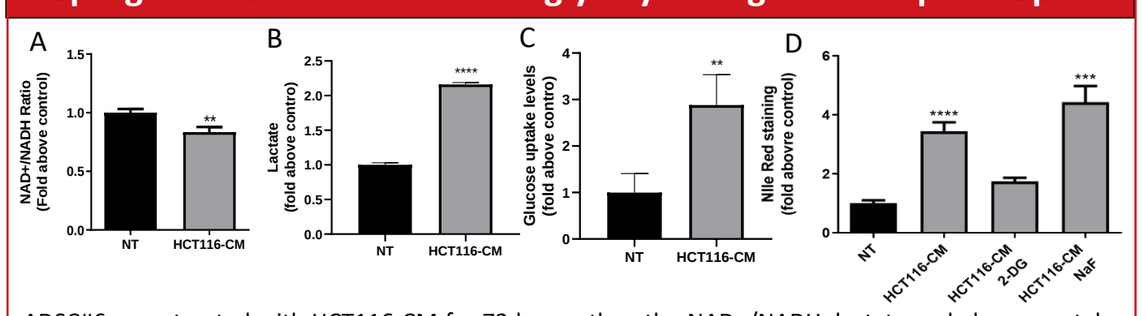
HCT116 and SW480 viability was evaluated by cell count using trypan blue after 2 weeks coculture with ADSC (ADSC#3 , ADSC#4, ADSC#6, ADSC#7) (A, B). HCT116 spheroids dimension was evaluated after 3 and 7 days of treatment with reprogrammed ADSCs conditioned medium from ADSC#3 and #6 (rADSC#3, rADSC#6) (C, D).
*, Student's T-test $p < 0.05$; **, Student's T-test p 0.01; ***, Student's T-test p <0.001; ****, Student's T-test $p < 0.0001$.

Mitochondrial footprint reduction in ADSCs is associated with mitochondrial network alterations



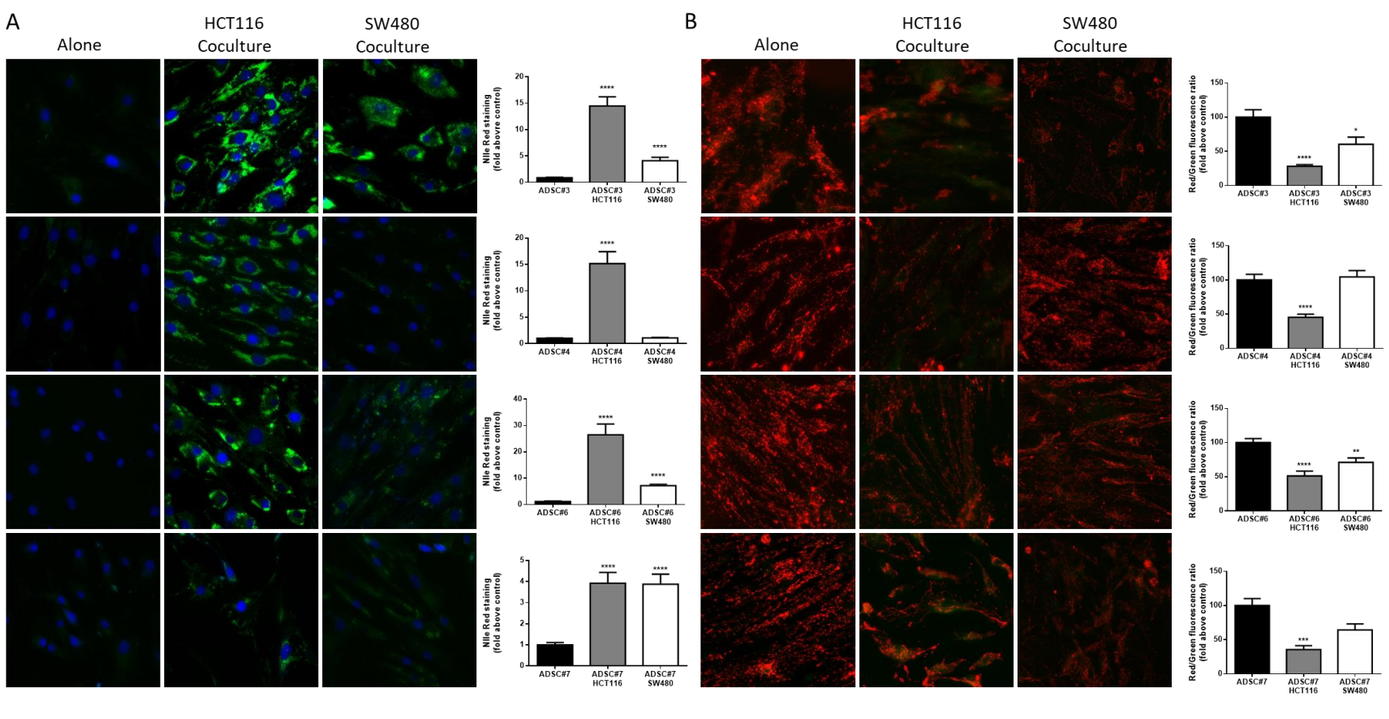
Mitophagy and mitochondrial footprint were evaluated in ADSC#4 after 72hrs treatment with HCT116 conditioned medium, normal medium was used as negative control. Pictures were acquired by confocal microscope. Mitochondria were stained using Mitotracker Red and LC3B was stained using LC3B primary antibody and Alexafluor 488 secondary antibody (green).Histogram showing quantification of Mitotracker and LC3B co-localization expressed as Pearson coefficient. Histogram showing quantification of mitochondrial footprint expressed as mitochondrial footprint area/total area. Data are presented as mean ± standard deviation from two independent experiments.; **, Student's T-test $p < 0.05$.

Reprogrammed ADSCs enhance glycolysis to generate lipid droplets



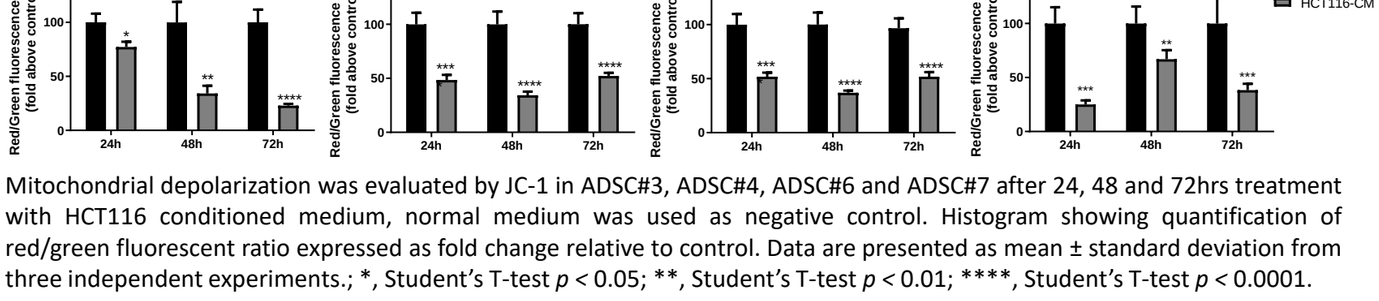
ADSC#6 were.treated with HCT116-CM for 72 hours, then the NAD+/NADH, lactate and glucose uptake levels were measured by NAD+/NADH assay kit (A-C). Neutral lipid accumulation was evaluated by nile red staining in ADSC#6 72hrs treatment with HCT116 conditioned medium alone or in combination with 2-DG and NaF. Data are expressed as fold change relative to control
*, Student's T-Test p<0.05; **, Student's T-Test p<0.01; ***, Student's T-Test p<0.001; ****, Student's T-Test p<0.0001.

ADSCs cocultured with CRC cells show lipid droplets accumulation and mitochondrial damage



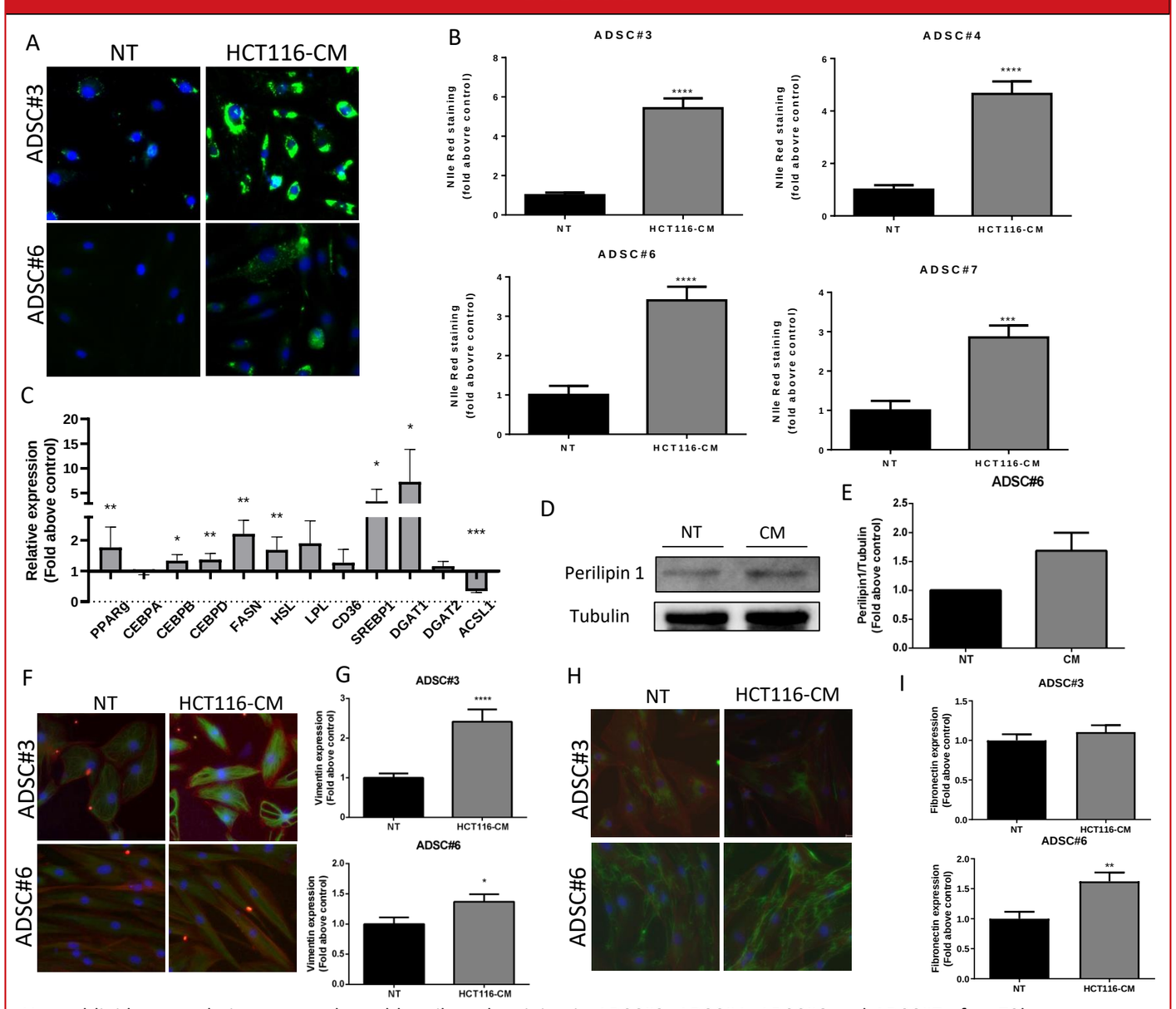
A) Neutral lipid accumulatio was evaluated by Nile red after two weeks coculture of ADSC with HCT116 and SW480. Representative images of ADSC#3, ADSC#4, ADSC#6 and ADSC#7 alone and in coculture. Histogram showing quantification of red/green fluorescent ratio as fold chance relative to control (**C**). Data are presented as mean ± standard deviation from two independent experiments, each performed in triplicate.; **, Student's T-test $p < 0.01$. ****, Student's T-test $p < 0.0001$.
B) Mitochondrial membrane potential depolarization was evaluated by JC-1 staining after two weeks coculture of ADSC with HCT116 cells. Representative images of ADSC#3, ADSC#4, ADSC#6 and ADSC#7 alone and in coculture. Histogram showing quantification of red/green fluorescent ratio as fold chance relative to control (**C**). Data are presented as mean ± standard deviation from two independent experiments, each performed in triplicate.; **, Student's T-test $p < 0.01$. ****, Student's T-test $p < 0.0001$.

HCT116 conditioned medium treatment induces mitochondrial depolarization



Mitochondrial depolarization was evaluated by JC-1 in ADSC#3, ADSC#4, ADSC#6 and ADSC#7 after 24, 48 and 72hrs treatment with HCT116 conditioned medium, normal medium was used as negative control. Histogram showing quantification of red/green fluorescent ratio expressed as fold change relative to control. Data are presented as mean ± standard deviation from three independent experiments.; *, Student's T-test $p < 0.05$; **, Student's T-test $p < 0.01$; ****, Student's T-test $p < 0.0001$.

HCT116 conditioned medium treatment induces adipogenic and fibroblastic alteration



Neutral lipid accumulation was evaluated by nile red staining in ADSC#3, ADSC#4, ADSC#6 and ADSC#7 after 72hrs treatment with HCT116 conditioned medium, normal medium was used as negative control. (A, B). Relative expression of genes associated to lipid metabolism expressed as fold change relative to control (C). Western blot analysis showing perilipin expression (D, E). Vimentin and fibronectin expression evaluated after 72h treatment with HCT116-CM in ADSC#3 and #6 (F, H). Graphs showing quantification of protein expression/nuclei as fold change relative to control (G, I). *, Student's T-test $p < 0.05$; **, Student's T-test p 0.01; ***, Student's T-test p <0.001; ****, Student's T-test $p < 0.0001$.

Conclusions

Our results evidence a strong interplay between ADSCs and cancer cells. Whereas naïve ADSCs conditioned medium can inhibit cancer cell proliferation, ADSCs co-cultured with cancer cells display metabolic reprogramming by lipid droplets accumulation, mitochondrial depolarization, and sustain tumor growth. At the same time, ADSCs treated with HCT116 conditioned medium show lipid metabolism and mitochondrial alterations, suggesting a mechanism of metabolic reprogramming.

UPO AGING PROJECT
UNIVERSITÀ DEL PIEMONTE ORIENTALE